The 112th Abbott Nutrition Research Conference

July 26-28, 2011

Columbus, Ohio

Pregnancy Nutrition and Later Health Outcomes

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Welcome

Abbott Nutrition invites you to review the *Proceedings from the 112th Abbott Nutrition Research Conference: Pregnancy Nutrition and Later Health Outcomes.* We gathered a team of worldwide experts to discuss their most recent research findings. Together our goal is to improve health care for mothers and their babies.

The 112th conference explored developmental programming and the impact of maternal nutrition and health on near-term growth and development of the offspring as it relates to the propensity for cardiometabolic diseases later in life. This developmental programming model asserts that the choices pregnant women make and the conditions their offspring face prenatally and in the immediate postnatal period potentially have great impact on health and disease in later years.

This publication offers 15 presentation summaries and 5 discussions from the conference. Key research insights focus on under- and overnutrition during pregnancy, early programming mechanisms and the role of the placenta, epigenetic markers, gestational diabetes, and maternal obesity and effect on infant body fat composition. Further insights focus on the optimal design of cohort studies, the impact of pregnancy nutrition on infant cognition, bone development, and immune response, and lifestyle interventions during pregnancy. Under- and overnutrition in India, China, and the Chile/Latin American and Caribbean (LAC) region, addressed by prominent researchers in these countries, conclude the conference proceedings.

We hope you find these summaries insightful in your practice, and that they reinforce the critical impact of proper maternal nutrition on the short- and long-term health of the mother and child.

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Pregnancy Nutrition and Later Health Outcomes

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Pregnancy Nutrition and Later Health Outcomes

The 112th Abbott Nutrition Research Conference was held in Columbus, Ohio, on July 26–28, 2011. This report contains summaries of presentations given by the following contributors:

Pregnancy Nutrition: The Impact of Under- and Overnutrition During Pregnancy

Lucilla Poston, PhD
King's College London, UK
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Nutrition in pregnancy presents a window of opportunity to influence the health of the mother as well as that of her child in infancy and later in life. Dr Poston offers an overview of the adverse effects of both undernutrition and overnutrition (ie, obesity) on mother and child. With respect to obesity interventions, Dr Poston suggests that approaches that focus on improvement of maternal glucose tolerance and consequent reduction of macrosomia may be more effective than strategies to limit gestational weight gain.

Overview of Mechanisms of Early Programming During Pregnancy

Susan Ozanne, PhD
University of Cambridge, UK
Department of Clinical Biochemistry

Extensive studies during the last 20 years have revealed that both low birth weight (especially when followed by accelerated postnatal growth) and high birth weight are associated with metabolic disease later in life. Dr Ozanne describes three categories of mechanisms by which a phenomenon that occurs in early life can have long-term effects on the function of a cell and therefore metabolism of an organism years later. The main goals now in the early programming field are to build on these findings and to translate them into ways to improve human health through development of preventative and intervention strategies.

Early Life Nutrition and Epigenetic Markers

Mark Hanson, PhD
University of Southampton, UK
Division of Developmental Origins of Health & Disease

Epigenetic processes are fundamental to development because they permit a range of phenotypes to be formed from a genotype. Such processes confer Darwinian fitness because the changes are induced in part in response to external signals that indicate to the developing organism critical aspects of the environment in which it will live after birth. Unbalanced nutrition—both undernutrition and overnutrition—is a part of that environment. Hanson demonstrates how nutrition can have graded epigenetic effects on a wide panel of genes throughout development. The author indicates that epigenetic changes measurable in early life may serve as valuable biomarkers of later risk of noncommunicable diseases and lead to interventions to reduce risk of those diseases.

The Role of the Placenta in Early Programming

Yuan-Xiang Pan, PhD
University of Illinois, Urbana-Champaign, USA
Department of Food Science & Human Nutrition

The placenta, the sole transport mechanism between mother and fetus, regulates the transport of all nutrients between maternal and fetal circulation. This organ is at a marked risk for the accumulation of ectopic fat in obese pregnancies, which are linked to increased risk for birth complications and a variety of adult onset diseases. Dr Pan describes the molecular mechanisms that connect maternal obesity and fetal outcomes and study results demonstrating that in utero exposure to a high-fat diet may program the gluconeogenic capacity of offspring through epigenetic modifications, which could potentially lead to excessive glucose production and altered insulin sensitivity in adulthood.

Optimal Design of Cohort Studies for Maximum Learning

Keith Godfrey, PhD, FRCP
University of Southampton, UK
Division of Developmental Origins of Health & Disease

Animal studies show that early life exposures can induce developmental plastic responses, with major long-term consequences for a wide range of metabolic pathways relevant to human health. Well-designed cohort studies are needed to define which exposures underlie the link between impaired human fetal development and susceptibility to later non-communicable diseases. Dr Godfrey explains the important differences between observational cohort studies and randomized controlled trials and describes key elements to consider when designing and interpreting observational studies.

Impact of Maternal Obesity on Long-Term Health Outcomes

Bert Koletzko, MD, PhD
University of Munich, Germany
Department of Pediatrics

In the USA, one in three women aged 20–39 years is obese. Obesity markedly increases health risks during pregnancy and accounts for as many as half of maternal deaths (UK data). Epidemiological studies, animal models, and clinical intervention trials provide ample evidence for long-term programming effects of nutritional and metabolic factors during sensitive, limited periods of early development, affecting health, well-being, and performance up to adulthood and old age. Nutritional studies, for instance, have shown that maternal and fetal overnutrition and undernutrition during pregnancy increase health risks for the offspring. Dr Koletzko argues that even better data are needed from observational studies and particularly from randomized controlled intervention trials that explore effective behavioral, nutritional, and other interventions to inform policy and practice.

Impact of Maternal GDM and Obesity on Mother and Fetus

Patrick Catalano, MD
Case Western Reserve University, USA
Department of Reproductive Biology

The worldwide increase in obesity among women of reproductive age signals a shift of the global disease burden from acute infectious disease to chronic diseases such as diabetes and atherosclerotic vascular disease, with their associated increase in health care costs. Dr Catalano discusses the causes and outcomes of the pattern of obesity/insulin resistance/gestational diabetes in pregnant women. Citing inflammation as a contributing factor in this pattern, Dr Catalano indicates that current research is examining lifestyle and dietary factors relating to decreasing inflammation in pregnancy and thereby improving maternal insulin sensitivity and fetal growth.

Insights From Body Composition Studies

David Fields, PhD
University of Oklahoma, USA
Department of Pediatrics

In females of childbearing potential the prevalence of obesity is approaching 30% and appears to be trending upwards. This rise in the percentage of overweight/obese pregnant mothers is worrisome given the negative long-term health consequences incurred by their offspring. Dr Fields describes the current state of the literature linking maternal obesity and offspring body composition early in life and reviews whole body composition techniques used in pediatric populations that allow accurate, quick, and reliable estimates of whole body composition in infants as young as 5 days old. These techniques provide the opportunity to study the link between maternal BMI and offspring body composition early in life, which could yield valuable insights into innovative strategies for obesity and diabetes prevention programs.

Impact of Pregnancy Nutrition on Cognition

Cristina Campoy, MD, PhD
University of Granada, Spain
Department of Pediatrics, EURISKITOS Excellence Centre for Pediatric Research
Health Sciences Technological Park

Nutrition plays an important role in supporting structural and functional growth of the human brain from conception, through childhood and adolescence, and into adulthood. Dr Campoy describes emerging techniques and tools to evaluate the role of nutrition in pregnancy on brain development, and discusses the controversy in current published studies that address this topic. She concludes that well-designed supplementation studies with long-term follow-up are needed to examine new confounding factors and combine new methodologies to learn more about optimal nutrition during early life, promote optimal neurodevelopment, and prevent deficiencies and other pathologies.

Impact of Pregnancy Nutrition on Offspring Bone Development

Stephanie Atkinson, PhD, FCAHS
McMaster University, Canada
Department of Pediatrics

Several factors other than maternal vitamin D may independently predict neonatal bone mass in offspring, including season of birth/ultraviolet B exposure, maternal smoking, maternal calcium intake, lower fat stores, and more vigorous physical activity during later pregnancy. Dr Atkinson discusses the role of nutrition during pregnancy on long-term outcomes including sarcopenia and osteoporosis, the association of protein intake and fetal bone health, and the critical periods during pregnancy for bone growth and mineralization. Future investigations in humans will help establish science-based recommendations targeted to pregnant women for intake of vitamin D, calcium, protein, and other nutrients that are important to bone health during fetal development and later in childhood.

Impact of Perinatal Nutrition on Neonatal Immune Response

Susanna Cunningham-Rundles, PhD
Weill Cornell Medical College, USA
Department of Pediatrics

Newborn exposure to colonizing commensal bacteria, environmental antigens, bioactive dietary substances, and potential pathogens has the potential to cause long-term effects on health. Differences in maternal and neonatal nutritional status are recognized as a source of variation in health outcomes and an avenue for early intervention. Dr Cunningham-Rundles describes the differences between immunogenic and tolerogenic responses on immune development in early life. She also discusses the role of nutrition during pregnancy, its influence on immune development and prevention of allergy, and the potential use of polyunsaturated fatty acids in preventing hyperinflammatory responses in neonates.

Lifestyle Intervention Trials During Pregnancy

Barbara Abrams, DrPH, RD
University of California, Berkeley, USA
Division of Epidemiology

Understanding the physical, psychological, social, cultural, and financial barriers that women face in addressing weight control during pregnancy and designing appropriate responses to women's experiences and concerns will improve future behavioral interventions for pregnant women. A variety of intervention strategies, including counseling and education about weight gain, healthy eating, physical activity, and monitoring of weight gain, are utilized to improve maternal dietary intake and physical activity. Dr Abrams discusses the results of the Fit for Delivery Study and other behavioral intervention trials that suggest that it is possible to moderate gestational weight gain through methods that are implemented in the clinical setting.

Undernutrition and Overnutrition During Pregnancy in India: Dual Teratogenesis

C. S. Yajnik, MD, FRCP
King Edward Memorial Hospital, Diabetes Unit, India

A study conducted by Dr Yajnik in India has shown that a mother's abnormal micronutrient levels (low vitamin B_{12} and high folate) can predict newborn adiposity (thin Indian babies with a high level of intra-abdominal fat) and a child's increased risk for developing diabetes later in life. This low B_{12} level is attributed to the common practice of vegetarianism in India, while the high folate is possibly because of the prescription of high-dose folate in obstetric practice. This apparent epigenetic effect shows how India must deal with both undernutrition and overnutrition in pregnant women. Dr Yajnik also describes future perspectives and actions to address these issues.

Nutritional Status of Pregnant Women in China

Chunming Chen, Professor
Chinese Center for Disease Control and Prevention, China
International Life Science Institute Focal Point

This overview looks at the nutritional status of pregnant women in China, describing the issues related to both undernutrition and overnutrition. Professor Chen reviews the importance of iron deficiency in various stages of pregnancy and looks at the deficiencies of other micronutrients, such as vitamin C, vitamin A, vitamin B_{12}, vitamin B_2, and folate, in both anemic and nonanemic women during their 3rd trimester. She also discusses anemia prevalence, maternal nutrition and its relationship to obesity risk during adult life, and the benefits of multinutrient supplementation in reducing the rate of low-birth-weight infants. She concludes by offering future perspectives and actions to address the issue of improving nutrition for pregnant women.

Challenges of Addressing Overnutrition and Undernutrition During Pregnancy in Chile/Latin America

Francisco Mardones, MD
Pontificia Universidad Católica de Chile, Chile
Department of Public Health

Challenges of both overnutrition and undernutrition face pregnant women in the Chile/Latin American and Caribbean (LAC) region. Dr Mardones focuses on the need for agreement on maternal anthropometric classification of nutritional status and weight gain guidelines in the LAC region. Recommendations for the optimal weight gain for pregnant women differ from the norm in the United States, mainly because the latter weight-gain guidelines were not determined proportionally for the individual height of each woman. This inhibits use in the LAC region because women show an important variability in height—1 SD of mean height is about 6 cm. Dr Mardones also discusses the importance of body composition and birth length, discussing studies that show that birth length strongly predicts adolescent height and that birth weight's effect disappears when adjusting for birth length. He also reviews the effect of birth weight as an indicator of fetal growth and its relationship to general mortality, obesity rates in school-age children, and metabolic syndrome factors.

Acronyms and Abbreviations

AA . . . . . .arachidonic acid

ADP . . . . . .air-displacement plethysmography

AGA . . . . . .appropriate for gestational age

ALSPAC . . . . . .Avon Longitudinal Study of Parents and Children

aMRI . . . . . .anatomical magnetic resonance imaging

ANT . . . . . .Attention Network Test

BMC . . . . . .bone mineral content

BMD . . . . . .bone mineral density

BMI . . . . . .body mass index

BOLD . . . . . .blood-oxygenation-level dependent

CKIα . . . . . .casein kinase I-alpha

CNS . . . . . .central nervous system

CpG . . . . . .cytosine-phosphate-guanine

CRP . . . . . .C-reactive protein

cVEP . . . . . .cortical visual evoked potentials

DBP . . . . . .vitamin D binding protein

DHA . . . . . .docosahexaenoic acid

DRI . . . . . .Dietary Reference Intake

DVL . . . . . .Dishevelled

DXA . . . . . .dual-energy X-ray absorptiometry

EAR . . . . . .estimated average requirement

EEG . . . . . .electroencephalography

ELISA . . . . . .enzyme-linked immunosorbent assay

EPA . . . . . .eicosapentaenoic acid

ERG . . . . . .electroretinogram

ERP . . . . . .event-related potentials

FADS . . . . . .fatty acid desaturase

FAT/CD36 . . . . . .fatty acid translocase CD36

FATP1 . . . . . .fatty acid transport protein 1

5-MTHF . . . . . .5-methyl tetrahydrofolate

fMRI . . . . . .functional magnetic resonance imaging

FPL . . . . . .forced-choice preferential looking

FZD . . . . . .Frizzled

GALT . . . . . .gut-associated lymphoid tissue

GDM . . . . . .gestational diabetes mellitus

GSK3β . . . . . .glycogen synthase kinase 3-beta

HDL . . . . . .high-density lipoproteins

HF . . . . . .high fat

HNF4α . . . . . .hepatocyte nuclear factor 4-alpha

HPL . . . . . .human placental lactogen

HtHcy . . . . . .hyperhomocysteinemia

IADPSG . . . . . .International Association of Diabetes and Pregnancy Study Groups

ID . . . . . .iron deficiency

IDA . . . . . .iron-deficiency anemia

IGF-1 . . . . . .insulin-like growth factor-1

IL . . . . . .interleukin (eg, IL-6, IL-8, IL-10)

IOM . . . . . .Institute of Medicine

IUGR . . . . . .intrauterine growth restriction

K-ABC . . . . . .Kaufman Test

LAC . . . . . .Latin American and Caribbean

LBW . . . . . .low birth weight

LCPUFAs . . . . . .long-chain polyunsaturated fatty acids

LPS . . . . . .lipopolysaccharide

LRP5/6 . . . . . .lipoprotein receptor-related protein 5/6

MEG . . . . . .magneto-encephalography

MLR . . . . . .multiple logistic regression

MMN . . . . . .multi-micronutrient

MPC . . . . . .mental processing composite

MRI . . . . . .magnetic resonance imaging

mRNA . . . . . .messenger ribonucleic acid

MTHFR . . . . . .methylenetetrahydrofolate reductase

NCD . . . . . .Non-Communicable Disease (Alliance)

NCDs . . . . . .noncommunicable diseases

NK . . . . . .natural killer

NEFA . . . . . .nonesterified fatty acid

NTD . . . . . .neural tube defects

1C . . . . . .one carbon

OP . . . . . .obese prone

OR . . . . . .obese resistant

PC . . . . . .phosphatidyl choline

PCM . . . . . .protein-calorie malnutrition

PDX1 . . . . . .pancreatic and duodenal homeobox 1

PE . . . . . .phosphatidylethanolamine

PET . . . . . .positron emission tomography

pg . . . . . .picogram

PMNS . . . . . .Pune Maternal Nutrition Study

PPAR . . . . . .peroxisome proliferators-activated receptor (alpha and delta)

pQCT . . . . . .peripheral quantitative computed tomography

PUFAs . . . . . .polyunsaturated fatty acids

PYGO . . . . . .pygopus

RCT . . . . . .randomized controlled trial

RDA . . . . . .Recommended Dietary Allowance

RM . . . . . .Rosso and Mardones Chart

RXRA . . . . . .retinoid X receptor A

SGA . . . . . .small for gestational age

TCF/LEF . . . . . .T-cell factor/lymphoid enhancer factor

TG . . . . . .triglyceride

Th . . . . . .T-helper

TLR4 . . . . . .toll-like receptor 4

TNF-alpha . . . . . .tumor necrosis factor alpha

TOBEC . . . . . .total body electrical conductivity

TPN . . . . . .total parenteral nutrition

Treg . . . . . .regulating T cells

25OHD . . . . . .25-hydroxyvitamin D

UL . . . . . .upper limit, upper intake level

UNICEF . . . . . .United National International Children's Emergency Fund

UVB . . . . . .ultraviolet B

VDR . . . . . .vitamin D receptor

VEP . . . . . .visual evoked potential

WHO . . . . . .World Health Organization

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National and Global Evidence Linking Pregnancy Nutrition and Health Outcomes

Pregnancy Nutrition: The Impact of Under- and Overnutrition During Pregnancy

Lucilla Poston, PhD

Optimization of maternal nutritional and fetal status has been a public health goal since records began. The association of low-calorie intake with low birth weight is perhaps best evidenced by reports from the famine-stricken populations of Leningrad and southern Holland during World War II.[1,2] Since the beginning of the 20th century, with the awareness that gestational weight gain was a proxy for maternal nutrition, efforts have been made to ensure adequate gestational weight gain. The effects of fetal nutritional excess also are exemplified by excessive fetal growth in response to suboptimal glucose control in women with gestational diabetes, but recently increasing trends in maternal dietary caloric excess and obesity have led to considerable concern over the myriad of associated adverse maternal and fetal health outcomes. Importantly, we are now aware that maternal nutritional status may have unforeseen influences on the developing child that extend to increased risk of disease in later life. This association, embodied in the Developmental Origins of Disease Hypothesis, has given renewed vigor to investigations of maternal nutritional status in pregnancy.

Protein-Energy Malnutrition

Women in developing countries remain at risk of protein-energy malnutrition and associated low birth weight, major causes of neonatal mortality and morbidity. A recent meta-analysis of women from developed and developing countries suggests that balanced protein-energy supplementation can lead to a 31% (95% CI 15%–44%) reduction in delivery of infants who are small for gestational age.[3] Data from some of the studies in the meta-analysis are shown in Fig 1.

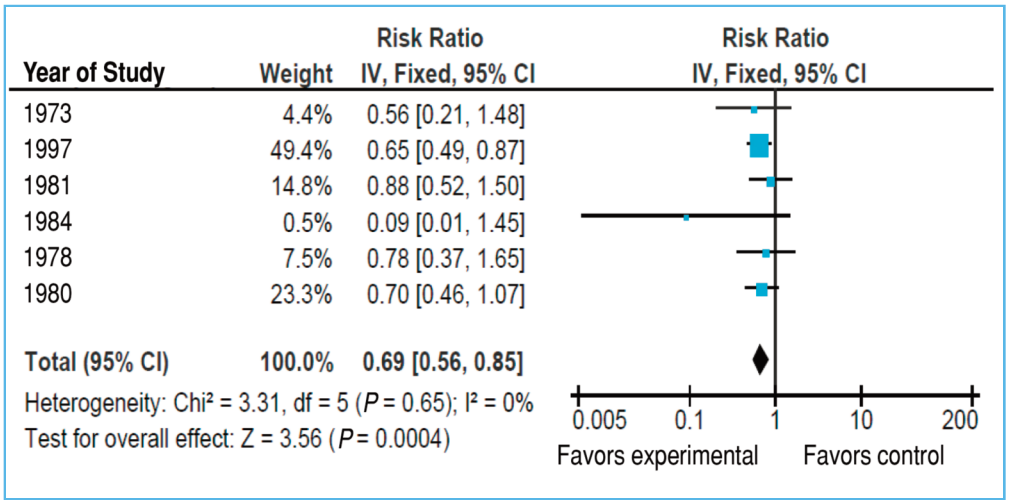

Fig 1. Effect of balanced protein energy supplementation during pregnancy on risk of small-for-gestational age births.[3]

Source: Imdad A, Bhutta ZA. Effect of balanced protein energy supplementation during pregnancy on birth outcomes. *BMC Public Health*. 2011;11(suppl 3):S17.

These findings suggest that this intervention should be scaled up in developing countries. There is hitherto no information, however, as to whether these benefits may reduce risk of disease in the child in later life.

Micronutrient Deficiencies

Pregnant women in both developed and developing countries are at risk of micronutrient deficiency. In developing countries where the incidence of anemia is high, iron/folate supplementation is an effective treatment, but iron and multivitamin supplements have only a modest influence on reducing low birth weight.[4] Iodine deficiency, which is associated with adverse influences on cognitive development and increased infant mortality and morbidity, remains a problem in some parts of the world.[5]

The influence of inadequate periconceptual folate intake on risk of neural tube defects is well known, but increasing evidence for the role dietary folate insufficiency plays in fetal growth restriction, for example, in pregnancy in adolescents,[6] (Fig 2) and in preeclampsia[7] suggests that folate supplementation should be considered throughout pregnancy.

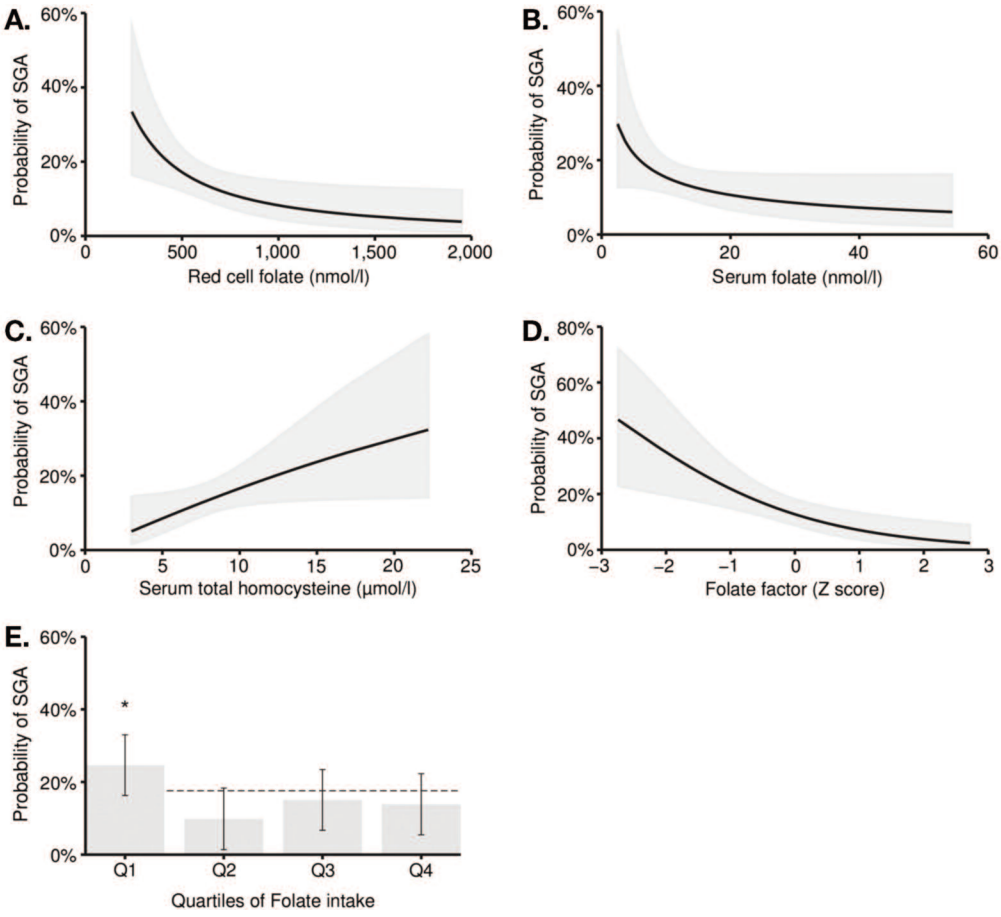

Fig 2. Folate status and folate intake measured in pregnant adolescents during the 3rd trimester and relations with small-for-gestational age (SGA) birth.[6] Shaded areas represent 95% CIs. Data adjusted for confounding variables (multiple logistic regression). A: Relation between red blood cell folate and SGA birth (n=263). Curve obtained by multiple logistic regression (MLR) of log-transformed biomarkers. B: Relation between serum folate and SGA birth (n=280). Curve obtained by MLR of log-transformed biomarkers. C: Relation between serum total homocysteine and SGA birth (n=290). Curve obtained by MLR of log-transformed biomarkers. D: Relation between folate factor score and SGA birth (n=290). Folate factor score defined by factor analysis of red blood cell folate, serum folate, and serum total homocysteine. Curve obtained by MLR of log-transformed biomarkers. E: Relation between quartiles of folate intake and SGA birth (n=288). Subjects in the lowest quartile of folate intake (<187 µg/day) were more likely to deliver an SGA infant than were subjects with higher intakes (odds ratio: 2.71; 95% CI: 1.28, 5.71; *P*=0.009, energy-adjusted). Columns represent the probability of SGA birth, determined by MLR of folate intake and SGA birth, adjusted for energy intake. Error bars represent 95% CIs. The dashed line indicates the incidence of SGA birth in the sample as a whole.

Source: Baker PN et al. A prospective study of micronutrient status in adolescent pregnancy. *Am J Clin Nutr.* 2009;89:1114-1124. Reprinted by permission of the American Society for Nutrition.

A new randomized controlled trial (the FACT trial) is addressing the potential for prevention of preeclampsia by folate supplementation in early pregnancy. Although there is little evidence of a prolonged effect of folate on childhood risk of disease, a recent study of Indian mothers and their children suggests that folate status in pregnancy may have an independent influence on cognitive function in young children.[8] Since folate metabolism plays an important role in gene methylation status and epigenetic regulation of gene expression, influences of maternal dietary folate on later risk of disease may occur through persistently altered gene methylation status in the child. Further studies are required to address the genome-wide or candidate gene methylation status of children born to folate-deficient and folate-replete women.

Vitamin D insufficiency in pregnant women is prevalent worldwide and observational studies have suggested links with gestational diabetes and preeclampsia.[9] While a relationship between vitamin D insufficiency and increased risk of infant rickets is well established, it now appears that maternal vitamin D status may have consequences for a child's bone density in later life.[10,11]

Obesity

Obesity in pregnancy represents an increasing challenge to health care professionals. Obesity is associated with increased risk of miscarriage, thromboembolism, preeclampsia, gestational diabetes, and a high cesarean section rate, among many other problems (Table). An infant born to an obese mother is more likely to develop congenital malformations, to be born large for gestational age, and to die in stillbirth.[12]

Table. Maternal and Fetal Risks Associated With Maternal Obesity

Maternal Risk	Fetal/Infant Risk
Gestational diabetes	Macrosomia
Preeclampsia	Shoulder dystocia
Venous thromboembolism	Brachial plexus damage
Genital infection	Intrauterine death
Urinary tract infection	Spina bifida
Wound infection	Heart defects
Postpartum hemorrhage	
Induction of labor	

An intervention is needed that reliably reduces the risk of these complications, in both mother and child. One approach is to devise strategies to limit gestational weight gain in obese pregnant women; however, a meta-analysis of recent small studies suggests that the strategies that have been tried have been unable to achieve the weight-gain restrictions recommended by the USA Institute of Medicine (5–9 kg).[13] However, approaches that specifically concentrate on improvement of maternal glucose tolerance and consequent reduction of macrosomia may be more apposite, although these require much larger studies to achieve adequate power. Such a study, the UPBEAT Trial, is now underway in the United Kingdom.

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Q & A

Q: Thank you, Dr Poston, for a wonderful presentation to kick off this meeting. I would like to add to your skeptical view on using gestational weight gain as the holy grail. Some data from a large national trial that was recently published in Germany showed that obesity prevalent among 70,000 children was positively related to gestational weight gain. The effect size, however, was small—1 kilogram of weight gain on average with only 1% increase in obese prevalence in the child population. To be realistic, how many kilograms could we ever change in terms of gestational weight gain? This would have a very small effect on child obesity. On top of that, the effect was only seen in normal-weight pregnant women. There was no effect in obese women.

I also have a question. I noticed in your pilot trial, you saw no effect of the glycemic load change on gestational age at birth. I am sure you are aware of the data that has been published on a small controlled trial last year in which the investigators reported a remarkable increase of 1½ weeks in gestational age related to glycemic load compared to a low-fat diet. Is there any reasonable hypothesis why there should be such an effect?

Dr Poston: I do not know why you would get an alteration in gestational age. On the other hand, obese populations have a high incidence of preeclampsia, which can reduce gestational age at delivery. But we need to distinguish between elective preterm delivery and spontaneous preterm births: The births among women with preeclampsia would not necessarily be spontaneous preterm births.

Overview of Mechanisms of Early Programming During Pregnancy

Susan Ozanne, PhD

More than 20 years ago the first epidemiological studies revealed a relationship between patterns of early growth and subsequent risk of diseases such as type 2 diabetes, cardiovascular disease, and the metabolic syndrome. Extensive studies during this time have revealed that both low birth weight (especially when followed by accelerated postnatal growth) and high birth weight were associated with metabolic disease later in life.

Evidence also shows not just that metabolic conditions are associated with patterns of early growth but also that similar relationships exist between birth weight and nonmetabolic parameters such as mental health and immune function. Studies of identical twins, individuals who were in utero during periods of famine, discordant sib pairs, and animal models have provided strong evidence that the early environment, including nutrition during fetal life, plays an important role in mediating these relationships.[1] The concept of early life programming therefore is widely accepted.

However, the mechanisms by which a phenomenon that occurs in early life can have long-term effects on the function of a cell and therefore metabolism of an organism many years later are only starting to emerge. Insight into these molecular mechanisms has primarily come from the study of animal models including those established in nonhuman primates, sheep, pigs, rats, and mice. A major strength of studying a range of diverse species is that it allows the identification of molecular mechanisms that are conserved between species and therefore likely to represent fundamental mechanisms that are likely to be important in humans. However, most studies have been carried out in rodents as these allow mechanisms to be addressed across the life course of an organism.

It has become increasingly apparent that a single mechanism cannot explain all the observed programming phenomena. Based on current knowledge, the potential mechanisms can be categorized into three groups. These three mechanistic categories clearly are not mutually exclusive, and there is likely to be interaction among them (Table).

Overview of Mechanisms of Early Programming During Pregnancy

Table. Proposed Mechanisms of Early Programming

Programming Mechanisms
1. Permanent structural changes
2. Epigenetic programming of gene expression
3. Accelerated cellular aging

One of the earliest proposed mechanisms was related to effects mediated by permanent changes in the structure and consequently the function of critical organs. It was suggested that during a critical period of development of an organ, exposure to a suboptimal level of a nutrient or hormone that is essential for appropriate development of that organ would permanently alter the structure and function of that tissue. Examples are low levels of nutrients early in life leading to a permanent reduction in pancreatic beta cell mass and renal nephron numbers, which can influence risk of type 2 diabetes and hypertension respectively.[2,3] Suboptimal levels of the hormones insulin and leptin in early life also can permanently influence the structure and, consequently, function of the hypothalamus, which plays a key role in regulation of energy balance and thus can influence risk of obesity.[4,5]

More recently focus has been directed toward the potential role of persistent alterations in epigenetic modifications (eg, DNA methylation and histone modifications) leading to programmed changes in gene expression that form the basis of cellular memory.[6] Several transcription factors including hepatocyte nuclear factor 4 alpha (HNF4α), pancreatic and duodenal homeobox 1 (PDX-1), and peroxisome proliferators-activated receptor alpha are susceptible to programmed changes in gene expression through such mechanisms. Fig 1 illustrates the role of HNF4α in the beta cell and consequently type 2 diabetes.

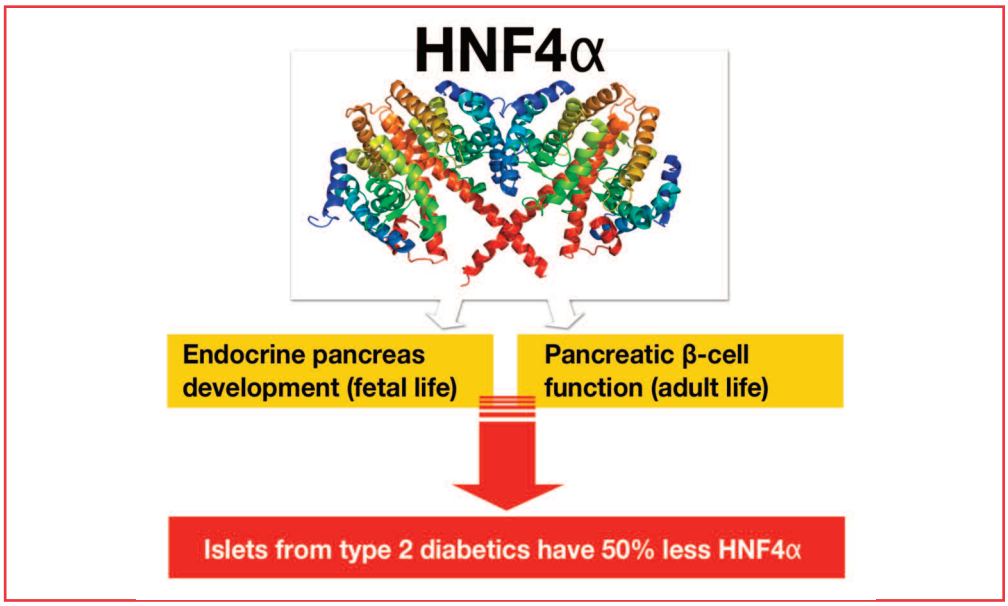

Fig 1. Mechanisms of programmed changes in gene expression through persistent alterations in epigenetic modifications: hepatocyte nuclear factor 4 alpha (HNF4α)→type 2 diabetes.

Source: http://en.wikipedia.org/wiki/File:Protein_HNF4A_PDB_1m7w.png.

Transcription factors are particularly attractive targets of developmental programming because a whole network of other genes can be modulated through modulation of their expression.

A permanent effect of early exposures on the regulation of cellular aging has been suggested as another mechanism that can link a suboptimal early environment and long-term health. Increases in oxidative stress leading to macromolecular damage, including that to DNA and specifically telomeres, can contribute to such effects by leading to premature cell senescence.[7] Mitochondrial dysfunction resulting from either defects in mitochondrial copy number or defects in mitochondrial complex activities could provide the source of the oxidative stress.[8]

The main goals now in the programming field are to build on these findings and to translate them into ways to improve human health through development of preventative and intervention strategies. However, there are still major challenges that need to be addressed to achieve such goals. It is clear, certainly from animal models, that maternal overnutrition and undernutrition during pregnancy can affect not only the health of the mother but also the long-term health of the baby. However, modulation of diet during human pregnancy is not straightforward and it

should be noted that maternal diet does not equate to fetal diet. Therefore, in many instances an apparently well-nourished or overnourished mother can deliver an undernourished baby as a result of poor placental function. Furthermore, although initial focus in human studies was directed toward high or low birth weight as a proxy for exposure to a suboptimal in utero environment, it is now apparent that birth weight is a very crude index of in utero experiences and that not every suboptimal environment influences fetal growth. Therefore, a need exists to identify at-risk individuals through good molecular markers (see strategies in Fig 2). These could be genetic, protein, or epigenetic factors, which to be clinically useful would have to be present in clinically accessible material such as blood, urine, placenta, or umbilical cord. The latter two are particularly attractive because they are available very early in life, thus providing the maximum time for intervention.

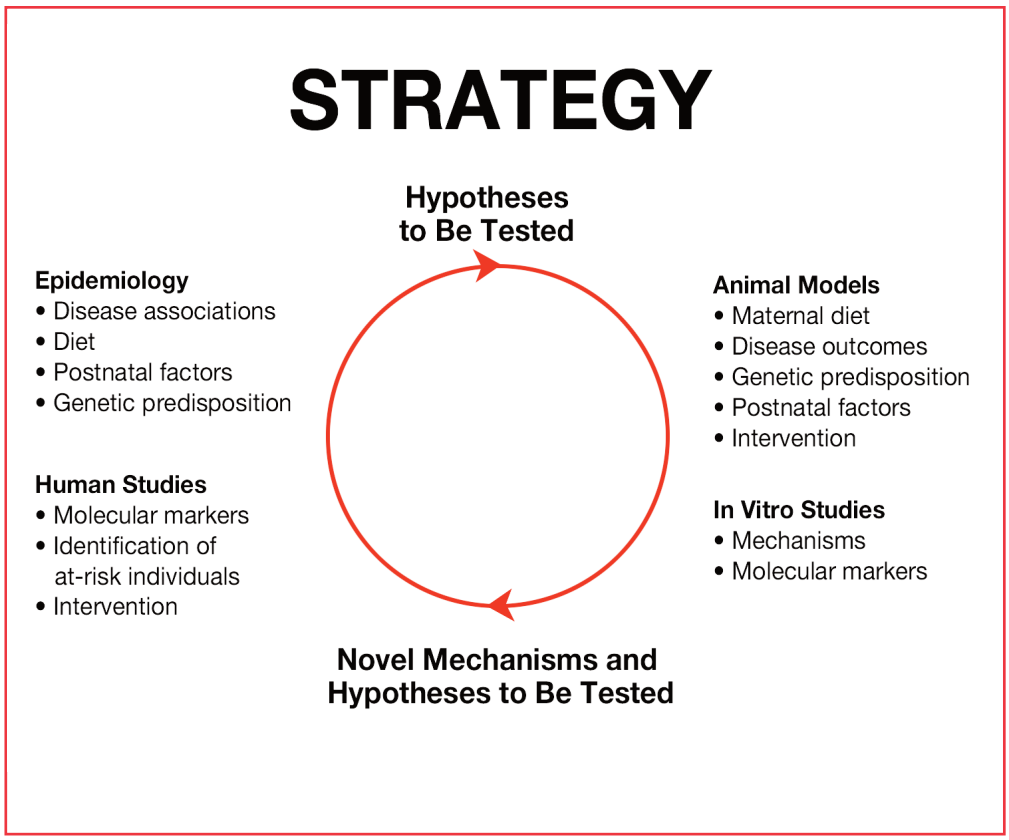

Fig 2. Strategies for identifying individuals at risk for certain metabolic diseases.

The insight gained from mechanistic studies may enable the design of targeted intervention studies and ultimately personalized medicines. Most of the diseases associated with a suboptimal environment are heterogeneous conditions. For example, type 2 diabetes is a phenotype that can result from a wide variety of factors that influence pancreatic beta cell function and/or insulin action. Therefore, it may be naïve to assume that the same medication will be suitable for all causes of type 2 diabetes. Mechanistic insight into the pathways that mediate the effects of a suboptimal early environment on type 2 diabetes risk could help identify rational drug targets.

Understanding environmentally driven processes provides more tractable targets for intervention than those driven by genetic processes. Therefore, further progress on understanding the pathways and mechanisms underlying early life programming offers the potential to help combat the burden of many common diseases faced by modern society.

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Q & A

Q: You mentioned that 50% of diabetics had a low level of hepatocyte nuclear factor 4 alpha. Did this track with disease progression or treatment?

Dr Ozanne: These were all postmortem samples from an array analysis from individuals who, I think, died in traffic accidents. The array just compared what genes were differentially expressed. The criterion was the classification of diabetes, not taking into account stage or treatment.

Q: I am still trying to work through methylation. You showed there was no effect in the promoter region but you did see it in the enhancer region, correct?

Dr Ozanne: I saw a small difference in methylation in the promoter region, but there was not methylation in the enhancer region.

Q: How well do you think we understand methylation, and how does that translate to, for instance, interventions?

Dr Ozanne: I think we still understand very little about DNA methylation and its regulation, particularly how we get gene-specific effects. We get changes in methylation at one region genome and not another. We do not really understand normal development and normal differentiation. We do not understand how that arises. It is even more difficult to interpret how it happens from a programming perspective. We do start to see differences in the promoter region later, with aging.

Q: How far are we from a pharmacologic or other type of intervention?

Dr Ozanne: I would say we are in the very early stages. Even in the cancer field, where the epigenetic mechanism has been seen, the epigenetic changes that have been recognized were much longer. We are even not at the stage where we can target those well from a disease perspective.

Q: Do you have data and animal models in which you have demonstrated the effects in the mitochondrial dysfunction associated with outcomes? Also, are there any data on the effects of nutrition modification and modifying mitochondrial dysfunction as a consequence?

Dr Ozanne: That is a good question. The answer to the first question is that we have not shown cause and effect, but certainly we have shown associations. So in collaboration with Dr Poston at King's College London, we have shown that there is a defect in complex two, three linked activities, in the low-protein offspring in the kidney. And we have shown that this happens before we see differences in telomere shortening. We see the defect but we cannot, at this stage, prove that it is causing the telomere shortening. We are looking at potential interventions to address that.

Early Life Nutrition and Epigenetic Markers

Mark Hanson, PhD

Epigenetic processes are fundamental to development because they permit a range of phenotypes to be formed from a genotype. Across many phyla such epigenetic processes confer Darwinian fitness because the changes are induced in part in response to external signals that indicate to the developing organism critical aspects of the environment in which it will live after birth. Environmental features such as abundance and quality of food or the number of predators and related stress levels may be critical in determining the chance of the offspring surviving to reproduce, given that from an evolutionary point of view this is more important than longevity or health. Epigenetic processes are increasingly recognized to be complex[1] and include DNA methylation, changes in structure of the histone proteins around which genomic DNA is wrapped, and noncoding RNAs (Fig 1).[2]

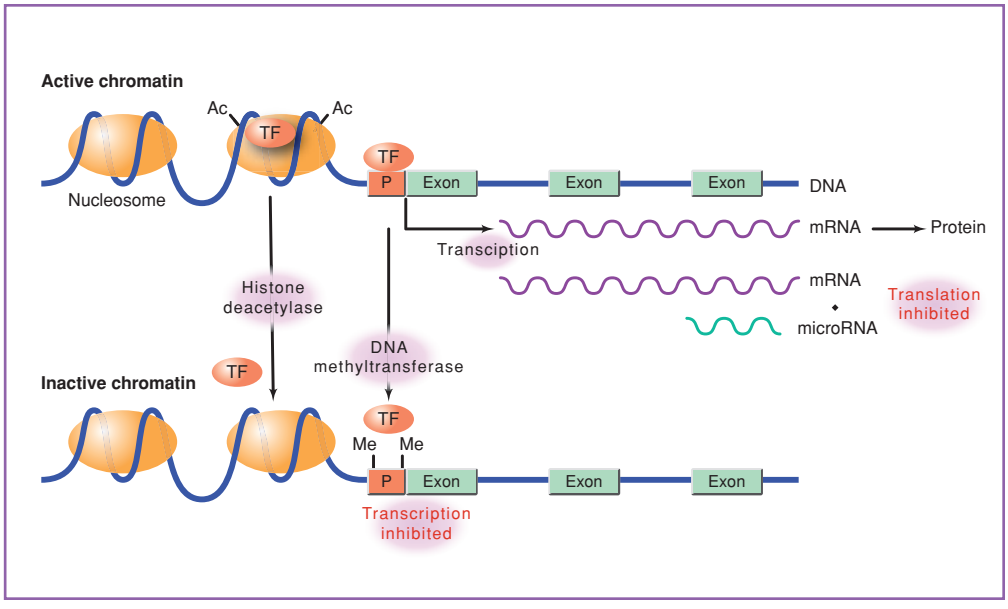

Fig 1. Complexity of epigenetic processes.[2]

Source: Gluckman PD et al. Mechanisms of disease: Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med*. 2008;359:61-73. Reprinted by permission of the Massachusetts Medical Society.

When epigenetic changes affect the promoter regions of genes they can alter the access of transcription factors to these regions and thus produce effects on gene transcription. Nutritional signals during development have been shown extensively to induce such epigenetic effects in animal models. Because such effects alter metabolic control systems, they can influence an individual's response to later challenges such as an obesigenic lifestyle and thus the risk of obesity and noncommunicable disease including cardiovascular disease and diabetes.[3,4]

The evolution of such epigenetic processes probably did not equip humans for the modern world, especially that now experienced in developed societies.[5] An unbalanced diet can lead to malnutrition even in those who have access to plentiful food. In developed societies many women consume poor-quality diets, resulting in nutritional deficiencies on one hand, or overweight and obesity on the other. While maternal undernutrition remains a major problem in developing societies, maternal overweight, excessive weight gain in pregnancy, and gestational diabetes are growing concerns. Thus unbalanced nutrition has effects on development across a broad dietary spectrum.

In addition, a range of studies now shows that such epigenetic processes can be passed beyond the subsequent generation, raising questions about the importance of nongenomic inheritance in demographic and secular changes in disease risk. Accumulating evidence also shows that epigenetic effects can be passed via the paternal line, presumably because not all epigenetic marks are erased in the packing of DNA into the sperm and because even the small amount of sperm cytoplasm contains some small noncoding RNAs, which can have effects on the oocyte or zygote. Indeed, a range of studies now indicates that very early embryonic development is sculpted by epigenetic processes. These extend beyond merely imprinted genes (where the expression of the allele depends on the parent of origin). Environmental factors such as nutrition can have graded epigenetic effects on a much wider panel of genes throughout development. These perceptions are important because they suggest that nutritionally induced epigenetic processes may start to operate even before a couple know that they have conceived.

Animal studies reveal how maternal nutrition affects the phenotype of the developing offspring, via epigenetic changes in endocrine, cardiovascular, and metabolic control that prepare the offspring for a predicted postnatal environment. Where developmental cues (nutritional, endocrine, etc) turn out to be inappropriate, or the environment changes between generations, the phenotype is mismatched.

Under these conditions experimental animals exhibit many of the features and risk factors equivalent to human noncommunicable disease, such as obesity, reduced muscle mass and bone density, fatty liver, high blood pressure and vascular endothelial dysfunction, and insulin and leptin resistance. Such “mismatched” offspring also show altered appetite, hyperphagia and changes in food preference, altered stress responses and anxiety, reduced learning, and changes in the timing of puberty. Some data indicate possibly similar effects in mismatched human subjects.[5] It is important to note that such effects are not pathological in themselves, but are manifestations of altered responses to the postnatal environment, including nutrition. This is not to say that pathological changes in phenotype are not induced by more severe undernutrition or overnutrition, but these occur at the extremes of the range and are more equivalent to teratogenic effects.

To pursue epigenetic concepts in the human, we have utilized prospective cohorts of children who had undergone detailed phenotyping and for whom we had previously measured aspects of the mother’s diet in pregnancy in detail. We focused on possible epigenetic effects at the promoters of candidate genes, selected on the basis of animal data and the previous literature, using an array-based discovery procedure followed by replication in separate cohorts. We extracted DNA from umbilical cord tissue stored at birth to gain information about the effects of prenatal nutrition on epigenetic processes. Such tissues may be invaluable where the epigenetic changes occurred during early development and so may be expected to be manifest in all somatic tissues. For the retinoid X receptor A (RXRA), which is involved in responses to fatty acids, metabolic control, and tissue differentiation, we found a highly significant correlation between mother’s carbohydrate intake in early pregnancy and methylation at a specific cytosine-phosphate-guanine (CpG) dinucleotide in the RXRA gene, and also between this methylation and the child’s adiposity at 6 or 9 years of age (Fig 2).[6] The effects were replicated in two separate cohorts.

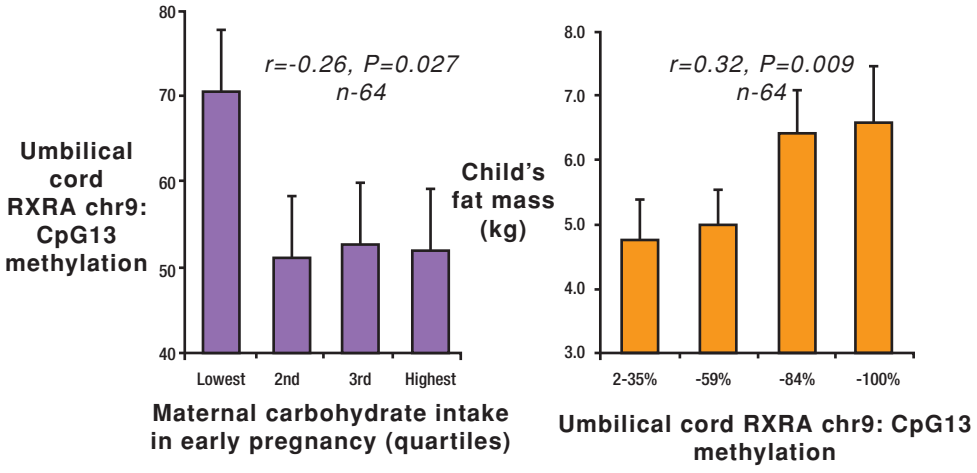

Fig 2. Data from Southampton prospective cohort showing strong correlation between level of DNA methylation at one CpG in the RXRA gene and mother's carbohydrate intake in early pregnancy (left) and child's fat mass at age 9 years (right). These effects were independent of mother's BMI or child's birthweight.[6] CpG = cytosine-phosphate-guanine (dinucleotide), RXRA = retinoid X receptor A, BMI = body mass index

Source: Godfrey KM et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*. 2011;60:1528-1534. Copyright 2011 American Diabetes Association. Reprinted with permission from the American Diabetes Association.

Further studies are required to determine the timing of a critical window after birth during which it may be possible to restore epigenetic changes in offspring exposed to a suboptimal environment before birth, although again animal studies provide proof of principle that either endocrine interventions in the neonate[7] or dietary interventions after weaning[8] produce effects on epigenetic processes induced prenatally, although the underlying mechanisms are not known.

In summary, the results of a range of animal studies, and of the first few investigations in humans, give hope that epigenetic changes measurable in early life may serve as valuable biomarkers of later risk of disease. It is not known whether biomarkers that have been measured to date lie on the causal pathway to greater risk of disease. Nonetheless, they can serve as better measures of prenatal environment than, for example, birth weight, which can be affected by a complex interaction between genetic and environmental factors and which is not necessarily affected by unbalanced nutrition. These biomarkers may be helpful in devising new interventions in early life aimed at reducing later risk of noncommunicable disease, and possibly in monitoring the efficacy of such interventions. This will be important

because a “one-size-fits-all” intervention is unlikely to be efficacious and may increase risk in certain individuals.[9,10] In view of the importance of meeting the challenge of noncommunicable disease globally,[11] the development of such biomarkers is a priority.

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Q & A

Q: Dr Hanson, you demonstrated that low protein leads to hypomethylation of peroxisome proliferators-activated receptor alpha (PPARα), while low carbohydrate leads to hypermethylation of retinoid X receptor A (RXRA). How do heterodimers PPARα and RXRA work if you have two variants with low protein or carbohydrate?

Dr Hanson: Do not forget that this is the low-protein, isocaloric model, so there is actually increased carbohydrate. So we should expect that the results in the animal study would be opposite of the human study, and the data show that. I do not want to duck the question, but we have to be careful when extrapolating from animal data to humans, from one species to another, because they all have different adaptive strategies, different metabolism, and different levels of maturity at birth and during their early postnatal life. This is proof of principle, however, and we see in the rat a metabolic phenotype in the offspring that we can correct. We have to do the human studies to see whether such changes actually relate to disease risk.

Q: How do you envision the functional interpretation of epigenetic biomarkers? How might the RXRA hypermethylation in the umbilical cord risen? From stem cells?

Dr Hanson: It looks hopeful. Some data suggest that changes in RXRA in the direction that we see are associated with changes in adipocyte number and differentiation. Of course, we are still not sure whether what we are seeing in the umbilical cord is just the shadow of things in other parts of the body or whether we might only see it in that particular tissue. It is correlation, not causation, at this point.

Q: I am interested in the temporal relationship between the protein restriction in your animals' fathers and conception. How long do these males have to be on low protein to have the effect that you saw?

Dr Hanson: These are not our data, but I recall that it was a few weeks [Carone et al. *Cell.* 2010;143:1084-1096]. Presumably the diet is affecting either the small RNAs or indeed the DNA packaging into protamine at the time the sperm are formed, because we know that even though textbooks say epigenetic changes are erased in the sperm during those processes, we know they are not. There could be enough of a signal to the transcriptional machinery in the zygote toward developing a phenotype even from that early point in life. That is what these data seem to show. Much stronger data have come from Skinner's group using endocrine disrupters

[Anway et al. *Science*. 2005;308:1466-1469]. They show changes in the male lineage through four or five generations. I think it is interesting that this dietary challenge produces such an effect.

The Role of the Placenta in Early Programming

Yuan-Xiang Pan, PhD

Placenta Functions

The placenta functions as the sole transport mechanism between mother and fetus for all essential nutrients and substances, as well as waste products (Figure).

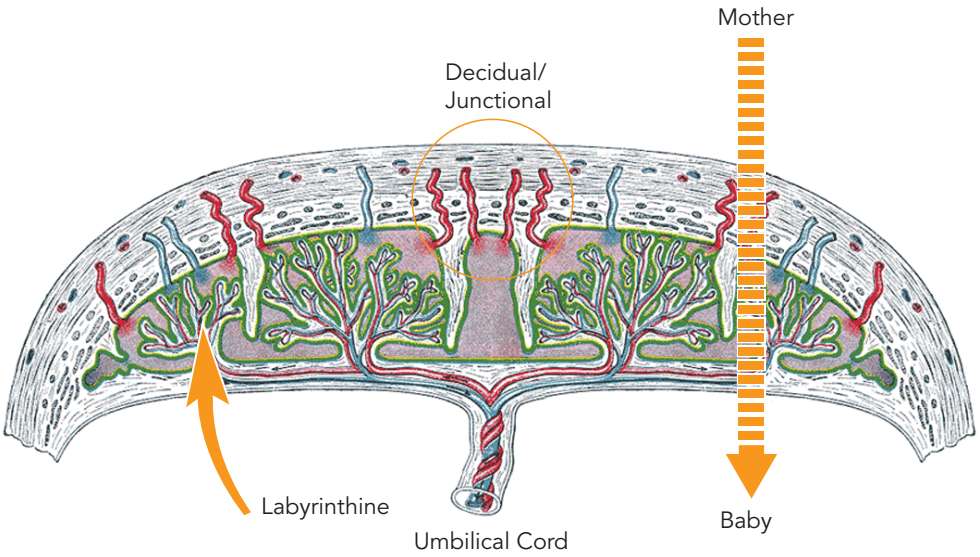

Figure. Placental structure.

The placenta is responsible for transporting oxygen, carbon dioxide, water, and any other essential nutrients. Respiratory gases pass freely from maternal to fetal blood, as do other lipophilic substances, and depend primarily on concentration differences of these substances between mother and fetus. Hydrophilic substances, such as ions, amino acids, and glucose have poor diffusion capacity across the placenta and require transport proteins and ion channels in order to become available to the fetus.[1] The amount of placenta required for adequate fetal development varies across species. The appropriate fetal-to-placental weight ratio in humans is approximately 6:1, while rats have a ratio of 10:1.[2] The rat's ability to produce more fetus per gram of placenta is thought to be due to the rodents' placental countercurrent blood exchange. In this arrangement, maternal and fetal

capillaries are in parallel to each other, and blood flows in opposing directions.[3] Humans have the less-efficient multivillous arrangement, resulting in the need for a larger placenta.[2]

The rates of gestational obesity have been increasing at the same rapid rate as the obesity epidemic in the general population.[4] These statistics are alarming not only because obese pregnancies are accompanied by various birth complications, but also because human and animal studies have shown that obese pregnancy programs offspring for a variety of adult onset diseases. The placenta regulates the transport of all nutrients between maternal and fetal circulation, and is at a marked risk for the accumulation of ectopic fat in instances of obese pregnancy. Although the connection between fetal outcomes and maternal obesity has been irrefutably established, the molecular mechanisms remain undefined. As the principal link between mother and child, the placenta has become an important factor in unraveling these mechanisms.[5,6] Poor placentation has been associated with intrauterine growth restriction (IUGR) as well as small-for-gestational-age (SGA) offspring, gestational diabetes, and possible risks of late-onset diseases.[7-9]

Signaling Pathway

Canonical Wnt signaling is transduced through Frizzled (FZD) receptors and acts through the complexing of nuclear β-catenin with the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, Legless family docking proteins, and pygopus (PYGO) family coactivators to enhance the transcription of target genes.[10-15] Canonical Wnt signaling occurs following the binding of a Wnt molecule and the downstream phosphorylation of Dishevelled (DVL) by casein kinase I-alpha (CKIα) and its binding to Frat. The subsequent assembly of the FZD-DVL and lipoprotein receptor-related protein 5/6 (LRP5/6)-Axin-FRAT complexes releases β-catenin from CKIα and glycogen synthase kinase 3-beta (GSK3β) phosphorylation and allows it to travel to and accumulate in the nucleus.[16,17]

Because of its rapid growth and vascularization, Wnt signaling may have a vital role in the development of the placenta. In fact, of the 19 known Wnt ligands, 14 have been found in the placenta, and 8 out of the 10 FZD receptors have been detected in placental tissue.[18] Numerous studies have explored Wnt signaling in placental tissues and cell culture experiments. β-catenin, the key mediator of canonical Wnt signaling, has been implicated in trophoblast adhesion, survival, and differentiation.[19-21] Other studies have shown that Wnt7a and TCF/LEF1 are required for chorioallantoic fusion,[22,23] and that Wnt2,[24] Wnt3a,[25] Wnt5a,[26] Wnt10b,[26] Fzd5,[26] Sfrp4,[27,28] and Rspo[29,30] may be essential for proper placental vascularization and

growth. Additionally, DKK1, a potent inhibitor of Wnt signaling, has been shown to be involved in trophoblast invasion and migration.[31] Therefore, it appears that many components of the Wnt signaling pathway may have a strong link to the poor fetal outcomes associated with inadequate placental development.

Effects of Obesity on Placenta and Offspring

We examined the mechanisms behind the effect of maternal obesity on placental lipid accumulation and metabolism. Pregnant Obese Prone (OP) and Obese Resistant (OR) rat strains were fed a control diet throughout gestation. Placentas were collected on gestational d21 for analysis of fat accumulation as well as β-catenin and DKK1 localization. Additionally, DKK1 was overexpressed in JEG3 trophoblast cells, followed by treatment with nonesterified fatty acids (NEFA) and Oil Red O stain quantification and mRNA analysis to determine the relationship between placental DKK1 and lipid accumulation. Maternal plasma and placental NEFA and triglyceride (TG) were elevated in OP dams, and offspring of OP dams were smaller than those of OR dams. Placental DKK1 mRNA content was 4 fold lower in OP placentas, and β-catenin accumulation was significantly increased as well as mRNA content of fat transport and TG synthesis enzymes, including peroxisome proliferators-activated receptor-delta (PPARδ), fatty acid transport protein 1 (FATP1), fatty acid translocase CD36 (FAT/Cd36), lipin 1, and lipin 3. There was significant lipid accumulation within the decidual zones in OP but not in OR placentas, and the thickness of the decidual and junctional zones was significantly smaller in OP than in OR placentas. Overexpression of DKK1 in JEG3 cells decreased lipid accumulation and the mRNA content of PPARδ, FATP1, FAT/CD36, lipin 1, and lipin 3. Our results indicate that DKK1 may be regulating placental lipid metabolism through Wnt-mediated mechanisms.

Livers of OP offspring had increased TG content and lipid accumulation compared to offspring of OR dams. Additionally, hepatic DKK1 mRNA content was significantly decreased in OP livers compared to OR livers, and treating rat hepatocyte cells with NEFA showed that DKK1 mRNA was also decreased in NEFA-treated cells. Analysis of the DKK1 promoter in fetal livers showed a pattern of histone modifications associated with decreased gene transcription in OP offspring, which supports our gene expression data. Our results demonstrate that offspring hepatic DKK1 is epigenetically regulated via histone modification in the current model of gestational obesity, and future studies will be needed to determine whether these changes contribute to excessive hepatic lipid accumulation in offspring of obese dams.

Effects of High-Fat (HF) Diet on Placenta and Offspring

In a mouse model of gestational HF feeding, placental weight was unaffected by HF diet, accompanied by increased placental glucose and amino acid transport,[32] while another study using the same model demonstrated that a gestational HF diet led to mixed placental inflammation and oxidative stress (possibly due to increased lipid peroxidation), as well as cellular necrosis and vasculopathy.[33] A recent review of animal models of gestational HF feeding[34] concluded that despite its not being as clear as black and white, there appears to be a risk of diabetes development in offspring of HF-fed dams, independent of maternal obesity. We investigated the effect of a maternal HF diet on fetal genes in the liver that control the production of glucose, and the potential regulatory mechanisms of these genes in an OR rat model to separate the effects of gestational diet and weight. By utilizing an animal model in which dams were not sensitive to the obesigenic effects of HF intake, we intended to confirm that HF feeding alone would have a negative impact on fetal development. In our study, placental DKK1 mRNA content was 4 fold lower in OP placentas.[35] Offspring of HF-fed dams were significantly heavier and had significantly higher blood glucose levels compared to offspring of control-fed dams, which we suggest is a result of their enhanced gluconeogenic capacity in response to the gestational HF diet. While maternal gluconeogenesis and plasma glucose were not affected by the HF diet, offspring of HF-fed dams had significantly higher mRNA contents of gluconeogenic genes in addition to the elevated plasma glucose.

Our results demonstrate that in utero exposure to a HF diet has the potential to program the gluconeogenic capacity of offspring through epigenetic modifications, which could potentially lead to excessive glucose production and altered insulin sensitivity in adulthood.

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Q & A

Q: I have two questions. First, do you think there is a threshold effect between when the response moved from being adaptive to being pathological? You could argue that the change is in fatty acid deposition and that the transport of fatty acid deposition in the placenta is actually adaptive in that it gets good nutrition across to the offspring. It occurs to me that some obese mothers actually have a relatively growth-retarded baby, possibly due to placental malfunction. Does your animal model mimic that in any way?

Dr Pan: Our model shows a 40% decrease of the functional zone in the placenta. The offspring of the obese mothers are actually smaller than those in the other group. If we looked at mothers before pregnancy, I am not sure we would see that what they are lacking was twice as high. We know the obese dams are heavier than obese-resistant dams but we did not do body composition tests to see how much fatter they were.

Q: Is this model pathological rather than macrosomia within the normal range?

Dr Pan: Yes.

Q: Second, in our high-fat model we showed that if we give the mother but not the offspring a statin in late pregnancy, we reverse or prevent most of the typical effects on the offspring later—for instance, changes in appetite and ability to run around and perform daily functions. If you gave a statin to one of your models, could you prevent those placental changes you see?

Dr Pan: That is a possibility. One thing that interests us about this pathway is that it is cell/cell interaction. Potentially we can give a nutrient or drug during pregnancy or earlier to modify the signaling pathway and potentially lessen the impact. For example, we believe that in obese pregnancy, it does not matter what the mother eats. As long as the diet or lifestyle does not change the obese general environment, the impact to the placenta will remain. But if we can use other things such as drugs to reverse that, then the cell/cell interaction will change and potentially we can reverse anything that happened in the placenta because of the change of the pathway.

Q: In your model, you used nonesterified fatty acids and triglycerides. Why did you not use high-density lipoprotein (HDL) particles? We know that HDL is important in vascular function. Dr Hanson mentioned statins, but HDL does the same things to reverse cholesterol.

The topic of hypertension also is important with regard to placental function. Hearing fatty acids and triglycerides discussed without glycoprotein that is related to them diminishes the effect of what you see as relevant to humans.

Dr Pan: One reason we did not use triglycerides in our study is that the placental structures of rat and human are basically permeable to fatty acids, but not to other big molecules. Triglycerides will not go through circulation to the fetus. We do not believe any other form of fatty acid will be seen by trophoblasts and other fetal tissue unless it is sent by the placenta.

Q: Several animal models of high-fat feeding show reduced birth weight. Interestingly, a subgroup of pregnant obese women has small babies, and their risks are greater than that of macrosomic babies. It is interesting to know what the difference is. In human pregnancy we are, perhaps incorrectly, assuming that a small-for-gestational birth occurs because of the inflammatory response early on and reduced placental growth. And those women are predisposed to preeclampsia. My question is about maternal glycemia. You have shown no effects of a high fat diet on maternal glucose and you are attributing the neonatal high glucose to Dickkopf-related protein 1 signaling. Have you rigorously assessed glucose homeostasis in those animals by doing, for example, an oral glucose tolerance test? Overfed mothers often have normal plasma glucose but abnormal resistance in glucose homeostasis.

Dr Pan: We do not have a metabolic lab in our facility to do those kinds of tests.

Q: We have fed rodents a high-fat diet and without the associated obesity we have not seen an effect on fasting glucose, but we do show remarkable insulin resistance and hypertension.

Dr Pan: We do not have any kind of glucose timing going on in our facility. In our model, we do not see gestational glucose increase in either obesity-prone or obesity-resistant mothers. We believe that one of the reasons the obese mothers have smaller babies is that the glucose supply is not adequate.

Q: You can look at the insulin proxy measure without doing a full glucose tolerance test.

Dr Pan: We did measure some of the markers in tissue but did not see significant change in our model.

Q: I meant in terms of plasma rather than tissues.

Dr Pan: Okay.

Discussion

Barbara Marriage, PhD, RD

Dr Catalano: Some recent literature has discussed iron supplementation in women during pregnancy and shown an increased risk of gestational diabetes. Another issue is related oxidative stress. Could this be a factor in placental function? Dr Poston, can you comment?

Dr Poston: There is a potential gestational diabetes increase related to the free iron and oxidative stress. Iron supplements should be given only to women who have demonstrated anemia.

Dr Godfrey: Although we tend to concentrate on nutrient preventive effects, which are important, the placenta also has an important role during pregnancy. We know that the placenta offers the fetus the big vascular circuit and that this alters the fetal cardiovascular system. And we know that altering loading of the fetal cardiovascular system has long-term effects on the coronary reserve. I think this is a ripe and important area. However, we need to think about timing when we assess nutritional effects on the placenta because broadly speaking, nutritional influences may be greater early in pregnancy than later in pregnancy.

Dr Christina Sherry [Abbott Nutrition]: Dr Ozanne, you briefly mentioned the role of adenosine monophosphate-activated protein kinase with exercise. What are your thoughts on exercise prescription during pregnancy?

Dr Ozanne: Clearly exercise improves insulin sensitivity, and both animal and human studies suggest that the insulin resistance of obese mothers is important. So targeting that, whether we do it by exercise or by pharmaceuticals, could be an important intervention.

Dr Sherry: What are your thoughts on whether exercise may have epigenetic modifications through a similar or different vein than diet?

Dr Ozanne: Nutrition and exercise may act through common epigenetic pathways to modify insulin sensitivity. We do not know about during pregnancy but we do know from a Swedish study that exercise results in epigenetic modification of one gene, the transcriptional coactivator gene PGC-1 alpha. So there are data that suggest that exercise does modify epigenetic markers, certainly particular genes. What precise range of genes, we do not know.

Dr Poston: In our intervention, we are promoting exercise because it is one way to improve glucose tolerance without weight loss. I think that is important in the gestational weight-gain side of the story; it is possible to improve glucose homeostasis without necessarily losing a lot of fat mass.

Dr Catalano: A colleague of mine studied exercise in pregnancy longitudinally, looking at women before pregnancy and during pregnancy [Clapp JF et al. *Am J Obstet Gynecol.* 2000;183:1484-1488]. Not surprisingly, he found that those women who continued to exercise through pregnancy had babies that were not as heavy because they were not as obese, ie, they had less fat mass. But an interesting finding was that women who started to exercise and then stopped in the 3rd trimester because of physiological issues had larger placentas and heavier babies. And this was serious exercise as opposed to walking, close to 50% to 60% VO_2 max. My colleague theorized that hypoxia in pregnancy prevents increased growth of the placental vascularization, improving nutrient transport. Therefore, when the women stopped exercising, their placentas were programmed to increase nutrient transport, and those women ended up having heavier, fatter babies.

Dr Yajnik: The particular activity and level of daily physical activity in pregnant women in India is phenomenal. The average pregnant woman weighs 42 kg. They do not exercise in the gym, but they work at home with the animals on the farms. This is very strongly related to growth retardation in their babies, so in one part of the world exercise may have benefits, but in an undernourished population, exercise is detrimental.

Dr Poston: I was referring to women with a good nutritional status and who are obese.

Dr Godfrey: On the flip side of the coin, almost none of the women in the UK have high levels of strenuous exercise during pregnancy. And in the obese population, the pre-intervention levels of exercise are extremely low.

Dr Yajnik: It seems that certain types of activities such as bending are strongly related to growth retardation.

Dr Hanson: Dr Godfrey, did the Southampton Women's Survey not show that the children of women who are able to walk faster, to undertake more vigorous exercise, have lower bone density [Crozier SR et al. *Am J Clin Nutr.* 2010;91:36-43]?

Dr Godfrey: The thought is that if a woman walks fast, she exerts strength through her bones and thus retains calcium rather than allowing it to leach across to the fetus.

Dr Poston: I think we have to go with the guidelines in the United Kingdom and the United States stating that mild-to-moderate activity in pregnancy is safe and should be encouraged. Current evidence indicates that mild-to-moderate exercise in developed countries is good for pregnancy outcome. I would not like for this audience to think that severe exercise can increase miscarriage rate and so on.

Dr Hanson: This was not severe exercise.

Dr Godfrey: These were women who reported walking faster than average.

Dr Marriage: Did the researchers look at their calcium, vitamin D, and nutritional intake?

Dr Godfrey: We used ultrasound to look at the mothers' bones and found that those women who exercised, who walked faster, tended to retain their calcium in their own bones.

Dr Robert Miller [Abbott Nutrition]: Dr Poston, I want to go back to the model you started with in which you were challenged by undernutrition and then overnutrition. Did I hear correctly that there are 30 million undernourished babies?

Dr Poston: Thirty million babies born small for gestational age.

Dr Miller: And there are how many total births worldwide? There are 24 million in India and 16 million in China, so I think the global figure is around 70 million. So 30 million is nearly half. Then around 8 million are obese. So the 32 million in the middle should be optimally nourished. Is there a definition of optimal or ideal in the middle?

Dr Poston: Not by those criteria because they are based on birth weight. I think we have to be careful about that. It would be nice to be able to define optimal nutrition, but that would be enormous work.

Dr Hanson: I am not happy with this overnutrition and undernutrition dichotomy. I think we probably should think in terms of balanced nutrition and unbalanced nutrition. A lot of women in this country and certainly in mine have plenty of food but they still have a poor, unbalanced diet. Another caveat is that we are talking about pregnancy and nutrition, but mostly we have talked about mothers when what we really want to know is what the baby is getting. That is a different story.

Dr Poston: That is right. I hope I made it clear that even in cases of nutritional excess there can be micronutrient deficiency. Teenage girls often have huge calorie

excesses, but many of them also have marked micronutrient deficiencies. It is hard to define undernutrition and overnutrition.

Dr Miller: Dr Hanson, this relates to what you said about plasticity. Could you map plasticity temporally in conception, gestation, and then birth? Is there any way of knowing what that looks like in a global perspective? If so, we could understand when, over time, we can have the greatest impact.

Dr Hanson: That is a good question. I do not think we know for each tissue, but I suppose we would tend to map it for the whole organism in terms of an exponential or reciprocal decline in plasticity, and accompanying that, an increase in the risk of disease through an inability to respond and be plastic to new challenges. But the timing of that would depend on organs and history that goes back over more than one generation.

Dr Godfrey: Plasticity is generally greater earlier on, but it is tissue-specific. The velocities for maximal growth of the femur bone are different than those of, for instance, the kidney and the placenta. Bones have periods of high velocity between 13 and 19 weeks' gestation, and then again during puberty.

Optimal Design of Cohort Studies for Maximum Learning

Keith Godfrey, PhD, FRCP

Animal studies have shown that early life exposures can induce developmental plastic responses, with major long-term consequences for a wide range of metabolic pathways relevant to human health. As a result well-designed cohort studies are needed to define which exposures underlie the link between impaired human fetal development and susceptibility to later noncommunicable diseases. The design of such studies needs to focus on core exposures and outcomes, taking into account the likely intermediary mechanisms. Collection of appropriate biological samples will enable definition of the epigenetic and other mechanisms that underlie developmental effects on noncommunicable diseases, aiding characterization of interventions. Such cohort studies need to be combined with a new generation of randomized intervention trials if we are to define the effects of maternal lifestyle, diet, and body composition on biological endowment.

Differences Between Observational Cohort and Intervention Studies

A strength of observational cohort studies is their temporal sequence, in which incident cases of disease or the development of particular phenotypes allows examination of whether a cause or risk factor precedes the effect. This reduces the risk of some potential biases, for example, lowering the risk of reverse causality or recall bias. A further strength of observational cohort studies is the breadth of analyses they can allow. New hypotheses can be tested after cohort studies have commenced and repeated measures can be ascertained over time. Cohort studies enable both multiple exposures to be related to one disease or phenotypic outcome, and examination of multiple phenotypic outcomes of a given exposure, enabling assessment of risks and benefits.

Cohort studies, however, have a number of weaknesses that need to be acknowledged. Compared with case control studies, they need to be sizeable for adequate power. Data ascertainment is often retrospective in phases, raising the possibility of recall bias, and their long duration makes them expensive and prone to changes in current practice and exposure over time. They are not useful for rare-disease outcomes and multiple testing increases the possibility of chance findings.

Compared with controlled interventions, observational cohort studies have the potential for biases of various types, including measurement, selection (particularly if losses to follow up are high), referral, observer, performance, and detection biases. Confounding is another critical issue, as it is likely that groups exposed and unexposed to a particular risk factor will differ in ways other than the risk factor under consideration. As such it is important to measure potential confounders, and undertake stratified and multivariate analyses that take these into account.

Despite their limitations, cohort studies can nonetheless give unique and important insights, informing both policy and the design of new intervention studies. Examples of insights arising from observational cohort studies undertaken by the MRC Lifecourse Epidemiology Unit include the following observations relevant to developmental influences on later health outcomes:

- The mother's prepregnant dieting behavior, health, and age influence the early trajectory of fetal development.[1]
- Many pregnant women are undernourished or vitamin-D insufficient, with lasting effects on offspring body composition.[2]
- Maternal obesity, gestational diabetes, and excessive pregnancy weight gain are common, with long-term effects on offspring adiposity.[3]
- The mother's dietary intake and parity are associated with offspring body composition.[4]
- The mother's diet influences fetal developmental plasticity and how she feeds her infant, and is itself influenced by education, smoking, and other children in the home.[5-7]
- Current infant feeding recommendations are likely to have long-term benefits, but are often not followed.[8]

Randomized Controlled Trials

Contrasted with observational cohort studies, strengths of randomized controlled trials include the unbiased allocation of subjects to treatment groups, and as a result confounders are generally equally distributed between groups. When such trials are blinded, the risk of most biases is low. In controlled experiments, with a clear time line in which intervention or treatment precedes outcome, it is easier to infer causality. While the evidence from randomized controlled trials is widely acknowledged as the gold standard on which to base policy, randomized controlled trials do have some weaknesses that often receive little emphasis. They are costly, particularly when the regulatory aspects are taken into account, and at times there may be ethical issues in relation to giving unproven treatments or, conversely,

withholding proven treatments. From the outset there is a selection bias, so generalizability may be uncertain, and randomization does not necessarily result in balanced groups. Attrition can be an issue and needs to be built into the power calculations. Also, highly controlled interventions generally test efficacy not effectiveness. Finally, it is critical to remember that these studies assess one "exposure" only, or a group of exposures, so given the cost and time involved they are generally not undertaken without a substantial evidence base accrued from cohort and other studies.

Key Elements to Consider When Designing and Interpreting Observational Studies

Study design is critical in observational cohort studies of pregnancy nutrition and later health outcomes, as shown in Fig 1 below.

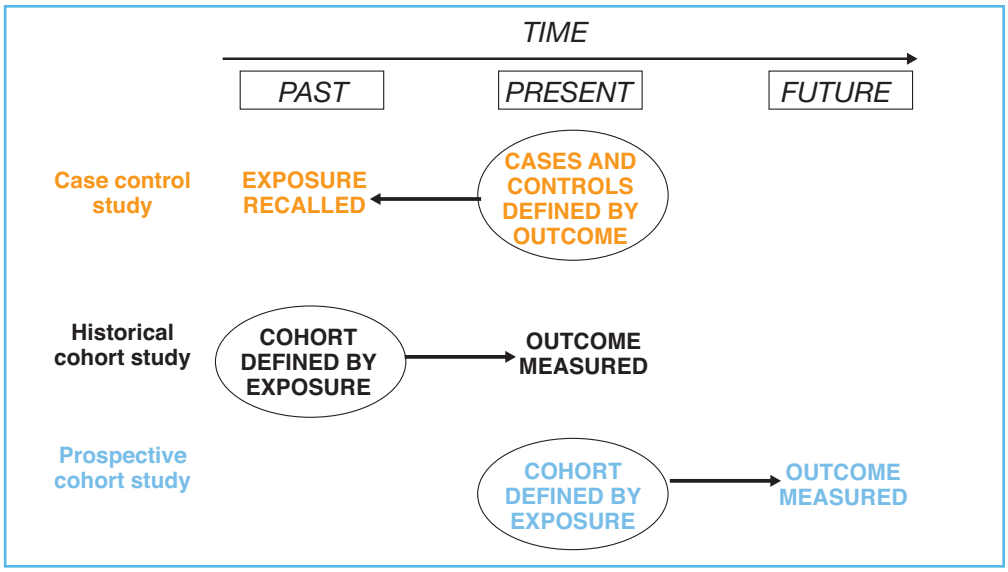

Fig 1. Key elements to consider when designing and interpreting observational studies.

While case control studies are valuable for rare outcomes and much less costly than other designs, retrospective recall of exposures is notoriously subject to bias and in isolation from other evidence provides a weak basis for recommendations.

In historical cohort studies, the cohorts are defined by a past exposure and outcomes assessed in the present time. Advantages include their lower cost and

results are available quickly, as soon as follow-up has occurred. Historical cohort studies are particularly useful if the disease has a long induction time, such as type 2 diabetes and coronary heart disease. Disadvantages of such studies are that exposure information may be of poor quality or incomplete, people or survival effects may be difficult to trace, and there is a risk of recall bias if any aspect of the exposure is recalled. Important examples of historical cohort studies include the Hertfordshire and Helsinki Cohort Studies,[9,10] which have provided pivotal early evidence in the field of developmental programming.

In prospective cohort studies exposures are defined in the present time and outcomes assessed in the future. Advantages of such studies include the capacity to ascertain detailed, good-quality exposure information and to collect biological samples enabling definition of biomarkers of later risk. Disadvantages are that they are expensive and can be prone to drop-outs. Investigators may have to wait a long time for hard outcomes and the relevance of intermediate outcome measures, such as childhood adiposity, may be uncertain. However, recent follow-up studies are starting to provide greater certainty; for example, follow up of 5000 Native Americans for 24 years showed that childhood obesity is associated with doubling of adult mortality before age 55 years.[11]

In designing prospective early life cohort studies it is important to realize that the "ideal" cohort study may be a flawed concept. There is a need to focus around the core hypotheses being addressed, as collection of quality data on all possible exposures and outcomes imposes substantial participant burden, increasing the risk of drop-outs and attendant biases. Such studies should commence in early pregnancy, or ideally preconception, as critical periods are often earlier than generally thought. New prospective cohort studies can certainly take advantage of methodologies developed for previous studies, but it is critical to appreciate that data collection needs to be context- and age-specific. This is particularly true for dietary and physical activity data; for example, in our Southampton Women's Survey[12] different dietary assessment tools had to be developed and validated for the various lifecourse stages at which we have assessed diet.[13] Exposure assessments should combine questionnaire and objective measures, for example, using accelerometry to measure physical activity and serum micronutrient concentrations to assess nutrient status.

Advances in metabolomics and in epigenetics have brought a greater emphasis on collection of biological samples for intermediary biomarkers. Different technologies require particular sample collection and processing protocols, so it is important to be as specific as possible regarding the purpose for which such samples are being collected. Even more important, however, is the choice of outcome measures.

These should include detailed assessments of body composition, including validated measures of fat mass, and not simply body mass index. We have reported, for example, that a shorter duration of breastfeeding was associated with greater adiposity measured by dual-energy X-ray absorptiometry at age 4 years, but there was no association with children's body mass index (Fig 2).[8]

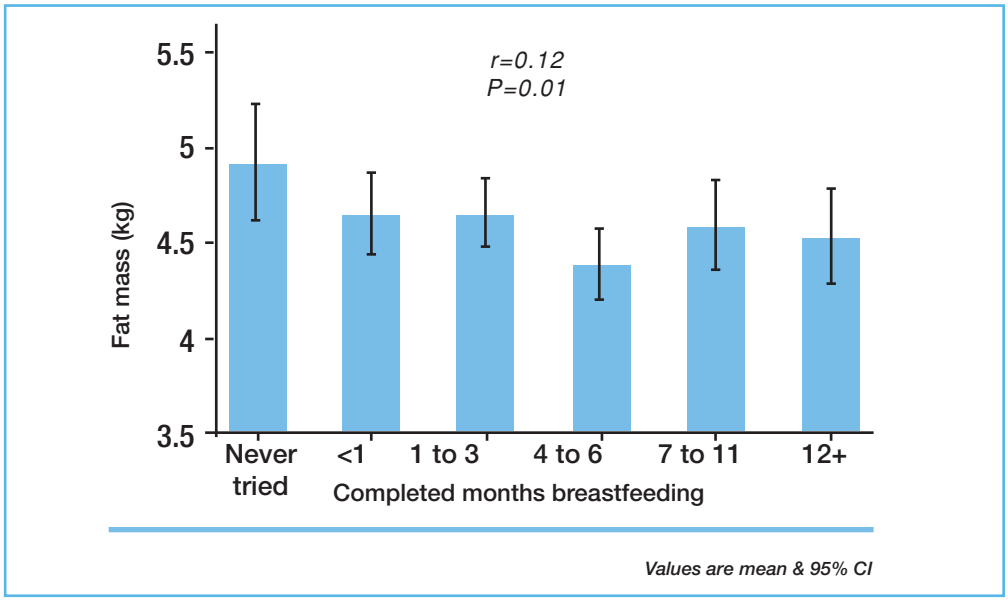

Fig 2. Shorter duration of breastfeeding associated with greater adiposity age 4 years.[8] No association with child's BMI.

Source: Robinson SM et al. Variations in infant feeding practice are associated with body composition in childhood: a prospective cohort study. *J Clin Endocrinol Metab*. 2009;94:2799-2805.

Preferably, outcome measures should take into account likely underlying mechanisms, such as assessing accentuated responses to a stress challenge as a link between early development and later cardiovascular risk. Wherever possible, outcome measures should be relevant to later health. A good example, with respect to cardiovascular disease, is to determine carotid artery intima media thickness, increasingly linked with later disease, rather than simply measuring blood pressure.[14]

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Q & A

Q: I would like to ask about cut-off points—for example, vitamin D less than 25 nmol/L or energy below 2150 kcal. You say there are even more gradations and perhaps a greater range. How do you interpret studies in that context?

Dr Godfrey: This question goes to the heart of what we are doing—the grade effects across the range of exposure. That might be the case for some exposures. Or are there effect thresholds? Typically in the first layer of analysis we tend to group subjects into quarters or fifths of the distribution. But those categories are not biologically driven; they are statistically driven. And you are right that we need biologically driven models. Typically those require a much more substantial number of subjects to be secure thresholds. To model the data, they often need to be combined with other study designs to say, for instance, that we know there is activation of the parathyroid hormone access in relation to vitamin D below a certain level.

In cohort studies, I think that we can make only a certain degree of progress, but it is critical to undertake those analyses. There is huge disagreement, particularly between the US and Europe, about what an appropriate vitamin D threshold should be. And 25 nmol/L was just one of the thresholds.

I hope the randomized controlled trial (RCT) we are undertaking will allow us to define thresholds with greater certainty. We do not think it is ethical to randomize subjects who are clearly vitamin-D deficient. So 25 nmol/L is an intermediate level of vitamin D. The RCT design does allow us to tackle those thresholds, at least to a degree.

Q: It takes a lot of effort to collect every little piece of data on every subject, and so we have missing cells when, for instance, a child wriggles during the dual-energy X-ray absorptiometry (DXA) or somebody forgets to write down the height or whatever. Could you speak about your views on the imputation of data and the limits of that when doing major analyses?

Dr Godfrey: Sure. There are statistical methods now for imputing missing values in long cohort studies, although they are not perfect. More important is to take care to get as few as possible wriggling children in our DXAs. We take steps to help keep them still, such as ensuring that they are fed before they go into the machine. For older children, we spend hours sorting out decent DVDs and other materials to keep them still during DXA. By doing these things, we end up with missing data in 3% of the neonates and in about 6% of 4-year-olds. You are right, however; they can be systematically different.

Q: I recently saw criticism of some research reports because the authors used imputed data, so I am trying to bring that issue to the forefront. As we have more and more of these longitudinal studies, I think that we will have to agree on the limits of use of this statistical maneuver. What is your thought on this?

Dr Godfrey: That is right. Imputed data can introduce bias, especially when the exposure or the interest is specifically linked to missing data.

Q: In these cohorts, you collect a lot of information from the mother and from the child. How important might it be to collect information from the father as well? Dr Hanson showed some results that suggest that the influence of the father might be relevant as well.

Dr Godfrey: I think it is more important than we imagine. We have been doing the best we can to get this information, but we need to do more. Part of the problem is that a small but important group of women has had bad experiences with the baby's father after conception, and they do not want us to ask him anything. The rest have surprised us. I thought, for instance, that the fathers would be pretty apathetic and not give us blood. We get blood from about 85% of the fathers. But you are right; ideally we should have preconception information on the fathers.

Q: One of the biggest trends in nutrition research in this country in the last 2 decades has been the advent of what has been called epidemiologic nutrition. Large cohorts such as that in the Nurses Health Study have been used to make definitive statements about what we should and should not eat in terms of outcomes such as cancer. I have heard very little from the scientific community, which should be pushing back and saying, wait a minute, we cannot make those kinds of statements from these data. What are your thoughts on that?

Dr Godfrey: I agree that we need to be cautious about utilizing data from even big cohorts like that in the Nurses Health Study. On the other hand, the alternative is not great either, particularly in disorders such as cancer with long induction times. Honoring the evidence from experimental studies or from case control studies is arguably worse. If evidence from the EPIC cancer studies in the UK and Europe and the Nurses Health Study in the US gives us similar messages, we probably have a reasonable basis on which to form policy. My links with policy makers suggest that if we do not provide them with any evidence, they just make policies in the absence of evidence. So we must do the best we can with the information we have, but with caution.

Q: I was going to ask the same question, but from a different perspective. I think one of the reasons we get into trouble with observational studies linking nutrients with outcomes is because people fish their data. This is tempting when we have so much information. We want to be creative and push the envelope, but then we run a risk. Right now we are looking at vitamin D. But during my career we also have studied vitamins A, B, C, and E. So far observational studies have almost never proven the truth about these vitamins. When you begin a study, what process do you go through to decide the questions you are going to ask?

Dr Godfrey: One reason we have made some progress is that we have links with experimental work in animals that allow us to "nail" the effects of a particular nutrient, group of nutrients, or bands of nutrients. I also think that fishing is important, but the fish have to be explicit. Fishing is how discoveries are often made. Take, for example, epigenetics. One reason we have made progress is we have a breadth of studies at our disposal at which we can look and replicate, or not.

But you are right. Vitamin A and lung cancer science is littered with examples of observations that have not stood the test of time. But where there is a clear biological basis for an effect, such as that of vitamin D on animal development, regulators for the food industry are increasingly seeing such evidence as important to the assessment of claims.

Q: You recently published a paper describing following mothers' weight. I do not remember how old the children were, but I think they were 16 years old. Are you going to continue to follow the mothers into midlife? You enrolled them when they were pregnant, but by following them into midlife you may learn a lot more about what happens to those mothers and about what is going to happen to those children. This seems like part of the lifecourse approach.

Dr Godfrey: We would love to do this, but it comes down to money. We have put in bids for such a follow up, but we have not made the case and we have not secured the money.

Q: Do you think that one of the ways to improve causality association is to use randomization? A genetic marker linked to the exposure of interest and association to the outcome would strain our beliefs of causality.

Dr Godfrey: I think this approach has unacknowledged weaknesses. For example, if we have a developed pathway, genetic polymorphism, the route through which we have levels may be slightly different from the route through which the general population gets to high levels. This may be a poor example, but I think that care still needs to be taken with respect to randomization.

Impact of Maternal Obesity on Long-Term Health Outcomes

Bert Koletzko, MD, PhD

Differences in nutritional experience during limited, sensitive periods in early life, both before and after birth, can program a person's development, metabolism, and health for the future. Epidemiological studies, animal models, and clinical intervention trials provide ample evidence for long-term programming effects of nutritional and metabolic factors during these sensitive periods that affect health, well-being, and performance up to adulthood and old age.

The term programming was introduced into the scientific literature by Günther Dörner in 1975, based on the analysis of data from both experimental and clinical studies.[1] The concept received broader attention when David Barker and colleagues published retrospective epidemiological studies documenting associations of weight at birth and at age 1 year, respectively, with the risks of hypertension, diabetes, and coronary heart disease in adulthood.[2] These observations raised the hypothesis that maternal and fetal malnutrition during pregnancy induce both fetal growth restriction and increased later disease risk. More recent data also suggest that fetal overnutrition as a consequence of maternal obesity, diabetes during gestation, and certain dietary habits increase health risks for the offspring.[3] Moreover, postnatal growth and nutrition, and in particular accelerated weight gain in early childhood, appear to induce adverse effects on long-term health and well-being.

Obesity before and during pregnancy is common in many populations around the world. In the USA one in three women aged 20–39 years is obese, with a body mass index (BMI) >30 kg/m^2.[4] A very high obesity prevalence also is found in the Middle East, Australia, and some European countries.[5] Moreover, obesity rapidly increases in many newly industrialized and even in low-income countries. Health risks for pregnant women are markedly higher with obesity, eg, spontaneous 1st-trimester and recurrent miscarriage, cardiac disease, preeclampsia, dysfunctional labor, gestational diabetes, thromboembolism, cesarean section, postcesarean wound infection, postpartum hemorrhage, overall severe morbidity, and maternal deaths. A recent review in the UK found about half of maternal mortality associated with pregnancy overweight and obesity.[6] Also, infants of obese mothers carry increased risks including stillbirth and neonatal death, prematurity, congenital abnormalities, and lower breastfeeding rates and duration.

Furthermore, maternal obesity can have severe long-term consequences for the offspring. The fuel-mediated hypothesis stipulates that an enhanced transplacental supply of glucose and fatty acids, along with elevated insulin levels, induces increased fetal growth, birthweight, and neonatal adiposity, along with adverse long-term effects on later health.[7] Hyperglycemia during pregnancy was found to be associated with increased neonatal adiposity, which predicts later BMI and body fat content.[8] Body weight gain prior to pregnancy, prepregnancy BMI, and gestational weight gain are all associated with infant birth weight, which predicts later obesity risk.[9,10] Many observational studies also have associated maternal BMI with the offspring obesity risk in childhood and adulthood.[11-13] These associations could reflect programming effects of the intrauterine environment, but also shared genetic factors, familial lifestyles, and socioeconomic factors, as well as other shared factors. A few studies have compared the association of offspring BMI with both maternal and paternal overweight and obesity, with closer associations with maternal BMI in some but not all studies.[14,15] Some evidence for causality arises from documented effects of bariatric surgery, with lower health risks in children born after surgery than in those born before surgery,[16] as well as from randomized controlled trials comparing the effects of routine care vs treatment of gestational diabetes on children.[17,18]

Maternal obesity may also program long-term child health through its adverse effects on intention to breastfeed, breastfeeding initiation, and breastfeeding duration. In a large cross-sectional survey of more than 9000 children participating in the obligatory school health examination in Bavaria, Germany, we found a much higher prevalence of obesity among children who had never been breastfed (4.5%) than in previously breastfed children (2.8%), with an inverse dose-response effect between the duration of breastfeeding and the prevalence of later obesity.[19] The protective effect of breastfeeding was not attributable to differences in social class or lifestyle. After adjusting for potential confounding factors, breastfeeding remained a significant protective factor against the development of obesity (OR 0.75, 95% CI 0.57–0.98) and overweight (0.79; 0.68–0.93) (Fig 1). Again, there was a clear dose-response relationship between breastfeeding duration and later risk of overweight and obesity, respectively.

Breastfeeding reduces overweight and obesity at school age in >9,000 Bavarian Children

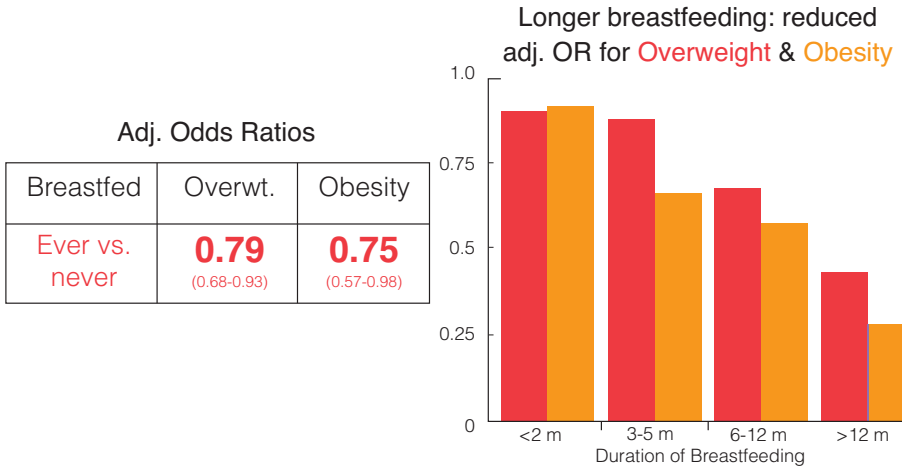

Fig 1. After adjusting for possible confounders, breastfeeding remained a significant protective factor against the development of overweight and obesity.[19]

A protective effect of breastfeeding also was found in several other studies, whereas some others found no benefit. However, systematic reviews and meta-analyses of cohort, case-control, or cross-sectional studies showed a modest but consistent protective effect of breastfeeding.[20,21]

The exploration of metabolic programming effects of maternal obesity on long-term health and on underlying mechanisms offers tremendous opportunities for early prevention of major health risks already during pregnancy, which could provide great benefits for promoting long-term health of the population. Because of the increasing public health importance and the transgenerational nature of the problem, obesity (more specifically, adiposity or body fat content) and associated disorders are the research focus of the new large multidisciplinary consortium *EarlyNutrition* supported by the European Commission's 7th Framework Programme with partners across Europe, the USA, and Australia (www.early-nutrition.org). *EarlyNutrition* will investigate the three principal hypotheses that are currently suggested to explain why early nutrition programs obesity and its comorbidities, ie, the fuel-mediated in utero hypothesis, the accelerated postnatal weight-gain hypothesis, and the mismatch hypothesis (Fig 2).

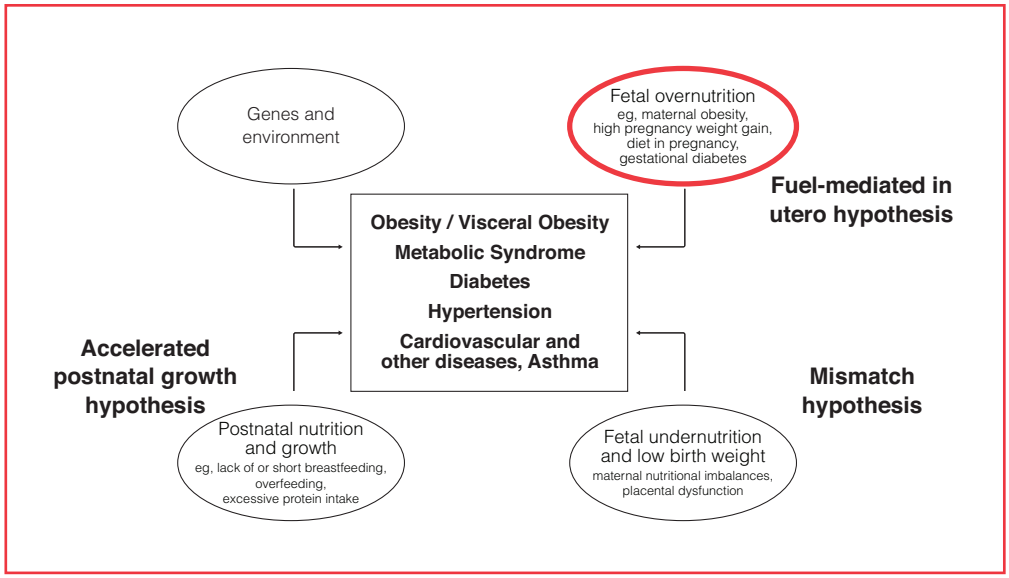

Fig 2. Key hypotheses linking early events and risk of obesity and other non-communicable diseases.[22]

The consortium will conduct experimental studies in animals, contemporary cohort studies, follow-up of existing randomized controlled trials (RCTs) in pregnant women and infants, as well as new RCTs with novel interventions. Through this integrated approach the roles of placental function, early fetal and postnatal growth patterns, the mother's prepregnancy nutritional status, pregnancy weight gain, physical activity, lifestyle, nutrition and fatty acid status, impaired maternal glucose tolerance, as well as the infant's early life nutrition and physical activity as determinants of the later health of the offspring will be explored. We aim to achieve better evidence for the impact of early metabolic programming on long-term health, well-being, and performance, with a focus on reduction of adiposity and associated disorders, characterization and validation of biomarkers for early growth patterns and later outcomes, and demonstration of effects of novel dietary interventions.

Below are several key conclusions:

- Obesity and hyperglycemia in pregnancy, and weight gain prior to pregnancy, are associated with adverse outcomes in many observational studies.
- Maternal bariatric surgery and treatment of gestational diabetes have reduced adverse outcomes in infancy.

- It appears prudent to aim at normalizing body weight in women of childbearing age prior to pregnancy, and to promote regular physical activity and a balanced health-promoting diet.
- Given the limited current level of evidence, better data are needed from contemporary, observational studies and particularly from randomized controlled intervention trials that explore effective behavioral, nutritional and other interventions, in order to inform policy and practice.
- In addition, further exploration of underlying mechanisms and of particularly susceptible subgroups is highly desirable.
- Timely progress in the field will be best achieved by close collaboration of public agencies, academia, industry, and small and medium enterprises. One example of such a collaboration is the European Commission FP7-funded collaborative research project *EarlyNutrition* (www.early-nutrition.org).

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Q & A

Q: You have shown that, in Denmark, birth weight is going up. Is this the result of better nutrition rather than increased obesity? Could it be that we cannot relate to those children who now have higher birth weights the same way that we did to previous generations?

Dr Koletzko: Birth weight is a very crude measure, and average birth weight is an even more crude measure. It does not allow us to really understand the complexity of the relationship. Increased mean birth weight can be a good thing if it means reduced early preterm births, intrauterine dystrophy, and small-for-gestational age births. Increased mean birth weight is less of a good thing if it means increased rate of macrosomia and neonatal adiposity increase. So we have to look at factors beyond mean birth weight in a population. In Denmark, the clear increase in large-for-gestational age babies is one of the key drivers of the increase of mean birth weight. We need to look into this in more detail.

Impact of Maternal GDM and Obesity on Mother and Fetus

Patrick Catalano, MD

Over the last 2 decades, the incidence of obesity in reproductive-age women has increased significantly. The increase has been observed not only in developed areas of the world, but possibly more important in developing countries as well.[1] The increase in worldwide obesity is a harbinger of a shift of the global disease burden from acute infectious disease to chronic diseases such as diabetes and atherosclerotic vascular disease, with their associated increase in health care costs.

One of the primary metabolic abnormalities associated with obesity and diabetes is increased insulin resistance. During pregnancy, obese women are at increased risk of the "metabolic syndrome" of pregnancy, ie, gestational diabetes mellitus (GDM) and hypertensive disorders such as preeclampsia.[2] Women developing GDM have both increased insulin resistance and impaired beta cell function, whereas obese women often have increased insulin resistance but are able to compensate with increased beta cell response resulting in normoglycemia.[3] The insulin resistance of pregnancy not only affects glucose metabolism but also lipid and amino acid metabolism, ie, "fuel mediated teratogenesis" as described by Freinkel.[4]

In one study, we examined longitudinal changes in maternal insulin sensitivity with a hyperinsulinemic-euglycemic clamp in women with a pregravid body mass index (BMI) <25, 25–30, and >30. We found that obese women were significantly less insulin sensitive (ie, more insulin resistant) than lean women (P=0.0001) and overweight women (P=0.004), particularly pregravid and in early gestation (Figure).[5]

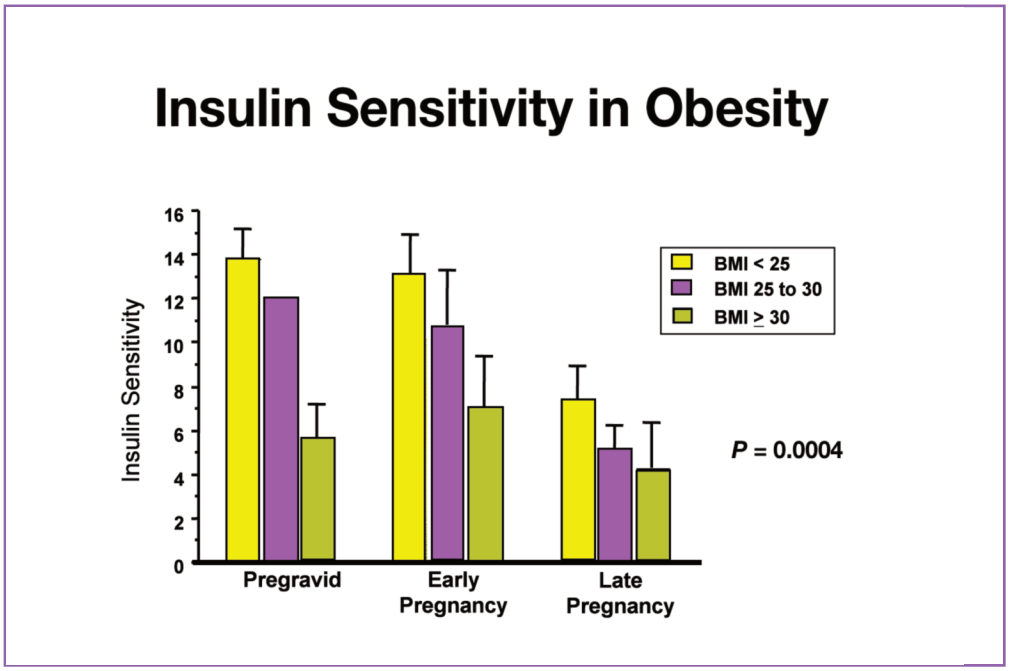

Figure. Longitudinal changes in insulin sensitivity in lean, overweight, and obese women, before conception (pregravid) and in early (12–14 weeks) and late (34–36 weeks) gestation.[5]

Source: Catalano P, Ehrenberg H. The short- and long-term implications of maternal obesity on the mother and her offspring. *BJOG*. 2006;113:1126–1133. Reproduced with permission of Blackwell Publishing Ltd.

Our research has focused on estimates of body composition to assess fetal growth, using anthropometrics, stable isotopes, total body electrical conductivity (TOBEC) and most recently air-displacement plethysmography (PEA POD®). At birth, infants of women with GDM often are larger than those of women with normal glucose tolerance because of increased fat and not lean body mass, even with the same weight.[6] Similarly, infants of obese women are larger at birth because of increased fat and not lean body mass.[7] Both GDM and maternal obesity are risk factors for later childhood obesity and related problems such as insulin resistance, glucose intolerance, and elevated blood pressure.[8]

Over 50 years ago, Jorgen Pedersen hypothesized that increased maternal glucose, which crosses the placenta in a concentration-dependent manner from mother to fetus (facilitated diffusion), results in increased fetal glucose concentrations and insulin response.[9] The combination of these two factors in women with diabetes results in fetal overgrowth, or macrosomia. More recently, investigators have

reported that maternal triglycerides are correlated with increased fetal growth and more specifically, adiposity.[10]

The increases in glucose and triglycerides during pregnancy are normal consequences of the physiology of pregnancy, ie, increased insulin resistance. However, when insulin resistance increases before conception, as seen in GDM and obesity, the physiologic changes in pregnancy are exaggerated. This results in greater nutrient availability to the fetus and subsequent fetal overgrowth. What then are the mechanisms by which insulin resistance increases progressively during pregnancy?

The insulin resistance of pregnancy improves significantly after delivery; therefore, placental factors are most likely responsible. Previously, placental hormones such as human placental lactogen (HPL) were assumed to be a factor. However, more recent research in both pregnant and nonpregnant women points to inflammation as the mechanism, resulting in dysfunction in postreceptor insulin signaling.[11-13] We studied 53 lean and 68 obese women who had a scheduled cesarean delivery to measure insulin resistance and inflammatory markers in the mothers and in umbilical cord blood.[14] Table 1 shows that obese women were significantly more insulin resistant and had significantly higher levels of several inflammatory markers than lean women.

Table 1. Maternal Systemic Inflammation in Obesity[14]

	Lean n=53	Obese n=68	*P* value
Pregravid BMI	22.0 ± 1.9	38.4 ± 6.3	0.0001
Plasma insulin (µU/mL)	11.8 ± 5.6	26.0 ± 14.6	0.006
Plasma glucose (mg/dL)	74 ± 7	79 ± 11	ns
Adiponectin (µg/mL)	10.7 ± 4.6	9.7 ± 4.0	0.0001
Leptin (ng/mL)	31.9 ± 20	72.1 ± 34.7	0.0001
IL-6 (ng/mL)	2.4 ± 1.4	4.6 ± 3.4	ns
TNF-alpha (pg/mL)	1.4 ± 0.9	1.3 ± 0.5	0.004
CRP (ng/mL)	8074 ± 6467	12433 ± 7918	

BMI=body mass index, IL-6=interleukin 6, ns=not sigificant, TNF-alpha=tumor necrosis factor alpha, CRP=C-reactive protein

Evidence indicates that in obese nonpregnant individuals increased inflammatory cytokines derived from adipose tissue may play an important role in insulin resistance.[11] During pregnancy the placenta is another potential source for cytokine production.

Preliminary data have shown that maternal circulating monocytes may be the source of cytokine production. In the placenta, macrophages also may contribute to the inflammatory milieu of obese pregnancy.[15] In gene array studies of placenta from women with obesity/GDM and type 1 diabetes, there is a differential expression of genes related to lipid and glucose metabolism.[16] These data support our hypothesis that both maternal glucose and lipids can be the substrate source of adipose tissue in the developing fetus, depending on the mother's metabolic profile.

As noted previously, at birth infants of obese women have increased body fat. Fetal adiposity is strongly correlated with fetal insulin resistance, as estimated by umbilical cord blood measures of glucose and insulin in women undergoing scheduled cesarean delivery.[14] The increases in fetal adiposity and insulin resistance are related to the increase in obesity and insulin resistance in later childhood. In a regression analysis, maternal pregravid obesity was the strongest risk factor for fetal obesity at birth and in childhood, even in women with well-controlled GDM (Table 2).[8]

Table 2. Maternal Pregravid Obesity as a Predictor of Childhood Obesity[8]

Pregravid BMI >30

OR 5.45 (95% CI 1.62–18.4, *P*=0.0006)

Including gender and group (GDM)

OR 7.75 (95% CI 1.51–37.74, *P*=0.01)

Maternal obesity

Accounts for 17.6% of the variance in childhood obesity

Treated GDM appears to be less of a risk factor for childhood obesity than maternal pregravid obesity

BMI=body mass index, (weight [k]/height [m]^2), GDM=gestational diabetes mellitus, OR=odds ratio

However, more research is needed in the time between birth and childhood in order to understand the effect of the modifiable factors on the individual's growth and development.

Finally, is there anything we can do during pregnancy to interrupt the cycle of maternal obesity and GDM that begets childhood problems of obesity and related metabolic dysfunction? Recently, two randomized controlled trials in women with GDM have shown improved neonatal outcomes at birth.[17,18] There is evidence that treatment of mild GDM can decrease the risk of macrosomia, fetal adiposity, and other related perinatal outcomes. However, only short-term studies have been conducted in children up to 5 years of age, without evidence of long-term benefit.[19,20]

The treatment of maternal obesity during pregnancy is not as well defined. All would agree that avoidance of excessive gestational weight gain during pregnancy is important for both mother and fetus.[21] Although the Institute of Medicine has recently revised the guidelines for gestational weight gain, many feel the guidelines do not go far enough, particularly in the 30+% of pregnant women who are obese.[22] Much research needs to be done in this area to determine both the short-term and long-term benefits of limited weight gain or loss in overweight and obese pregnant women. In the interim, our research group and others are examining lifestyle and dietary factors relating to decreasing inflammation in pregnancy and thereby improving maternal insulin sensitivity and fetal growth. Because of the effects of maternal metabolism on placental growth and gene expression, prevention—which ideally should begin before a planned pregnancy—by necessity should be initiated as early in pregnancy as possible.

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Q & A

Q: Dr Catalano, you talked a bit about looking at the new recommendations for diagnosis of gestational diabetes mellitus (GDM). A lot of times women are diabetic before they become pregnant and the condition just manifests itself in pregnancy. And other women actually have gestation-induced diabetes. Is there any thought on looking at those two groups? You have stated often that what a woman is in late pregnancy is the same as what she was before, and we may manage those who actually have GDM differently than those we just see and realize they are diabetic.

Dr Catalano: I think you are right. The trouble is that we all use classification schemes that we are bound to. So up until the paper from the International Association of Diabetes and Pregnancy Study Groups (IADPSG) came out, we would call these women gestational diabetics. We would lump together women with gestational diabetes at 12 weeks with women with mild glucose intolerance at 34 weeks. This is like preeclampsia. Women can have mild preeclampsia or preeclampsia at 28 weeks, which is devastating. We call both conditions preeclampsia. With the IADPSG criteria, we can go forward calling glucose intolerance in early pregnancy overt diabetes. If these criteria are accepted, we will be able to differentiate between the women who had undiagnosed type II diabetes beforehand and those with the delayed effect of beta-cell dysfunction because of the insulin resistance in late pregnancy.

On the other hand, if these criteria are accepted, who is going to pay for the results? I have a diabetes clinic now in which I hardly ever see 5% of the patients. Who is going to pay for taking care of 20%? A lot of work needs to be done, but at least the issue is being recognized.

Q: Can I just ask you a bit more about lean body mass? We have been looking at placental functioning in more detail, looking at some of the transport systems. We see that first, they relate back to maternal nutrition, but second, they relate specifically to neonatal and subsequent childhood lean body mass moreso than to fat mass. So my take on this would be definitely there are genetic influences operating across all of the compartments but that the environmental regulators of fat are different.

Dr Catalano: I agree that there is a genetic component to it. The data from India suggest that the issue may be that the lean body mass component is decreased.

Q: I think that may in part be environmental and nutritional in origin. When we use direct measures of mother's lean mass, we find that those predict offspring lean body mass. I think the fact that Indian women tend to be thin probably is due more to nutritional influences than genetic influences.

Dr Catalano: I want to bring up the issue that when we look at who is at risk, we find it is not so much how much fat a person has, it is how much fat that person has relative to how much lean mass. If a person has a tendency to put excess nutrients in the liver or in the muscle, the metabolic effect, enlargement, occurs because it happens to be in tissue that is more of a regulatory tissue than a storage tissue.

Insights From Body Composition Studies

David Fields, PhD

Twelve percent of children under 2 years[1] and 10% of 2–5 year olds are obese (National Health and Nutrition Examination Survey; body mass index [BMI] ≥95th percentile)[2] with ≈70% remaining obese in adulthood.[3] In females of childbearing potential the prevalence of obesity is approaching 30% and appears to be trending upwards.[4] This rise in the percentage of overweight/obese pregnant women is worrisome given the negative long-term health consequences incurred by their offspring.[5] Over the last 3 to 5 years, exciting technology has emerged allowing clinicians/researchers to measure whole body composition accurately, quickly, and noninvasively in infants as young as 5 days old. This development allows for the first time the opportunity to study the link between maternal BMI and offspring body composition early in life, which could yield valuable insights into innovative strategies for obesity and diabetes prevention programs. Therefore, the purpose of this presentation summary is to first review whole body composition techniques used in pediatric populations. With this in mind, the second purpose is to review the current state of the literature linking maternal obesity and offspring body composition early in life.

In pediatric populations, body weight or BMI percentiles define overweight/obesity risk. An important but often overlooked issue using this approach is that the quality of the weight is left unaccounted. The following two examples highlight this point (unpublished data; body composition measured by dual-energy X-ray absorptiometry [DXA]). In example 1, two 5-year-old girls have the same BMI (16.3 kg/m^2; 73rd percentile) but have a 5% fat unit difference in %fat (29.1% vs 24.5%) and a 26% difference in trunk fat mass (2,022 g vs 1,495 g) (see Fig 1).

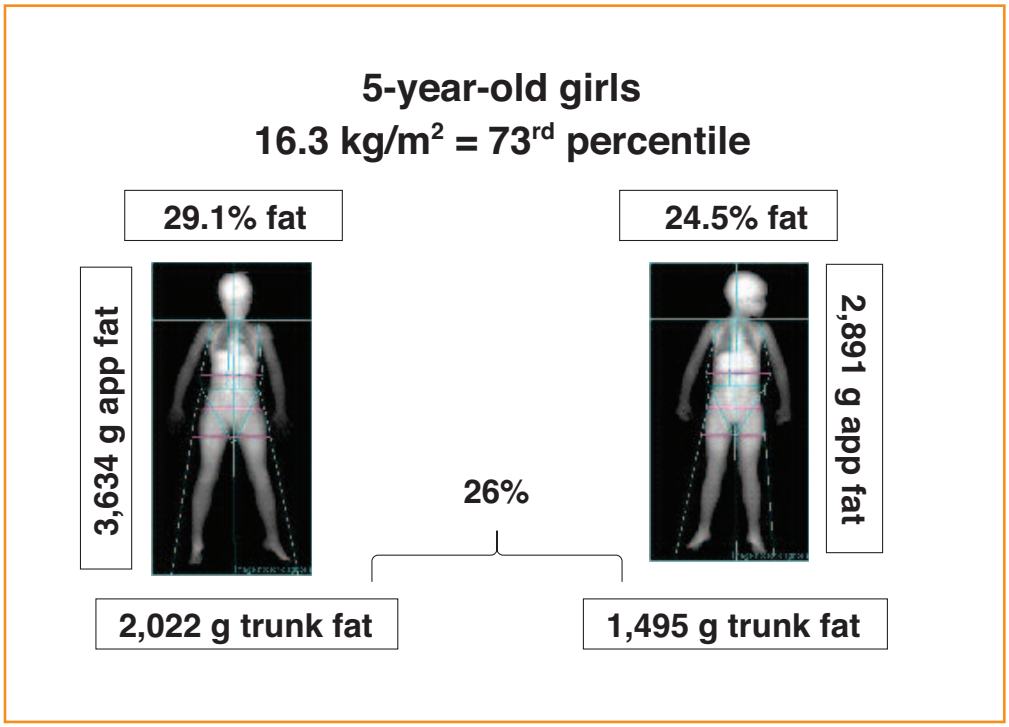

Fig 1. Dual-energy X-ray absorptiometry (DXA) of two young girls with the same BMI.

In example 2, two 6-month old girls weighing 6.68 kg have a 4% fat unit difference in %fat (30.9% vs 34.9%). Both examples demonstrate the importance for accurate body composition assessment. The measurement of body composition allows clinicians to individualize treatment plans in growth- and development-related matters in their patients, while giving researchers the ability to disentangle complicated observational data linking early life events with long-term morbidity.

Two commonly available research-based whole body composition techniques in pediatric populations are air-displacement plethysmography (ADP) and DXA. Of note, these techniques are not limited to pediatric populations but their application to pediatric populations is new.

The basic underlying principles of ADP originated in the early 1900s in Germany[6,7] with the foundational basis of a feasible, practical, accurate, and valid ADP described in detail by Dempster and Aitkens in 1995.[8] The first validated published data using ADP in an adult population also appeared in 1995.[9] In brief, ADP (BOD POD®; COSMED USA Incorporated, Concord, CA, see Fig 2) comprises a single unit dual-chamber plethysmograph consisting of both a testing and reference chamber with whole body volume determined by the ratio of the pressure amplitudes

between the two chambers.[8] In 2000, ADP was validated for the first time in children (≈10–12 yrs)[10] and in 2003, ADP (PEA POD® , see Fig 2) accurately measured body volume in infants <8 kg.[11,12] ADP is an attractive technology given its application across the spectrum of ages and body weight, ie, ADP can measure infants as small as ≈ 4 kg while accommodating morbidly obese individuals weighing >150 kg.

Fig 2. Air-displacement plethysmography: Body composition tracking systems.

Source: http://www.cosmed.com/index.php?option=com_content&view=article&id=971&Itemid=304&lang=en

DXA determines body composition by measuring attenuated X-rays emitted at two energy levels (40 and 70 kV), with the ratio between the low-energy and high-energy sources being 1.21 and 1.369 for fat and soft lean tissue respectively.[13] DXA is an exciting technology given its ability to provide regional body composition estimates. A recent paper indicated that DXA in newborn infants was reliable and was a reasonable reference tool for estimating body composition.[14] As with ADP, DXA can accommodate a wide range of ages and body sizes; however, some institutional review boards do not allow testing in young children given the small dose of radiation.

Parental obesity, especially on the maternal side, more than doubles the risk of the offspring being obese as an adult.[15-17] Maternal obesity conferring increased adiposity in their offspring is multifactorial with likely candidates being genetic and epigenetic factors, overnutrition, maternal BMI and gestational weight gain,

socioeconomic factors, and environmental factors to name but a few.[18] It is important to understand that maternal adiposity is not restricted solely to pregravid obesity, but also to gestational weight gain. Both contribute to increased maternal circulating glucose and fat concentrations, which lead to increased fetal insulin secretion and increased fetal growth. An excellent review article presents the body of the literature on the association between maternal obesity and offspring obesity throughout the lifespan.[4]

Briefly, and focusing narrowly on neonates, it has been shown within the first 2 to 3 weeks of life offspring from overweight/obese mothers (defined by a BMI of >25 kg/m^2) were significantly fatter (determined by ADP) on both an absolute (414 vs 264 g fat; $P<0.05$) and relative (13.6 vs 12.5 %fat; $P<0.001$) basis compared to offspring from normal weight mothers (see Table below).[19]

Table. Neonatal Body Composition at 3 Weeks[19]

	Normal (N=38)	Overweight/Obese (N=44)	*P* Value
Gestational age (wks)	39.5 ± 1.2	38.9 ± 1.0	0.03
Age at testing (days)	19.5 ± 8.5	19.8 ± 9.3	0.91
Birth length (cm)	50.7 ± 2.6	49.6 ± 2.6	0.08
Birth weight (g)	3433 ± 396	3368 ± 400	0.44
% body fat	12.5 ± 4.2	13.6 ± 4.3	0.00
Fat mass (g)	414 ± 264	448 ± 262	0.04
Fat-free mass (g)	3311 ± 345	3162 ± 343	0.03

Furthermore, increased fat mass in offspring from overweight/obese mothers is present as early as 72 hours postpartum.[20] Sewell et al[20] reported offspring from overweight/obese mothers were significantly fatter (determined by total body electrical conductivity) (11 vs 9.6 %fat; $P<0.01$) compared to offspring from normal weight mothers, while having significantly greater absolute fat mass (406 vs 331 g fat; $P<0.01$). Interestingly, Catalano and Kirwan[21] reported significant whole body composition (determined by total body electrical conductivity) differences starting at birth between offspring from normal weight and obese mothers (11.6 vs 13.1 %fat

[percent fat for entire body]; $P<0.001$; 384 vs 448 g fat [absolute amount of fat in body]; $P<0.01$). These relationships between maternal obesity and offspring adiposity persist unabated into adulthood with long-term obesity, diabetes, and cardiovascular morbidity certain.

In conclusion, over the last 10 years technological advances have given clinicians and researchers the opportunity to accurately, quickly, and reliably estimate whole body composition in infants as young as 5 days old. This is an important development given the link between maternal obesity and offspring adiposity and highlights the need for valid methods to unravel this knotty problem. Though the literature is developing, it appears maternal obesity increases the likelihood of their offspring being obese later in life with a significant impact present starting at birth.

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Q & A

Q: At birth, the lean body mass of a fetus is about 80% water. Do you think that the changes you see at 3 months could be a reflection of the change in water mass rather than bone mass or muscle mass? When we diurese that extra water in the fetus, we end up with a decrease in lean body mass.

Dr Fields: That is a good point. My data do not show that because I usually do not start dosing children until about 6 months of age. We probably should try to start earlier.

Q: From a functional point of view, it is probably important to know exactly where the fat is. Would you comment on such issues as looking at fat position in the liver, which drives you to discuss ultrasound and magnetic resonance imaging (MRI)—techniques other than those you described?

Dr Fields: That is where I want to go, but as everyone keeps saying, MRI equipment costs money, so I will need a big grant to get it. It is easy to do these sorts of tests in very young infants after you feed them. Compliance is good.

Q: I know you have measured skinfolds on neonates, as well. Will you compare your other methods with the skinfold measurements?

Dr Fields: I measure at least five sites: the abdomen, chest, thigh, tricep, and subscapula. The measurements are in my database, but I have not done much with them, nor have I compared this to other methods. We measure the mothers as well.

Q: Do you have any experience with ultrasound in utero?

Dr Fields: I am trying to collaborate with some of my friends in obstetrics because they do ultrasounds in utero. We have not done that ourselves yet.

Q: And in children postnatally?

Dr Fields: No, because it has been difficult to obtain the time in radiology. As a researcher, I want to know that I can bring a subject in at 8:00 and get it done. The radiology staff does not want to do that. Or they want me to come in Saturday at 7:00 in the morning. Clearly, that is not going to work.

Q: You showed that at 3 weeks there are already differences in lean body mass and fat mass in infants. Did you control for feeding practices?

Dr Fields: We looked at the data many different ways but we did not control for feeding practices.

Q: Do you believe there were more breastfed than formula-fed infants in your sample?

Dr Fields: In our sample, 60% were breastfed and 40% were formula fed.

Discussion

Raanan Shamir, MD

Dr Shamir: Dr Catalano, you looked at insulin influence on gene expression, showing that insulin in the placenta causes upregulation of the cholesterol pathway. We are not used to seeing this in medication cultures. I mean fibroblasts or macrophages. Did you follow this throughout pregnancy?

Dr Catalano: The trouble is getting placenta samples at different times during the pregnancy. That is why we started with delivery—the sample was easy to get. We went back to the 1st trimester, which was hard to get, but we were still able. I think the best way is to use a chorionic villus sampling. Do it in early pregnancy so we can have a baseline, look at the environment the woman is exposed to, and then repeat it again in late pregnancy. Whether the study is a randomized controlled trial or just observational, we can see the changes in the mother that did not affect gene expression.

Dr Shamir: Did you have a chance to look at both premature and full-term infants?

Dr Catalano: No, just full-term infants.

Dr Shamir: This is a very noble finding; usually insulin does not enhance cholesterol synthesis just by induction of the enzymes that are responsible for cholesterol.

Dr Catalano: Right. I think it may have to do with the lipid that is already in the placenta, and then not just the placenta tissue but the macrophages that also may be in there. So it may be coming from the cytokines.

Dr Pan: Dr Catalano, you mentioned using anti-inflammatory supplements to decrease low-grade inflammation. Did you get results from that?

Dr Catalano: No. It is ongoing so we have no results yet. It is interesting that these data about looking at aspirin as an anti-inflammatory relative to the issue of diabetes are in the adult literature but not related to pregnancy. We want to use something that we think is safe in pregnancy.

Dr Pan: We used an obese rat model to look at whether macronutrients can modify the inflammatory markers but we did not see any changes whether we increased fat, decreased fat, increased carbohydrate, or increased protein. As long as the weight stayed the same, the fat mass always produced those markers in our system.

Dr Catalano: We use a 3-day dietary recall to get dietary histories. We are trying to measure three concentrations so that we were blind to the results and the randomization. The thought is that we will be able to look at the results objectively and see if there is a dose response because I am not sure everyone took all the pills all the time. We also can look at macronutrients to see whether there is a relationship.

Dr Poston: Dr Catalano, you commented about the use of metformin in gestational diabetes mellitus (GDM). It has been used successfully to reduce macrosomia in women with GDM [Ijäs H et al. *BJOG*. 2011;118:880–885]. I share your concerns about the long-term influence on the child. We do not know anything about that. But that aside, two studies are going on in the United Kingdom in which obese pregnant women are being randomized to receive metformin or not. Do you think that is a sensible approach to improve pregnancy outcomes?

Dr Catalano: I can give my personal opinion only, and I do not think there is an official opinion. I think one study shows that metformin is equivalent to insulin, but not better than insulin. I have seen only some preliminary data, but no follow-up data. I know that insulin is not fun to take, but I know that it works and that it does not cross the placenta. It has been used for 60 years without any problems.

Dr Poston: A lot of obese pregnant women have normal glucose tolerance and do not have insulin resistance. Do you think giving insulin to sensitize women who have normal glucose tolerance, which is what happened in that study, is safe? Will that not induce a hypoglycemic response when they have normal insulin sensitivity?

Dr Catalano: The studies have been done only in women with GDM, but insulin resistance increases even in women who do not have GDM. I think induction of a hypoglycemic response is possible but unlikely because insulin resistance would have to be overcome.

Dr Poston: We should do a trial to find out.

Dr Shamir: But when we look at the data, we see it is not GDM. The lower the blood glucose level, the fewer the complications. It is not only an abnormal glucose tolerance test, if I understand you correctly.

Dr Catalano: But if we lower blood glucose levels too much, we can get into problems with fetal growth restriction. I think one researcher reported that when we push so hard that a woman's glucose level is 87 mg/dL, we increase the risk of her baby being born small for gestational age.

Dr Poston: That is my concern.

Dr Catalano: But I do not know whether we can do that with metformin.

Dr Yajnik: Just by treating GDM we introduce an element of growth restriction?

Dr Catalano: That is a hard question to answer. We may be. We talked about insulin resistance relating to carbohydrate and lipid, but it also relates to protein metabolism. There is no question that amino acid turnover changes in pregnancy, and if we give the pregnant woman insulin, we probably will suppress that to a certain degree. Could this have an adverse effect? I think the answer is yes, but I will not speculate.

Dr Yajnik: The reason I ask is that a follow up of children of the ACHOS study was published. These children were on average 150 g lighter than the control group at birth, and had a lower incidence of macrosomia and associated perinatal adverse outcomes [Crowther CA et al. *N Engl J Med*. 2005;352:2477-2486]. However, at 5 years of age, they had caught up with and were similar to the control group [Gillman MW et al. *Diabetes Care*. 2010;33:964-968].

So, I think an element of growth restriction was introduced in utero, which promoted rapid catch-up. It would prove interesting to see whether they have an earlier adiposity rebound, which is a risk factor for future diabetes. Long-term follow up of children in both of the GDM intervention studies is critical to answer the question of long-term benefits.

Dr Godfrey: Data about the effects of GDM treatment at age 7 years are quite reassuring in terms of beneficial effects without apparent detrimental effects.

Dr Catalano: I think we are talking about treating someone who does not have gestational diabetes.

Dr Poston: But the follow-up group was a small subgroup of the main trial, so it was not necessarily representative of the whole trial population.

Dr Yajnik: Agreed. Follow-up was only in South Australia?

Dr. Poston: Yes. About 200 subjects.

Dr Catalano: In our trial, we did not find any difference at age 3 or 4 years.

Dr Shamir: But the study has no nutrition data after birth. That is one of the difficulties.

Dr Catalano: Not for the whole cohort, but nutritional data were produced for some of the sub-cohorts. As you probably know, there is a grant to fund a follow-up study. I am sure we will get nutritional data then.

Dr Rueda: Dr Catalano, you described an interesting positive role of the microbiome reaching the placenta, modifying the inflammatory response and influencing different target outcomes, so this is perhaps a potential road to modifying the grade of inflammation at the maternal-fetal interface.

A recent publication showed that the microbiota colonizing the placenta is different in low-birth-weight infants than in normal weight infants [Fichorova RN et al. *mBio*. 2011;2:e00280-10]. Do you think a role might exist for the colonizing microbiota of the placenta, modifying the proinflammatory to anti-inflammatory profile there and, as a consequence, modifying functional outcomes in the infant? Also how about modifying obesity prevalence in the infant through this mechanism?

Dr Catalano: I have heard of that paper but have not read it. I think that this is an interesting issue in light of our discussion about genes or the environment expressing the placenta. Just as epigenetic changes occur, I would think that microbiome changes could occur, depending on what a person eats. A study out of Finland showed that a probiotic diet can decrease the risk of GDM and change the bacteria in the microbiome. What is the mechanism? I do not know anyone who is looking at this yet.

Dr Shamir: Jeffrey Gordon came out with the theory relating obesity and probiotics, but he changed the way he looks at this relationship. Now it is more the effect of nutrition on the microbiota than the cause and effect between the microbiota and obesity.

Dr Godfrey, you were talking about your randomized trial in which you supplement women with vitamin D. What is the supplementation dose you are using?

Dr Godfrey: We have trialed with two different doses of vitamin D. In an initial phase, we looked for a dose that produced a decent increment in vitamin D levels. We ended up using 1000 units.

Dr Shamir: Now people are talking about 2000 units as being the maximum dose.

Dr Godfrey: That is the reason why it was done on an ethics committee. We had to go through an initial phase of assessing the effects that we saw.

Dr Shamir: I like your comment about starting supplementation much earlier during pregnancy. We are conducting a study in which we are giving 400 units vs 2000 units during the 3rd trimester. Our institutional review board gave us trouble but finally cleared the 2000 units, but perhaps if we started earlier, we could have used smaller amounts of vitamin D.

Dr Catalano: What is the reference value for a normal vitamin D level?

Dr Shamir: There are no studies showing this. The only question is what is a safe dose?

Dr Catalano: Is there a biomarker that one could use such as bone density?

Dr Shamir: There is a high prevalence of low 25-hydroxyvitamin D (25OHD) levels in our population, so we are using 25OHD as a marker of whether we need to supplement. There are not any studies that tell us how much to increase 25OHD throughout pregnancy or what the effect would be on the newborn.

Dr Poston: A recent publication describes a large study looking at different supplement doses of vitamin D in pregnant women. The researchers gave the women up to 4000 units and found that 2000 were sufficient. A dose of 4000 units did not confer any extra benefit. Importantly, the researchers did not report any adverse effect throughout the whole of the study at any dose.

Dr Godfrey: I think there are concerns other than the immediate outcomes of high doses.

Dr Poston: Later outcomes.

Dr Godfrey: Exactly. For our institutional review board, we were tabled to enroll women with high vitamin D levels at the outset.

Dr Shamir: People now would be reluctant to give more than 2000 units.

Dr Yajnik: In the Pune Maternal Nutrition Study, we found the opposite association. Higher maternal vitamin D levels were associated with a smaller baby. Part of the reason is that the vitamin D status in these women who work in the farms is quite good, and vitamin D is partly a surrogate for maternal physical activity, which is considerably high. Thus, there was quite a bit of compounding by maternal activity.

Dr Atkinson: Other studies done by Prentice and colleagues in Gambia [Prentice A et al. *Acta Paediatr*. 2009;98:1360-1362] found no association between maternal vitamin D status in pregnancy (all mothers had serum 25OHD >50 nmol/L) and infant outcomes of birth weight, weight, length, or head circumference during the 1st year, or bone outcomes, including bone mineral content, bone width, or area or size-adjusted bone mineral content of the radius or whole body.

So, I think the jury is still out on the long-term effects of exposure to maternal vitamin D status in pregnancy.

Impact of Pregnancy Nutrition on Cognition

Cristina Campoy, MD, PhD

Evidence shows that early nutrition can influence later mental performance, cognitive development, and behavior. Nutrition plays an important role in supporting structural and functional growth of the human brain from conception, through childhood and adolescence, and into adulthood.[1] Most of the energy is spent on supporting the propagation of action potentials, followed by postsynaptic signaling and maintenance of resting potentials.[2] A steady supply of macronutrients and micronutrients also is important for the synthesis of neurotransmitters, as well as their receptors and transporters, for the renewal and maintenance of the axonal cytoskeleton and its myelin sheath, for the growth of synaptic spines, and, as such, neural plasticity, and for neuronal survival.[1]

During the short period of 9 months, the initial "mother" cell gives rise to more than 100 billion nerve cells and a brain that weighs approximately 400 g when the child is born. During the first 4 years of life, the brain continues to grow, reaching the size of 1200 g at 3 years, which is only approximately 200 g less than that of an adult's brain. During the next 10–15 years, brain growth continues, involving different brain compartments in a slightly different way. For example, the thickness of the different regions of the cerebral cortex changes between the ages of 5 and 18 years at different paces, with the regions important for reasoning, planning, and social communication maturing last.

The idea that the diet of mothers could have an influence on long-term mental performance has major implications for public health practice and policy development and for our understanding of human biology, as well as for food product development, economic progress, and future wealth creation. Nutrients might serve as critical signals, acting directly or via coupling mechanisms on "receptors" in sensitive tissues.[3] Some programming events might have immediate effects on structural development of the brain (eg, on dendrite arborisation or glial cell growth with long-term consequences).

Although many epidemiological studies have uncovered associations between various macronutrients and micronutrients and cognitive performance and mental health,[4] the results of randomized clinical trials (RCTs)[5] less often support causal effects of nutrition on the brain and cognition. It is argued that most RCTs are too

short (lasting only a few months), too focused on a single micronutrient, or too small to detect modest effect sizes against the heterogeneous genetic and environmental background of the participating individuals.[5] Furthermore, the primary outcome measures, such as rating scales of cognition and mental health or even some of the cognitive tests, are perhaps too insensitive or may have low test-retest reliability. In this context, the use of various approaches to directly measure brain structure and function seems appealing.[1]

Methodologies for the Assessment of Brain Development

A number of techniques are available for the assessment of nutrition-related variations in brain structure and function (Table). With the exception of positron emission tomography (PET), it is possible to apply all of the methods mentioned from childhood onward.

Table. Methodologies to Explore Brain Development

Neuropsychological tests	Electrophysiological recording	Neuroimaging
Study of different domains to assess: • Intelligence and mental performance • Psychomotor development • Behavior maturation	**Visual and auditory acuity:** • Sweep VEP • Transient flash VEP • Pattern-reversal stimuli VEP • Steady state VEP • HOTV visual acuity • Sonksen-Silver acuity system • Teller acuity cards **Scotopic ERG** **EEG** **EEG/ERP**	**Brain structure and function:** • aMRI • fMRI • MEG • PET

aMRI=anatomical magnetic resonance imaging, EEG=electroencephalography, ERG=electroretinogram, ERP=event-related potentials, fMRI=functional magnetic resonance imaging, HOTV=single letters that are presented to the child using the Electronic Visual Acuity System,[6-8] MEG=magneto-encephalography, PET=positron emission tomography, VEP=visual evoked potential

Neuropsychological Development Assessment

Neuropsychological development is a heterogeneous process in which several critical periods are involved.[9] Therefore, the effects of nutrition on mental performance will depend on maturation stages and are easier to detect during and after each critical period. In addition, the neuropsychological tests should assess the specific neuropsychological domains (perceptual, motor, attention, learning and memory, and executive functions), instead of global cognitive performance.[9,10]

Recent papers reported some methodological concerns in studies about the effect of nutrition on mental performance, cognition, and behavior.[9,11] First, global measures of mental performance are possibly not sensitive enough, so specific effects of the nutritional intervention are hidden or masked. Second, for the assessment of nutritional effects, the specific approach is theoretically driven according to a specific biological mechanism. Moreover, it is necessary to pay special attention to cultural factors in order to compare similar neuropsychological tests administered in several countries. Another factor to consider is the practice/learning effect, especially if the intention is to test some improvement after a nutritional intervention.[12,13]

These suggest that any one test is not enough to detect significant changes in brain development because of a specific nutrient supplementation. Therefore, each study should have carefully designed, specific neuropsychological tools combining different neuropsychological domains to evaluate the potential effect of a nutrient, always taking into account the biological mechanism involved in the specific nutrient-effect that is explored.

Electrophysiological recordings can help us understand the cognitive functioning in children, especially because they can provide information about the “when” and even the “where” in the brain that these functions are taking place (Figure).[14]

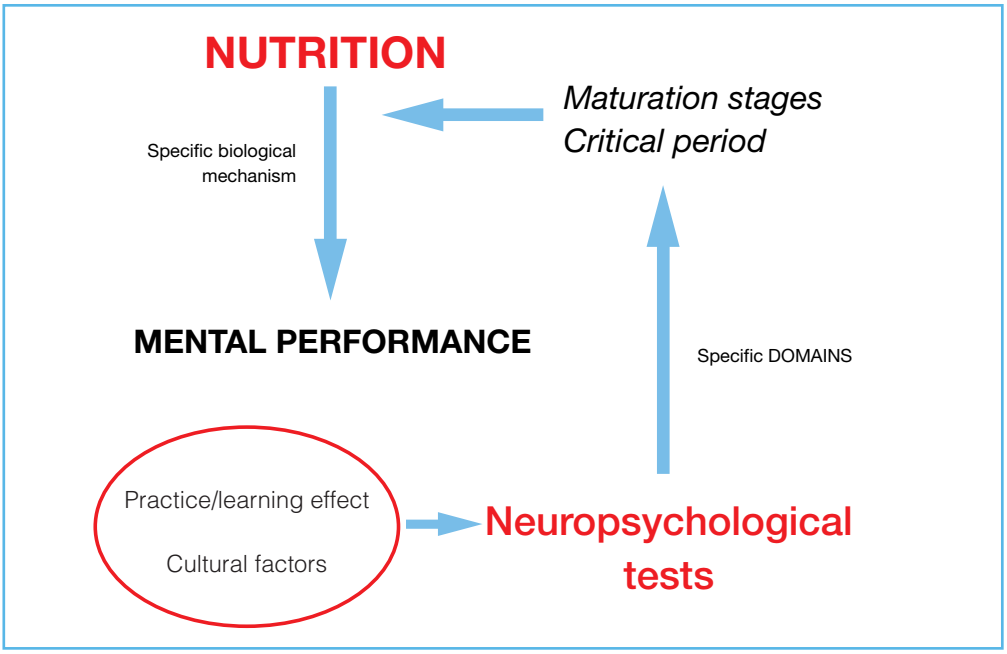

Figure. Electrophysiological recordings.

Visual Acuity

Visual development is incomplete at birth, particularly in premature infants. Maturation of the visual system, which includes neurological and ocular components, is influenced by many factors, such as prenatal and postnatal nutrition and postnatal visual stimulation.[15]

Evaluation by testing for visual acuity is performed in newborns (3–12 months), preverbal infants (12–24 months), and verbal infants.[16] Tests include preferential looking and visual tracking tests. Preferential looking is tested using static Teller acuity cards,[17-19] forced-choice preferential looking (FPL) or the Bébé vision test,[20,21] and other computerized infrared oculography, which allows testing in preverbal infants.[22] All methods employ a manual or automated system based on black and white patterns, usually vertical black and white bars or gratings on cards or a screen.

FPL, which is used in neonates up to 6 months of age, is based on the premise that neonates dislike boring visual stimuli and will tend to look at a pattern rather than a plain display. In FPL, infants are shown a display containing a pattern (the stimulus), and the observer decides where the stimulus is located, based on their observation of the infant's head and eye movements.[21] Because age causes the highest spatial

frequency resolved by a child to vary, the Operant Preferential Looking Test was adapted for use as a shorter screening procedure in children from as young as 6 months of age and up to 3 years, using the appropriate specific diagnostic-grating frequency for the age of the child.[23]

Visual evoked potential (VEP) testing[24] is used to assess communication between the eye and the brain and was adapted for use in preverbal infants as an automated test known as sweep VEP.[25] The sweep technique allows measurement of VEP in a few seconds, ensuring that the child's attention is maintained and stable fixation achieved for a sufficient time to complete the test, even in newborns.[25] However, as the test parameters are subjective and involve lengthy interpretation, sweep VEP mainly is used in the research setting.

An electroretinogram (ERG) is used to assess retinal function by measuring the difference in electrical potential between the anterior eye surface and the face in response to a visual stimulus.[26]

Electroencephalography/Event-Related Potentials (EEG/ERP)

EEG/ERP is a noninvasive method for assessing brain activity from scalp recordings. Researchers interested in cognitive development and brain functioning have used these techniques in their studies for decades. The applications of EEG/ERP are wide, including use to discriminate the effect of glucose or protein intervention on visual and auditory perception.[27,28] Other studies utilize EEG/ERP to analyze the effects of iron or iodine supplementation on working memory.[29] Some studies are exploring the effect of interventions with lemon balm, ginseng, or *Ginkgo biloba* on attention and vigilance, while other studies have explored EEG/ERP with other brain domains involved in problem solving, emotions, or decision making.

For instance, the development of attention networks were traced using the Attention Network Test (ANT)[30-32] and behaviorally[33] by EEG/ERP measures.[34] Both measures are proven to discriminate attention abilities as a function of age and training,[34-36] and even temperament.[37] Literature clearly indicates that both behavioral and EEG indices of ANT are a steady measure of the brain attention networks, especially in the case of the conflict resolution system. Within the NUTRIMENTHE EU Project, a protocol was developed to analyze the effect of early nutrition on attention and working memory at 8.5 and 9.5 years of age to detect long-term effects of dietary intervention with docosahexaenoic acid (DHA) and folate during pregnancy.

One of the most relevant parameters for analysis within the EEG/ERP examination is the N2 wave. This appears at the time that the individual is performing the action after processing the paradigm shown. The N2 is an early frontal negativity that is elicited during conflict and inhibition tasks[38] and is thought of as a marker for cognitive control processes, most notably conflict monitoring and detection.[39,40] The N2 is linked to affective, attentional, and cognitive factors that appear to play a role in the emergence of a range of mood and anxiety problems.[41,42] Thus, the N2 has the potential to serve as a neurophysiological marker for a biological diathesis of attention and temperamental factors associated with affective risk and resilience.[41,43] N2 also is useful for other cognition assessments (ie, using the working memory task developed in NUTRIMENTHE EU Project).

EEG/ERP also offered interesting neuroimaging, which helps locate exactly the place in the brain that is activated at the same time the children are trying to solve the paradigm proposed.

Neuroimaging Studies

Structural Magnetic Resonance Imaging (MRI)

MRI is a noninvasive technique that creates detailed three-dimensional pictures of the brain, each consisting of thousands of three-dimensional image elements called voxels. The most common structural MRI sequence produces T1-weighted images, which are characterized by a high contrast between gray matter and white matter. These images are analyzed using various computational algorithms that quantify, automatically and precisely, many different features, such as thickness of the cerebral cortex, volume of gray and white matter, and tissue densities.[44] In addition, other MRI sequences allow one to assess the microstructure of white matter (diffusion tensor imaging and magnetization transfer imaging) or the chemical composition of brain tissue (magnetic resonance spectroscopy).[1]

Functional Magnetic Resonance Imaging (fMRI)

For imaging brain function, the most commonly measured MRI parameter is the blood-oxygenation-level dependent (BOLD) signal. The BOLD signal reflects the proportion of oxygenated and deoxygenated blood in a particular brain region at a given moment. A strong correlation between the amount of synaptic activity and regional cerebral blood flow is why the BOLD signal is a good, albeit indirect, measure of brain function.[45] In the majority of fMRI studies, changes in BOLD signal are measured in response to various sensory, motor, or cognitive stimuli.

Therefore, only brain regions that respond to a particular set of stimuli are examined using the given paradigm.

Structural MRI and resting EEG procedures are used in healthy, unsedated infants as young as 7 days old,[46,47] while fMRI and event-related EEG and MEG (magneto-encephalography) are feasible on subjects aged approximately 5 years or older. In large studies (n=>100) and RCTs, structural MRI is the best imaging modality for evaluating the long-term effects of nutrition on the brain. In RCTs with modest sample sizes (n=<100), a combination of structural MRI and fMRI with EEG would provide the most comprehensive assessment of brain structure and function and, hence, offer insights into possible brain mechanisms underlying the effect of nutrients on cognition and mental well-being. Overall, brain imaging offers a rich armamentarium of acquisition and analysis tools for the quantitative in vivo assessment of the effects of nutrition on the human brain.[1]

Update of Findings

A lack of clarity and little consensus still remain regarding the role of several nutrients, such as proteins, long-chain polyunsaturated fatty acids (LCPUFAs), B vitamins, minerals and other nutrients in neurodevelopment, mental performance, and mental illness. Many animal studies have demonstrated that changes in dietary nutrients can alter brain morphology, as well as its biochemical functions, but human studies have not offered clear evidence of the specific effect of each nutrient during early life, except in some specific cases.

Protein

Protein deprivation can cause direct deleterious effects on the brain, such as loss of brain weight, altered hippocampal formation, impairment of neurotransmitter systems, and changes in protein phosphorylation.[48] Undernourished children (under 3 years of age) usually have lower development, behavior, and school achievement, and supplementation studies have shown benefits on their development.[49] These benefits are attributed to energy and protein supplementation rather than to micronutrients.[50] No data are available from large prospective follow-up studies on the possible effect of different levels of protein intake early in life on later neurodevelopment for well-nourished infants. Breastfed infants have lower protein intake and show lower insulin-like growth factor-1 (IGF-1) levels.[51] Children who were breastfed in the early weeks of life had a significantly higher IQ in childhood compared to formula-fed children exposed to high protein intakes during the first 12 months of life who had higher plasma concentrations of total and free IGF-1.[52,53]

IGF-1 is involved in brain development, ranging from neuroprotection after neuronal damage to neurogenesis, myelination, synaptogenesis, and dendritic branching.[54-57] Furthermore, overexpression of the IGF-1 gene determines an increased brain size,[58] whereas targeted IGF-1 gene deletion stunts brain size.[59] These studies prove that IGF-1 is associated with brain development in childhood, but detailed investigation of influencing factors still is needed.

Long-chain Polyunsaturated Fatty Acids (LCPUFAs)

Animal models have shown that maternal diets deficient in n-3 and n-6 LCPUFAs alter the accretion of these fatty acids in the neonatal cortex, which results in changes in neurotransmitter metabolism and learning impairments.[60] DHA deficiency leads to reduced dendritic arborisation[61] and impaired gene expression for regulation of neurogenesis, neurotransmission, and connectivity.[62,63]

A growth spurt in the gray matter of the human brain occurs during the 3rd trimester of pregnancy and the first postnatal months, with a large increase in the cerebral content of arachidonic acid (AA; 20:4 n-6) and DHA.[63] Adverse outcomes associated with insufficient intake of long-chain omega-3 fatty acids during pregnancy include intrauterine growth retardation, delayed or suboptimal depth perception,[64] adverse neurodevelopmental measures,[65] residual deficits in fine motor skills, speed of information processing in infants,[66] and irreversible deficits in serotonin and dopamine release. Higher maternal intake of DHA results in higher maternal plasma levels and thereby increased DHA transfer to the fetus.[67] Thus it seems an appropriate prenatal and postnatal supply of LCPUFAs is essential for normal fetal and neonatal growth, neurological development, and functional maturation, including learning and behavior. However, the long-term effects of LCPUFA supplementation on the neurocognitive outcome and mental performance of these infants remain unclear.

A large observational study described beneficial effects on cognitive development in children whose mothers consumed seafood during pregnancy.[65,68] Supplementation studies during pregnancy and/or lactation have not demonstrated clear evidence of beneficial effects of LCPUFAs on visual acuity (ERG and VEP), stereoacuity, or neurodevelopment.[69-77] Only three trials have reported long-term effects of supplementation to date.

Helland et al report a better performance in the Kaufman Test (K-ABC) in the supplemented group compared to control at 4 years of age, but this effect was not observed in IQ at 7 years. The authors also report a significant positive correlation between IQ at 4 years and DHA levels in infant plasma at the 4th week of life, as

well as an association between maternal DHA levels in 35 weeks of gestation and IQ in children at 7 years of age.[71,72] The NUHEAL trial reported no differences in neurological outcome of children at 4 years and 5½ years between children born to mothers receiving fish oil supplements and those who did not, but they did demonstrate better neurological optimality scores in children at 5½ years with increasing DHA levels in cord blood.[78] Moreover, children whose mothers had higher DHA percentages in erythrocyte phosphatidylethanolamine at delivery were more likely to have a Mental Processing Composite score of the K-ABC over the 50th percentile at 6.5 years.[79] Despite these contradictory results, a sufficient availability of DHA during the perinatal brain growth spurt is widely considered mandatory for normal cognitive, visual, and motor development.[80]

Recently, it was demonstrated that polymorphisms of FADS1 and FADS2 (fatty acid desaturase gene variants) are closely related to important changes in fatty acid metabolism linked to brain development.[81] Koletzko et al[82] showed a consistent and significant association of rare single-nucleotide polymorphism alleles with lower amounts of DHA in red blood cell phospholipids of pregnant women. A modulation of DHA status during pregnancy by frequently occurring FADS genotypes is possibly of major relevance for child outcomes.

As previously mentioned, several cohort and randomized control studies showed positive but also null associations between LCPUFA intake and status in the prenatal and postnatal period and developmental outcomes in early childhood. However, more studies are required to explore the effects of FADS gene variants in populations with different ethnic backgrounds, lifestyles, and dietary habits, and to investigate in greater depth the interaction of FADS gene variants, diet, and clinical end points of such developmental outcomes.

Folate

Normal brain development and function also depend on the active transport of folate across the blood-brain barrier. Supplementation of pregnant mothers with folate significantly decreases the incidence of developmental defects, including neural tube defects (NTDs), conotruncal heart defects, and cleft lip/palate. Relative folate deficiency is not uncommon and does not appear as harmful in most pregnancies. The risk appears the greatest if the mother's homocysteine levels, which are genetically influenced, are elevated. Hyperhomocysteinemia (HtHcys) is related to fetal toxicity because of DNA alterations, hypomethylation, or insufficient synthesis of DNA because of structural damage in the genes implied in the DNA synthesis.

The most common mutation of the enzyme methylenetetrahydrofolate reductase (MTHFR) gene is 677C→T. The 677TT homocygosis determines a low MTHFR activity, which is related to NTDs and HtHcys. Vitamin B_{12} and folate deficiencies are common during childhood[83] and may have an acute effect on the central nervous system (CNS) via hypomethylation, inhibiting the synthesis of methionine and forming S-adenosylmethionine. This in turn inhibits methylation reactions throughout the CNS involving proteins, membrane phospholipids, and DNA and metabolism of neurotransmitters, such as dopamine, norepinephrine, serotonin, and melatonin.[84] Another potential mechanism of how folate could relate to mental performance is facilitating DHA accretion by the fetus.[85]

Probably the most significant effect at present related to folate status during pregnancy and behavior is the emerging results from Generation R within the NUTRIMENTHE EU Project. Children of mothers who did not use folic acid supplements during the 1st trimester of pregnancy had a higher risk of total problem behavior. Use of a folic acid supplement protected from both internalizing and externalizing problems, even when adjusted for maternal characteristics such as age, national origin, educational level, and psychopathology.[86] In addition, data from the Generation R Study recently have shown that low levels of maternal folate are associated with smaller head circumference and smaller transcerebellar diameter in the fetus.

In conclusion, well-designed supplementation studies with long-term follow-up, examining all new confounding factors and combining the new different methodologies, are required to advance the knowledge of optimal nutrition during early life, promote optimal neurodevelopment, and prevent deficiencies and other pathologies.

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Q & A

Q: One of the reasons that the Institute of Medicine's Committee on Weight Gain and Pregnancy was worried about having obese pregnant women not gain enough weight or to even lose weight was an old finding that ketonemia might impair cognitive development in the child. Have you heard of that hypothesis?

Dr Campoy: Yes.

Q: Can you talk a little bit about that because it is really murky. To my knowledge, the research basically stopped in the 1970s or 1980s, and we found really insufficient information either way. I just wonder how you might approach studying that given that ketonemia is pretty hard to measure in the mother.

Dr Campoy: Yes, it is a very interesting topic. It is shown that obese adolescents have problems in the development of executive functions, which are the last domain in the steps of brain development. This alteration is linked to the metabolic and inflammatory changes in obese patients [Verdejo-García A et al. *Obesity* (Silver Spring). 2010;18:1572-1578]. The most critical windows for brain development are

established during prenatal life. It is shown that babies born from diabetic mothers could have an impaired neurodevelopment. After the diagnosis of gestational diabetes, the women are treated in the 3rd trimester of pregnancy, including pharmacological treatment as needed, and principally dietetic treatment and physical activity recommendations. This intervention is shown to have beneficial effects for the mother and fetus development.

What happens with a child born from an obese mother? More than 30% of obese pregnant women develop gestational diabetes (diabesity), becoming the worst metabolic situation for brain development. But in the case of obese pregnant women, actually no treatment is implemented and no dietary advice is regularly recommended. Sometimes the obese mothers are only advised about recommended weight gain during pregnancy. So, the metabolic and inflammatory status is maintained and probably these insults will determine an abnormal fetal programming effect, which is similar to that already seen during diabetic pregnancies, phenotypically manifested by newborns large for gestational age. However, we suspect that brain development also is affected in the offspring of obese mothers. The problems eventually are no longer as studied.

Obese mothers normally have a very bad diet, and so most of them have lipid and micronutrient deficiencies, such as folic acid or iron, among others. Significantly lower levels of serum iron and transferrin saturation (the ratio of serum iron to total iron-binding capacity) were found in obese as compared to nonobese adult volunteers, and fat mass was shown as a significant negative predictor of serum iron concentration [Menzie CM et al. *J Am Diet Assoc.* 2008;108:145-148].

Anemia during pregnancy is linked to brain development impairment, but the long-term effects were not studied extensively. Limited evidence suggests that iron supplementation during pregnancy may positively influence children's psychomotor development during the first 2 years of life, whereas it does not seem to alter their mental development or behavior [Szajewska H et al. *Am J Clin Nutr.* 2010;91: 1684-1690].

It is necessary to analyze iron supplements during pregnancy individually, because supplemental iron during pregnancy will increase the oxidative stress and could cause even worse problems for fetal neurodevelopment. This is a topic for new studies.

We actually are involved in assessing this topic in the cohort of the PREOBE Study. We are exploring the effects of obesity and diabetes, and mothers' nutritional status during pregnancy on their offsprings' neurodevelopment. The assessment of these

effects on neurodevelopment is very difficult, because many other confounder factors are involved. It also is necessary to examine long-term outcomes. At present, data from other studies already published are inconsistent.

Q: The comprehensive review of the broad spectrum of methodology that you presented perhaps illustrates the difficulty that we have. We do not really know what to measure and how. While it is very plausible that metabolic insults during the very rapid development of the brain early on should have an impact on long-term structured function, we really have very limited understanding of that. We obviously have some examples of dramatic insults, malnutrition of B_{12} deficiency in vegans, or high saturated fat or protein dietary intake.

If you think about the variation of normal, such as introduced by variation of nutritional habits, we really have very little knowledge and understanding as to how that would impact brain development and function.

What is the opportunity moving forward? Should we learn from the whole story of the omega-3 fatty acids, where the field was advanced by animal experimentation, where we had specific hypotheses, where we had chemical hypotheses, omega-3 fatty acids, resins, and photo receptors, and then we had primate studies showing effects and specific studies in humans based on that, which I think draw forward?

Or should we embark on broader fishing expeditions where, for example, we collect metabolic profiles in pregnant women or at-birth infants and then we take that to the broad spectrum of outcome markers in children or adolescents, and then based on that, we formulate hypotheses, or what is your vision? How do we progress in this sort of murky area?

Dr Campoy: Well, it is a really interesting question, because it is a major topic and a major problem. It is really difficult to assess the effect of nutrition during pregnancy and early life on long-term neurodevelopment, based only on the results of different neuropsychological tests available in each country. This could achieve important data, but with the results possibly influenced by many other confounder factors. Actually, new technologies will give us the opportunity to advance in the knowledge of how early nutrition impacts on brain development. However, there is still very little data of reference. For example, only a few studies are available with fMRI or with MRI in children.

If we use nutritional and metabolic biomarkers combined with these new techniques for neurological assessment, we could have a chance. MRI could allow us to analyze structural changes in the brain, depending on early nutrition and fMRI

functional changes, such as after nutritional interventions. The combination of these techniques (neuropsychological tests battery, VEP, EEG/ERP, MRI, fMRI, etc) will help us in future studies to understand the structural and long-term functional effects of early nutrition on brain development.

Future studies to demonstrate the long-term effects of early nutrition on brain development, using these methodologies, should include not only normal and healthy pregnancies, but also babies born to mothers with very common metabolic diseases, such as diabetes or obesity. Studies in children or adolescents also are needed, and the new procedures will prove extremely useful in understanding the brain effects after nutritional interventions.

Impact of Pregnancy Nutrition on Offspring Bone Development

Stephanie Atkinson, PhD, FCAHS

Osteoporosis is increasingly recognized as a disease with its roots in early life events and exposures. Bone accretion in early life may set the stage for peak bone mass achieved in adolescence, and suboptimal peak bone mass is a well-established predictor of fracture risk. Factors known to influence bone status include genetic inheritance, fetal exposures such as maternal smoking, body composition, physical activity, and nutrition during pregnancy. Because bone growth and bone mass accretion continue throughout childhood, it is important to take the lifestyle influences of diet and activity of the child into account, as well as diseases and therapeutic drugs that may directly or indirectly have adverse effects on skeletal growth.

Fetal/Neonatal Programming of Bone Status

Several candidate genes are proposed to elucidate the genetic basis of adult bone mass, such as the vitamin D receptor (VDR), the gene encoding for type 1 collagen, and the gene for estrogen receptor.[1] Paternal genotype influences fetal skeletal growth, because paternal height and skeletal size and volumetric bone mineral density (BMD) independent of maternal factors predict intrauterine bone mass of newborn infants.[2] Father's bone mass was significantly correlated to child bone mass at 6 years.[3]

Emerging evidence supports the concept that intrauterine exposure to specific adverse environmental factors may operate via fetal "programming" of candidate endocrine systems that influence skeletal metabolism, such as the growth hormone/insulin-like growth factor 1 (IGF-1) axis,[4] which regulates cell proliferation and growth in bone as well as other organs. Retrospective epidemiological cohort studies have established linkages between poor fetal and early infancy growth and reduced bone mass in adults, or greater risk of hip fracture after the 6th decade.[5] Fetal programming of vitamin D metabolism to its active hormone form (1,25-dihydroxyvitamin D) also is implicated from epidemiological research.[6]

Maternal Nutrition: The Basis of the Dietary Reference Intakes for Calcium and Vitamin D

In the recently revised United States/Canada Dietary Reference Intakes (DRIs) for calcium and vitamin D,[7] values were established for pregnancy for the estimated average requirement (EAR), Recommended Dietary Allowance (RDA), and upper intake level (UL), making them the same as for nonpregnant women of similar age. The calcium demand by the fetus, especially in the 3rd trimester when fetal accrual of bone reaches peak velocity,[8] is accommodated by natural physiological responses that double the maternal intestinal absorption of calcium, owing to doubling of the synthesis of the active metabolite 1,25-dihydroxyvitamin D via a nonparathyroid hormone mechanism that upregulates the renal 1-alpha-hydroxylase enzyme.[9] Vitamin D binding protein (DBP) also is upregulated during pregnancy. Whether transplacental transport of calcium is vitamin D-dependent or not remains controversial.

The report from the Institute of Medicine[7] seemed to base the recommendation for calcium intake in pregnancy on research in animal models in which vitamin D-deficient pregnant rats and VDR-null mice demonstrated a rise in intestinal calcium absorption during pregnancy, regardless of the absence of calcitriol or its receptor for action. The epidemiological evidence supporting a role for maternal dietary vitamin D and possibly calcium in fetal, neonatal, and adult bone outcomes was largely discounted in the deliberations of the review panel in revising the DRIs for calcium and vitamin D.

The RDA for vitamin D was set at 600 IU/day for all individuals 1–70 years of age, with no increase for pregnant or lactating women.[7] For calcium, the RDA was 1300 mg/day for ages 14–19 years and 1000 mg/day for 19–50 years.[7] Evidence discounted in the DRI report included several prospective cohort studies that have focused on maternal nutrition during pregnancy as a predictor of offspring bone health up to 16 years of age. Such cohort studies were not considered, partly because they do not sufficiently account for confounders, such as lifestyle factors passed on from mother to child. The DRI panel did not appear to consider the hypothesis that intrauterine programming is a contributing factor to osteoporosis in later life and that maternal suboptimal vitamin D status during pregnancy is a key-related component of potentially adverse environmental exposure for the fetus.[10]

Maternal Pregnancy Factors Influencing Bone Outcomes

Several factors other than maternal vitamin D intake may independently predict neonatal bone mass in offspring, including season of birth/ultraviolet B (UVB) exposure,[11] maternal smoking,[12] maternal calcium and protein intake,[13,14] lower fat stores, and more vigorous physical activity during late pregnancy (Figure).[12,15] This paper will focus on the relationship between modifiable nutritional factors during pregnancy and outcomes of bone mass during fetal life and beyond the newborn period.

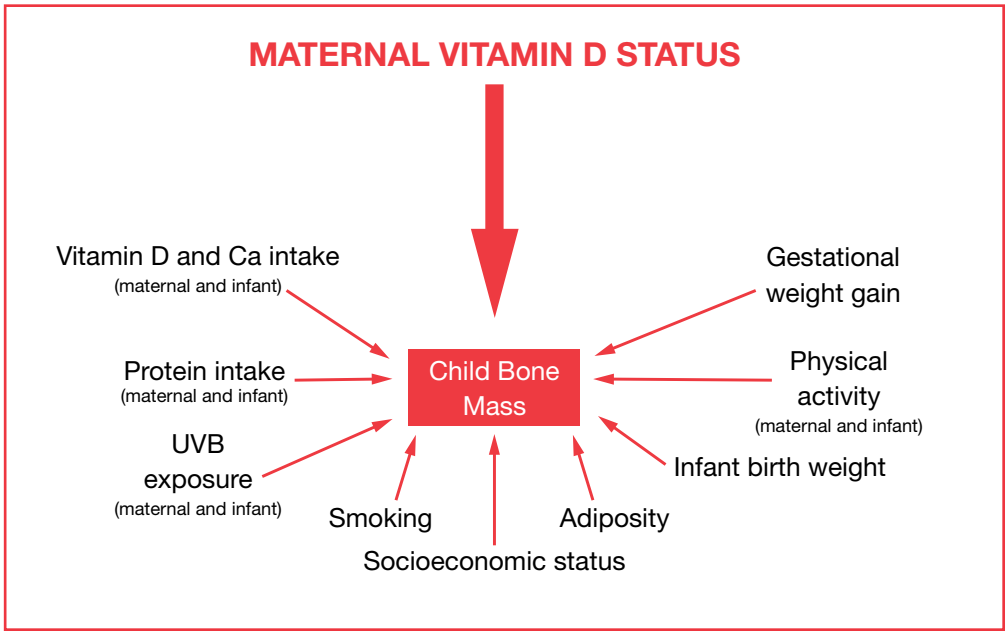

Figure. Potential maternal and infant influences on programming of child bone mass.

Maternal Vitamin D Status and Bone Health of Offspring

Observational studies have demonstrated that offspring of vitamin D-deficient women are born vitamin D deficient,[16] have reduced intrauterine long-bone growth and slightly shorter gestation,[17] lower bone mass at birth,[18] lower fetal long-bone growth,[19] lower infant bone mass,[18] and risk of neonatal rickets.[20]

Maternal vitamin D status may program a larger body size in utero.[17,21-23] This also could explain any effect on bone mass, because a larger body size potentially means having a larger skeleton with longer and/or thicker bones that contain higher mineral content. Maternal vitamin D deficiency (serum 25-hydroxyvitamin D [25OHD] <28 nmol/L) during late pregnancy is linked with reduced knee-heel length

at birth, indicative of reduced intrauterine long-bone growth.[17] Fetuses of vitamin D-deficient mothers had a pattern of femoral growth that resembled childhood rickets, including an increased splaying index and metaphyseal cross-sectional area measured by 3-D ultrasound at 19 weeks of gestation.[19]

Although femur length was not affected, a higher velocity of femur growth was observed in mothers with higher vitamin D status. During pregnancy, a lower dairy intake in the mother predicted a shorter fetal femur length in the 3rd trimester,[24] and lower UVB exposure predicted reduced birth length.[21,23] Small size at birth was observed in infants of mothers with lower vitamin D status.[25] Evidence of effect modification by infant FokI genotype for the vitamin D receptor was suggested by observations of lower birth weight in infants of vitamin D-deficient mothers.[26]

The question of whether constrained bone growth is long lasting is partially addressed by studies where maternal UVB exposure during pregnancy positively correlated with the child's height until as late as 9 years of age.[22,23] Consistent with this, a study in guinea pigs showed that the effect of vitamin D metabolites on longitudinal growth in utero is possibly not reversible, even with sufficient postnatal supplementation with the vitamin.[27]

Information on bone size and mass derived from whole body and specific bone sites using dual-energy X-ray absorptiometry (DXA) in neonates and young children provides some insights into the possible role of maternal diet in offspring bone health. Size at birth, which sometimes is a function of maternal prepregnant body mass and/or diet during pregnancy, and early infant growth are possible determinants of later bone status. A recent systematic review[28] conducted in 14 retrospective and longitudinal studies explored the relationships between birth weight and weight at 1 year of age and later bone mass. Meta-analysis revealed that higher birth weight and weight at 1 year predicted significantly greater bone mineral content (BMC) of the lumbar spine and hip, but not whole body in adulthood.[28] However, no impact of birth weight on areal or volumetric bone density was noted. In a single study, fetal femur length also predicted whole-body bone size and mass at 4 years of age.[29]

Vitamin D status during pregnancy is also a determinant of bone mass in the offspring. Mothers in the United Kingdom with lower UVB exposure (assessed by using local meteorological data) in their 3rd trimester had children with lower bone mass at 9.9 years of age.[23] Similarly, children of mothers who were vitamin-D deficient during pregnancy by serum measures had significantly lower whole-body BMC and lower lumbar-spine BMC at 9 years of age.[30]

Maternal vitamin-D status, based on intake from food and supplements, as well as serum 25OHD status, was explored as a determinant of bone size and mass using peripheral quantitative computed tomography (pQCT) at birth and 14 months of age.[31] Maternal serum 25OHD >35.6 nmol/L as compared to <35.6 nmol/L was associated with significantly higher tibia BMC and cross-sectional area in newborn infants, even after adjustment for z-score for birth weight, maternal height, and age.[31] At follow-up in the infants at 14 months of age, the cross-sectional area still was advantaged in the high-maternal vitamin-D group, but tibia BMC demonstrated catch-up in the low vitamin-D group.[32]

The recent publication of the one randomized clinical trial of vitamin D supplementation in pregnancy provides insight into the amount of vitamin D intake to achieve various levels of vitamin D status.[33] Of 494 women randomized in early pregnancy to vitamin D supplements of 400, 2000, or 4000 IU/day, 350 women were followed to term birth. Intake of vitamin D from food was about 200 IU/day and calcium was about 1000 mg/day. For the groups receiving 2000–4000 IU/day, >80% of subjects achieved a serum 25OHD of >80 nmol/L, and both maternal and cord blood 25OHD (Table) was significantly higher than for the group randomized to 400 IU/day vitamin D.[33] No adverse effects were reported for hypercalcemia, hypocalcemia, hypercalciuria, or parathyroid hormone level.

Birth Vitamin D Status

Maternal Supplement	Infant Birth 25OHD, nmol/L Mean ± SD
400 IU/day	45.5 ± 25.3
2000 IU/day	57.0 ± 24.5
4000 IU/day	66.3 ± 25.8
P value	<0.0001

❖ No clinical outcomes in infants reported; birth weight similar

Table. Vitamin D status at birth in infants born to mothers who received 400, 2000, or 4000 IU vitamin D/day during pregnancy.[33]

Source: Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011;26:2341-2357. Reproduced with permission of Blackwell Publishing Ltd.

One limitation of the study by Hollis et al[33] is that no clinical outcomes of infant growth (other than birth weight, which was similar across vitamin D-supplement groups) or bone size or mass were reported. In a previous study in 125 Gambian women who all achieved serum vitamin D in pregnancy >50 nmol/L,[34] no differences were observed in birth weight, infant length, or whole-body or radius-bone measures by DXA at 1 year of age between groups from mothers with a serum 25OHD status during pregnancy above or below 80 nmol/L. Thus, the need to achieve vitamin D status in pregnancy of more than 80 nmol/L in order to optimize bone size and mass outcomes in the offspring remains open for further investigation.

Maternal Calcium Intake and Bone Mass in Offspring

The high demand for calcium by the fetus during pregnancy necessitates sufficient calcium in the mother's diet.[35] Maternal consumption of higher amounts of calcium and milk during pregnancy was associated with higher lumbar-spine BMD in offspring at age 16.[36] Higher total body and spine BMC and/or BMD also were observed in children (n=698) in rural India at 6 years of age whose mothers had a higher frequency intake of calcium-rich foods, especially milk and milk products, during pregnancy, independent of parental or infant size and other confounding variables.[3] This aligns with observations that consumption of less than two dairy servings/day during pregnancy is associated with shorter femur length at 20–34 weeks of gestation, which is an indicator of fetal bone development.[24]

Cord calcium concentration (corrected for protein) was a determinant of child bone mass at 9 years,[30] indirectly indicating a potential role for calcitriol in placental calcium transport, perhaps through modulation of the transcription of placental calcium transporters.[37] Supporting evidence is that messenger ribonucleic acid (mRNA) expression of one calcium transporter isoform, PMCA3, predicted neonatal skeletal size, independent of several other maternal predictors.[38] However, the dependency of placental calcium transport on vitamin D sufficiency and the impact on programming of skeletal development require further investigation.

Maternal Protein Intake and Bone Outcomes in the Offspring

Maternal protein intake during pregnancy impacts fetal bone development. For example, a higher protein intake during the 3rd trimester is associated with higher whole-body BMD in the offspring.[39] It was observed that maternal protein

deprivation during pregnancy in rats delays mesenchymal stem-cell proliferation and differentiation in the skeleton, modifies growth-plate morphology,[40] and negatively impacts bone composition, length, and mechanical strength.[41]

The developing skeleton requires a continuous supply of amino acids for collagen formation and also for modulation of serum IGF-1 levels, which in turn impacts on homeostasis. Cord IGF-1 levels correlated positively with whole-body bone-mineral content of infants after adjusting for other independent predictors of bone mass.[42,43] Notably, cord-serum IGF-1 concentrations are directly related to protein intake of mothers in late pregnancy, thus maternal dietary protein inadequacy may impact fetal skeletal outcomes.[42]

Future Research

Further investigations in humans, preferably as randomized clinical trials, are needed to fully examine the influence of variations in maternal nutrition on fetal or infant bone outcomes, which would help to establish science-based recommendations targeted to pregnant women for intake of vitamin D, calcium, protein, and possibly other nutrients important to bone health during fetal development and later in childhood.

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Q & A

Q: Where was the study population from in the paper by Hollis et al [Hollis et al. *J Bone Miner Res*. 2011;26:2341-2357]?

Dr Atkinson: The study took place at the Medical University of South Carolina in Charleston. Of the 350 women studied, more than 60% of them were African American or Hispanic.

Q: Does anybody know what the average compliance is for taking multivitamins during pregnancy for the average American woman? How many take their multivitamins or prenatal vitamins? Does anyone have an idea?

Dr Atkinson: I cannot speak to America. In Canada, based on the most recent population-based survey by Health Canada, 40%–50% of nonpregnant women take mineral/vitamin supplements [Socio-economic status and vitamin/mineral supplement use in Canada. *Health Reports.* 2010;21(4). Statistics Canada, No. 82-003-XPE]. This study did not sample pregnant women.

Q: So do you think your study population of pregnant women represents all of Canada, that 100% are taking their multivitamins?

Dr Atkinson: In our study, more than 83% of mothers took prenatal or other multivitamins that provided 200–400 IU of vitamin D/day, and, in addition, some took single vitamin D supplements containing up to 1000 IU/day during pregnancy. However, our study population represents a middle socioeconomic class that may have different practices for use of supplements and level of food security than those of lower financial status. The study population reflects those in our community who are willing to join long-term studies.

We do not have very many smokers—only about 4% of the women were smoking during pregnancy, although some had smoked prior to pregnancy. In the last 3 years, we have seen a big push through Health Canada, our obstetrical society, and pediatric society about healthy lifestyle practices for nutrition and exercise that they advise mothers to adopt during pregnancy. Maybe our public health people are doing a good job and that is reflected in the nutrition practices that we observed in our study.

Impact of Perinatal Nutrition on Neonatal Immune Response

Susanna Cunningham-Rundles, PhD (with Michael M. Espiratu, MD, and Jeffrey Perlman, MD)

Shaped by fetal life, the neonatal immune system is immature at birth and must adapt rapidly to new environmental challenges. Newborn exposure to colonizing commensal bacteria, environmental antigens, bioactive dietary substances, and potential pathogens has the potential to cause long-term effects on health.[1,2] Differences in maternal and neonatal nutritional status are increasingly recognized as both a major source of variation in health outcomes and as an avenue for early intervention.

The historical view of neonatal immune response, based on specific studies in mice, was that an early encounter with antigen led to lack of responsiveness, or tolerance to the same antigen, but also conferred susceptibility to infectious organisms and poor response to vaccines. The later discovery of T-lymphocyte subsets and the effects of microbial pattern recognition receptors on innate immune cells has led to a very different understanding of why infants are vulnerable to infection, and also why some infants are susceptible to allergy, asthma, and even obesity and type 1 or 2 diabetes in later life.[3-8]

Current studies show that the newborn immune system has mainly naïve thymus-derived T lymphocytes, lacks memory T and effector B lymphocytes, and has a deficient T-helper (Th) type response characterized by low or absent production of interferon gamma. Although the T-cell response in newborns is now known to vary widely in individual infants, the cytokine response usually is dominated by interleukin-4 (IL-4) and IL-13, a pattern that is characteristic of Th-2 cells. The Th-2 cytokine phenotype forms in fetal life as part of maternal fetal regulation to avoid inflammation and must shift to a Th-1 type at birth. Failure to do so is potentially associated with allergic predisposition.[9,10]

Neonatal host defense depends on innate immune responses to environmental antigens that prime and mold the developing adaptive immune system.[11] However, recent studies show that the innate immune response also must undergo postnatal maturation. For example, expression of a key pattern recognition toll-like receptor 4 (TLR4) on neonatal monocytes is low compared to adults and is a cause of infants' delayed response to lipopolysaccharide (LPS), the dominant surface component on gram-negative bacteria.[12]

The differences between infant and adult immune response are not only greater in premature infants, but also often include a dysregulated cytokine response characterized by increased production of inflammatory cytokines that is not controlled by an equal anti-inflammatory response.[13,14] The preterm infant's susceptibility to unbalanced inflammation is related, at least in part, to reduced progenitors of regulatory T cells (Treg) in the naïve T-cell population (Fig 1).[15]

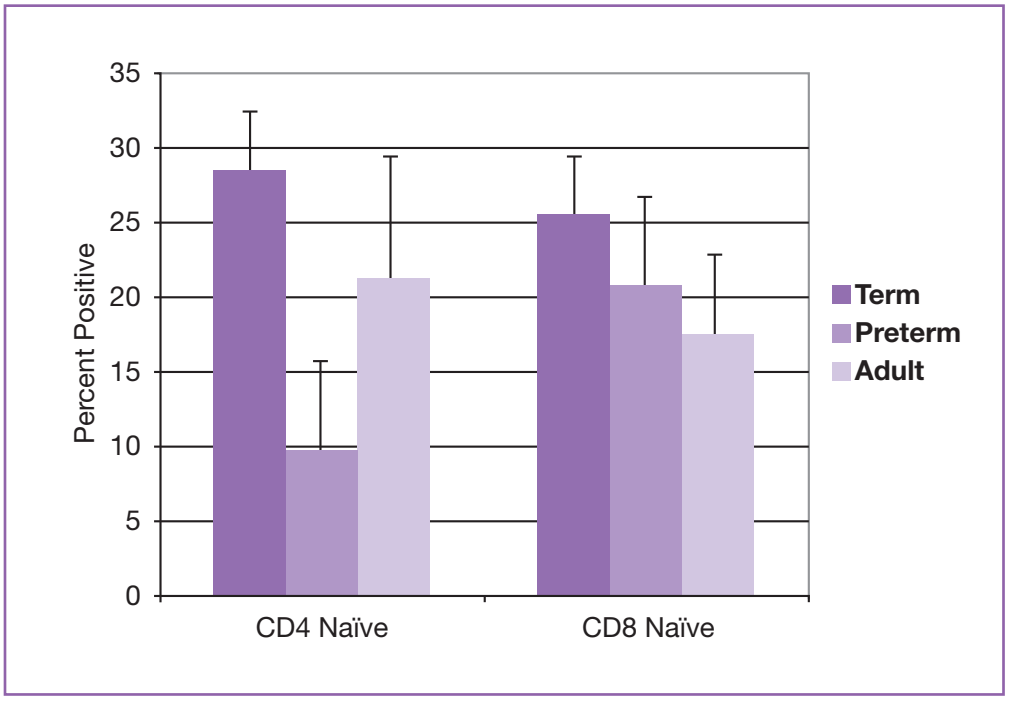

Fig 1. Naïve CD4+ T cells are reduced in preterm compared to term infants. Data show that the population of progenitor cells for T regulatory lymphocytes (CD4+CD25+Foxp3+ and Th-17 T cells) is lower in preterm compared to term infants (P <0.01). The data are shown as percent of cells positive for lineage and memory markers as determined by flow cytometry. This is one possible reason why preterm infants are more vulnerable to infection.[15]

While all of these problems are greatly magnified in the preterm infant who is small for gestational age (SGA), even late preterm infants who are appropriate for gestational age (AGA) and infants <1500 g show greater risk of infection at birth and greater morbidity from infection in early childhood.[16,17] Numerous studies indicate that low birthweight is associated with enhanced levels of proinflammatory mediators, overweight, obesity, and enhanced risk of type 2 diabetes in later life.[2,18] The Barker hypothesis that adult diseases have their origins in early development, such that prenatal undernutrition compromises later function, also is true for antibody formation and thymic function.[19]

Exposure to food (nutrients, antigens, and bioactive substances) and microbes primes immune response and can lead to beneficial adaptive changes, such as production of IgA and IgM that exclude antigens from crossing the gastrointestinal tract, and the timely development of suppressor mechanisms that are needed to mediate oral tolerance to foods and commensal bacteria. For example, cow's milk allergy, because of exposure to casein, sometimes is a serious problem, which causes diarrhea, impairs growth, and often is associated with other atopic conditions. The child's ability to outgrow allergy to cow's milk and become tolerant depends upon the development of casein-specific Treg cells.[20] The causes of allergic predisposition include both genetic and environmental factors.

Atopic Diseases, Allergy, and Asthma

The overall incidence of atopic diseases, allergy, and asthma increased dramatically during the 20th century. This is attributed to decreased early exposure to commensal bacteria and microbes as encapsulated in the hygiene hypothesis.[21] Current studies show that supplementation with probiotic lactic-acid bacteria reduces the development of allergic responses in children with inherited genetic risk and ameliorates response in milk-intolerant children.[22-25] Although more detailed studies are needed, nutrients are recognized as critically important for the development of the microbiota and energy homeostasis.[26,27] The development of future microbiome in the infant occurs during gestation and during early neonatal life, and interacts with genetic and epigenetic factors involved in fetal and neonatal programming.[28]

Protein-Calorie Malnutrition

The impact of primary maternal protein-calorie malnutrition (PCM) on neonatal vulnerability to infectious disease is well known. Much of the damage to neonatal host defense occurs through impact on the developing immune system, especially the thymus, often called the barometer of nutrition.[29] Malnourished children have lower levels of thymulin and deficient T-cell development. Zinc deficiency alone also can cause this.[30] Leptin, the adipocyte-secreted hormone that regulates weight centrally, regulates the thymus by increasing thymopoiesis and inhibiting apoptosis, and is decreased in malnutrition. Malnutrition enhances tumor necrosis factor and IL-1, IL-6, and IL-8 cytokine production, activates hepatic synthesis of acute phase reactant proteins such as C-reactive protein, and inhibits production of serum albumin and transthyretin. Shifts in storage pools of iron, zinc, and copper during the acute phase response because of transport by newly synthesized binding proteins such as ferritin, metallothionein, and ceruloplasmin lead to low levels of

these trace elements in blood. Although malnutrition is associated with reduced cytokine response to antigen in vitro, the levels of circulating proinflammatory cytokines in vivo are increased.[31,32]

Micronutrient imbalance or deficiency in the mother in the absence of PCM can alter the program of immune development in the infant (Table).[33] The strongest evidence for micronutrient programming effects comes from studies of vitamin A deficiency. Vitamin A is required for the homing of T cells into the gastrointestinal tract and promotion of antigen-specific Treg development. Retinol concentrations at birth are associated with atopic disease in childhood and later life.[34]

Table. Micronutrients Modify Neonatal Immune Response[7]

Zinc deficiency:
- Gestation—teratogenic effects
- Levels in milk affect neonatal T cells, natural killer (NK) cells, cytokines
- Persistence of defects after repletion

Iron deficiency:
- Prenatal stress may cause anemia, NK cell defects
- Deficiency increases iron and copper gene expression, decreases oxidative response genes (eg, vitamin C transporter)

Selenium:
- Deficiency may increase viral virulence

Vitamin C:
- Transporter variants cause preterm birth
- Improves response to infection

Vitamin D (deficiency is common):
- Low D_3 promotes infant allergy, atopy, inflammation; repletion (+ Ca) cures experimental inflammatory bowel disease
- Low D_3 increases infections

Vitamin A (deficiency is common):
- Deficiency worsens infection
- Essential for development of the gut-associated lymphoid tissue (GALT)
- Important for oral tolerance

Vitamin E:
- Maternal levels influence allergic sensitivity

Inflammatory Challenge Relates to Gestational Age

Current studies show that the newborn immune system is more vulnerable to inflammatory challenge in relationship to gestational age. Importantly, neonates appear to have a reduced compensatory anti-inflammatory response. Therefore, they are possibly at greater risk for inflammatory damage. Recent studies showed that neonatal cytokine response to bacteria is dysregulated in term and preterm infants compared to adults and has a tendency toward an uncompensated proinflammatory response.[15] Although a lower percentage of neonatal monocytes produced cytokine responses to a panel of microbes compared to adults,[35] the levels of cytokines IL-6 and IL-8 secreted in response to the same microbes actually were higher (Fig 2).[36]

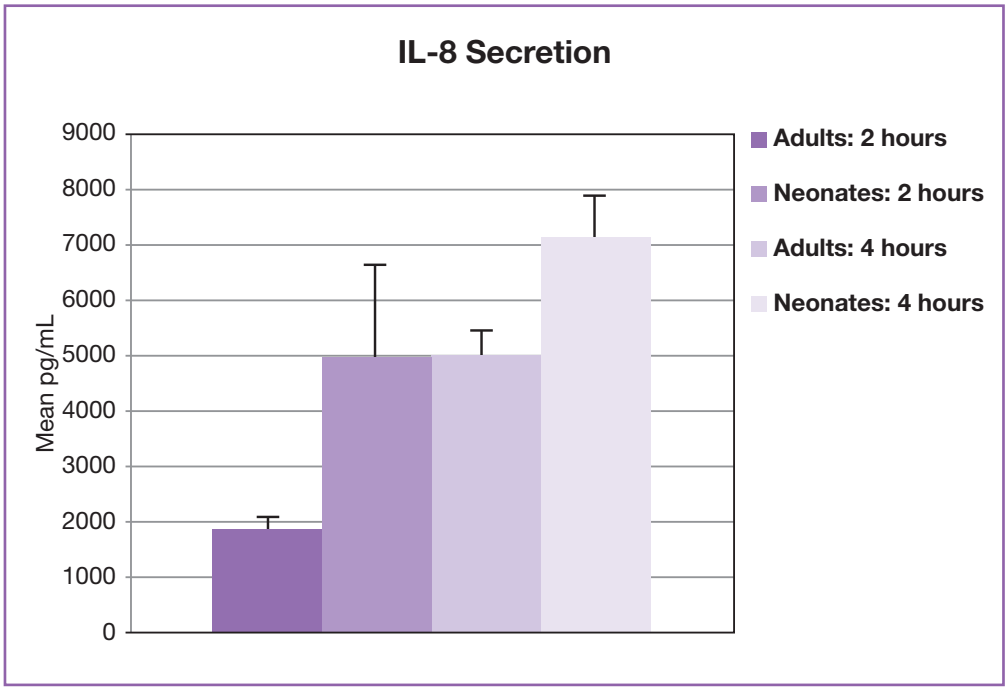

Fig 2. Neonatal cytokine secretion in response to *Escherichia coli.* Data show that neonates secrete more IL-8 at 2 hours in response to physiological heat-killed *E coli* than do adults (P <0.01). Data are shown as picograms (pg)/mL, as measured by enzyme-linked immunosorbent assay (ELISA). Increased levels of cytokines can lead to hyperinflammatory response.[35]

The Potential Role of Polyunsaturated Fatty Acids

Nutrient treatment may offer a physiological and safe approach to promote a balanced proinflammatory response and protect against overproduction of cytokines associated with preterm birth. The potential role of long-chain omega-3 polyunsaturated fatty acids (PUFAs) that are not synthesized de novo is of particular interest because of the evidence for anti-inflammatory activity when given perinatally.[37] Formulas given to preterm infants on total parenteral nutrition do not provide a significant source of either eicosapentaenoic (EPA) or docosahexaenoic acid (DHA). Preliminary data from our studies using the THP-1 human monocytic cell line showed that cytokine responses to LPS were significantly reduced compared to controls when cells were pretreated with EPA or DHA. Subsequent studies with full-term healthy neonatal cord blood also showed that PUFA treatment inhibited proinflammatory cytokine response.[38,39]

Summary

In summary, current studies show that postnatal development of the immune system requires priming signals and nutrient/micronutrient resources. Variation in neonatal response provides a sensitive reflection of genetic and epigenetic factors that interact with the evolving microbiome and are predictive of future response to environmental antigens, bioactive dietary substances, commensal bacteria, and potential pathogens. The need for controlled proinflammatory response and a shift from a Th-2- to a Th-1-dominated cytokine pattern after birth are required to engender tolerance, promote host defense, and avoid allergic responses. Birth weight, gestational stage, and nutrient sufficiency affect immune development.

Micronutrients and microbial encounter can protect against allergic predisposition, and nutrients influence the evolving microbiota, as well as immune development. Conversely, nutrient and micronutrient deficiencies at birth and an abnormal microbiota impair immune regulation and can deprogram immune development. Nutrient supplementation and probiotic treatment are valid approaches for postnatal use to regulate and support neonatal immune response and avoid a hyperinflammatory response.

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Q & A

Q: Can you elaborate a little on intervention, because the results are fine—the interplay between intestinal permeability and the exposure to antigens. Then you have the question of the timing, of when would you expose and when a neonate eats something. It depends on the immune system and comorbidity (potential pathogens) of the gut, and at various times, you will get different responses. Many problems come up with the interaction of immune cells and then the immune response to gut microbes, and there is now increased permeability. Could you elaborate a bit on this issue?

Dr Cunningham-Rundles: That is of concern. I do not know if you are thinking now about the use of probiotic organisms in early life. You would have some concerns about gut permeability there.

What we would propose doing, at least our next step, is preterm infant feeding TPN, total parenteral nutrition. We would propose using, at least in the beginning, DHA. I did not show you the data, but in our ramp-up studies, we discovered the DHA was more powerful at lower doses, so we proposed adding it as a supplement. The addition of DHA actually is beneficial, so it should not present a problem, and we can probably get that through the institutional review board.

I do not think about it having any problems with respect to gut permeability. It would definitely have a potential impact on immune cells as well.

Q: I was referring to cow's milk rather than to probiotics, because the studies are talking about exposure during the first 2 weeks of life and whether you would prefer, in order to create tolerance, that the gut is still permeable or not.

Dr Cunningham-Rundles: I do not really have an answer to that. Primarily, we still think of doing things once you have a problem. You assume that tolerance will develop.

People have different points of view about whether or not you should wait and let children outgrow cow's milk allergy or if you should intervene immediately and put them on another diet. That is another question.

In terms of when best to intervene with omega-3 fatty acids, I think you could give omega-3s as soon as you were giving any element of food other than mother's milk, which of course does contain some DHA.

Q: We have just completed a randomized control trial of increasing salmon intake during pregnancy, contaminant-free salmon and intervention.

We have looked at the innate immune responses to TLR4 and such, IL-10 production, and we did find quite a substantial effect of maternal supplementation with the provision of salmon on the IL-10 responses in the cord blood samples. I think it is potentially a pathway for us.

Dr Cunningham-Rundles: I might quickly add that omega-3 fatty acids are not anti-inflammatory. They are proinflammatory, but much less, so it is a balanced effect. You also get effects from incorporation into membranes. I am so glad that you have done that study, because that was needed, because it is good to do for the mother as well.

Discussion

Ricardo Rueda, MD, PhD

Dr Mardones: Dr Campoy, the results you presented on the effect of arachidonic acid on visual acuity were amazing. Would you comment on those specific results? How many women were involved?

Dr Campoy: These results are from the NUHEAL follow-up study, granted within the EARNEST EU Project framework. The recruitment took place from 2002 to 2004 in three EU countries (Germany, Hungary, and Spain). A total of 312 women were randomized into four groups. One group received 500 mg/day of docosahexaenoic acid (DHA) plus 150 mg of eicosapentaenoic acid (EPA), a second received 400 μg of 5-methyl tetrahydrofolate (5-MTHF), a third received both supplements, and the fourth received placebo. This supplementation began at 20 weeks of pregnancy and continued until delivery, and the mothers received follow-up during this period. After birth, the children received follow-up and still do today. They are 9.5 years old.

At 5.5 years of age, neurodevelopment in these children was examined using the Touwen test and cortical visual evoked potentials (cVEP). The NUHEAL trial reported no differences in the neurological outcome of children at 4 years and 5½ years between children born to mothers receiving fish oil supplements and those who did not. However, better neurological optimality scores in children at 5½ years were seen in those with increasing DHA levels in cord blood. Moreover, children whose mothers had higher DHA percentages in erythrocyte phosphatidyl-ethanolamine (PE) at delivery were more likely to have a Mental Processing Composite (MPC) score of the Kaufman ABC over the 50th percentile at 6.5 years. Results from the VEP examination at 5½ years demonstrate that DHA and particularly arachidonic acid (AA) concentrations in mother's phosphatidyl choline (PC) in red blood cell membranes at delivery determine the visual acuity and retinal maturation in their children at 5½ years of age.

Dr Mardones: How many children participated?

Dr Campoy: If I remember correctly, we have about 170 NUHEAL children participating at 5½ years of age. At the beginning, we saw significant correlations between AA and the different measurement points of the VEP, which we did not believe. However, reviewing the literature, we saw that Dr Sheila Innis and colleagues in Canada also demonstrated that AA is a major factor influencing long-term visual acuity, although they examined this in younger children. So, for us to

see these results in children at 5½ years of age was really interesting. The logistic regression performed in the VEP results of the NUHEAL children demonstrated that after taking into account all of the potential confounders, only mothers' AA in PC in red blood cell membranes at delivery remained, until the end of the analysis, as the most significant factor influencing long-term visual acuity.

Dr Godfrey: Just to clarify, was this looking into the trial data in an observational fashion because AA was not part of the intervention?

Dr Campoy: It was not part of the intervention.

Dr Godfrey: In those studies, were you able to control for the mother's IQ?

Dr Campoy: Yes, so it was surprising that AA was the major factor influencing visual acuity in the 5½-year-old children. The children born to mothers who had a lower level of AA were at a 7.7 times increased risk of having lower visual acuity and retinal maturation at 5½ years of age. This is an important result. We are now processing all these data.

Dr Steckel: My question applies to Dr Campoy's paper, as well as to several others. I did not get a good sense of what the study populations were. I have a sense that they are mainly in rich countries. Is it true that the populations are pretty well-off as a whole? Do we not have large numbers of poor populations or low-income groups that can be studied?

Dr Campoy: Do you mean in the data from the NUHEAL study? These mothers were recruited at three centers in Hungary, Munich, and Spain. The preliminary idea was that mothers in Hungary would have a low intake of fish and so DHA, while those in Germany would have a medium intake and those in Spain a higher intake. However, it was found that the women in Germany and Spain had almost the same intake of fish and those in Hungary a lower intake, but no differences were seen between them in social class or socioeconomic status.

Dr Steckel: My question is about some of the effects you are trying to measure. The statistical significance is a function of the variance of the explanatory variables, and the study population is fairly homogeneous. You will get high standard errors. It would prove useful to involve some groups that are very stressed. The Gambia study was mentioned here, but I think many populations around the world would give us more extremes and variation in the data, which would affect standard errors.

You could see interactions if a person is deficient in protein, for example, in an environment in which several micronutrients are deficient. The consequences could

differ significantly, so studies of populations that are generally in good health except for fish or omega-3 fatty acids would differ from those in populations that are highly stressed.

Dr Campoy: All the mothers are assessed prenatally for dietary intake, and we performed a food questionnaire assessment at 20 and 30 weeks. All of these data are published [Franke C et al. *Br J Nutr.* 2010;103:1648-1656]. We planned the study to see the differences in intake between the three countries, but not the social differences. However, mothers were asked about their socioeconomic status, type of job, etc. The study included mothers in all groups with a low, medium, or high intake of DHA. So, within the DHA-supplemented pregnant women groups, some mothers probably had a higher intake than recommended after the supplementation, but no significant effects were demonstrated after supplementations even higher than 2 g/day of DHA.

Dr Steckel: I suggest that it is perhaps useful to partner with people who work in the developing world. A number of interesting studies are going on, such as one in Ecuador in an Amazon hunter/gatherer population. This population exists under an extremely high disease level, and they are highly stressed. If you could get some of the measures you are interested in into that study, it would prove useful. It is hard to work in the developing world because of the costs, travel, and so on, but some good things could come out of that.

Dr Campoy: Do you know whether the researchers have the structure to measure these kinds of things?

Dr Steckel: I do not know, but they have an elaborate lab setup. They are bringing people out of the jungle and doing blood tests and several metabolic tests. If you are interested, I can give you contact information. I think some good studies are going on in Bangladesh and other countries that stretch the whole idea of the environment. Consider the Tsimane Health and Life History Project, an effort in the Bolivian Amazon directed by Michael Gurven, University of California at Santa Barbara, and Hilliard Kaplan, University of New Mexico.

I also think that, in some sense, the participants of this conference are the appropriate audience for people doing this kind of research because the consequences of deprivation on cognition may be much greater in the developing world than in the rich industrial world. I believe these people in the developing world are those we want to treat. This is where in the next 10, 20, or 30 years we will see a crisis of economic development because of the cognitive or metabolic limitations that arise from pregnancy onward. These are people Abbott Nutrition can help with

nutritional supplements. This population is huge—hundreds of millions or a billion people who live under conditions of extraordinary deprivation. We would want to know how they respond to infections.

Dr Campoy: In fact, the EU Commission asked the NUTRIMENTHE Consortium to produce a report at the end of the project concerning the important impact of nutrition on brain development and mental performance, which will cause exponential damage in the malnourished populations from developing countries. This hereditary damage will determine a relevant qualitative increase of the gap between developed and undeveloped countries, which will separate the latest definitively, even when the economic situation improves. We should take actions as soon as possible to give the developing countries an opportunity for the future.

Dr Mardones: Dr Atkinson, I was surprised that in most of your studies birth length was not considered as an outcome when you provided or supplemented vitamin D or calcium during pregnancy. Would you comment on that, please?

Dr Atkinson: I only can comment from my own personal experience. Unless we have a research assistant standing in the delivery room 24/7, it is difficult to get this information. In our longitudinal birth cohort study, if we are advised of the infant's birth, we measure the length using a length board within 24 hours. However, sometimes such measures are not possible, because mothers go home within 6 hours if the delivery is full term.

Dr Godfrey: In our observational studies, we have measured crown-to-heel birth length, and found it is a challenge to get accurate measurements. I think the ones we have obtained are good. Generally we do not see higher vitamin D status associated with the greater crown-to-heel lengths at birth, so we think it directly affects mineralization more than linear growth per se. Perhaps postnatally, you might see an effect on growth plate that manifests later on, but when we followed up at age 9 years, the children with lower vitamin D status are of the same height as those with a higher vitamin D status, but have reduced bone density and content.

Dr Atkinson: In fact, in the fetal measurement study, no difference in femur length was noted. Rather it was a significantly greater splaying index and metaphyseal cross-sectional area (a pattern of femoral growth that resembled childhood rickets) measured by 3D ultrasound in the fetus at 19 weeks of gestation that was associated with vitamin D deficiency in the mothers [Mahon P et al. *J Bone Miner Res.* 2012;25:14-19]. I agree that we do not have sufficient evidence to say that maternal vitamin D status influences length per se. However, your question was, why do we not measure it? In many cases, it is just impractical in the setting.

Dr Rueda: Dr Atkinson, there are recent reports concerning bone and muscle as a functional unit. In fact, results from Dr Cyrus Cooper in the University of Southampton describe how building bone mass and muscle mass early in life is perhaps an ideal way to prevent osteoporosis and sarcopenia later in life. In the future when we design studies in which we have an ingredient such as vitamin D that might affect not only bone but also muscle, should we consider bone and muscle as a functional unit and try to evaluate the effects on both functional outcomes together?

Dr Atkinson: This is a reasonable proposal, although relevant data are limited as of yet. In the Mysore Parthenon Study that was just published [Rishnaveni GV et al. *Am J Clin Nutr.* 2011;93:628-635], children born to vitamin D-deficient mothers (defined as serum 25OHD <50 nmol/L) had smaller arm-muscle area (thus smaller muscle size) at 5 and 9½ years.

Dr Godfrey: I think with appropriate training and research staff we can take good reliable measurements with a dynamometer in children as young as 4 years of age. I think that going forward, it is important to prove a link between nutrition and sarcopenia. In our studies of elderly populations, it is closely linked with frailty and a whole series of adverse outcomes. We do see associations between size at birth and later muscle mass and grip strength. How those come about we do not know.

Dr Rueda: Dr Campoy, while you mentioned some results about the effect of long-chain polyunsaturated fatty acids on cognitive development in children, results reported in the literature still are controversial. What is your opinion about the potential influence of desaturase polymorphism on these results? Today we know that conversion of precursors (linoleic and linolenic acids) into AA and DHA is not similar in all children, and consequently, desaturase polymorphism is perhaps a main factor influencing those clinical results.

Dr Campoy: I am not saying that nutrition, and specifically AA or DHA, will become the main and unique factors implicated in general neurodevelopment, but they are very important. These results from the NUHEAL cohort and those emerging from the NUTRIMENTHE Project show us that an important relationship between prenatal and postnatal nutrition and brain and behavior development do exist. Moreover, these studies point out that we probably need to define more detailed and focused procedures to measure brain development in each stage. More well-designed studies are needed to demonstrate in which specific areas of the brain a nutrient is needed for optimal development and how this depends on a critical window. We could determine how a deficiency will affect the individual, so we can assess the structural changes produced in the brain and the clinical or the neuropsychological symptoms.

In the case of IQ as a sole parameter to evaluate cognition, it is not so clear. The measurement of the IQ in a child will only offer us a very general score of intelligence. From my point of view, IQ is only one of the parameters needed to evaluate cognitive development. To define a child as more intelligent than another, it is necessary to explore many domains. The combination of them will offer us a real score. Even more, the IQ of a child is the result not only of accretion of different fats in the brain, but also the addition of many other nutrients and factors, such as fatty acid desaturase 1 (FADS1) and FADS2 genetic polymorphisms.

The ALSPAC (Avon Longitudinal Study of Parents and Children) study demonstrated that fish intake in the mother during pregnancy is related to offspring IQ, but no associations with maternal blood fatty acids after adjustment were found. Moreover, those children that were homozygous for FADS2 (GG) and were not breastfed had a lower IQ, even below 4 to 6 points with respect to those who received human milk.

We probably will need to include these genetic polymorphisms in future studies to define more precisely the role LCPUFAs (long-chain polyunsaturated fatty acids) have in brain development and also to explore parameters other than IQ, combined with new technologies, in order to measure the real effect of these nutrients on brain development. These new approaches would permit us to establish dosages and critical windows for individualized prevention and therapy.

Dr Koletzko: I fully agree that the effect of the fat polymorphism on both fatty acid levels and plasma, red blood cells, and tissues is a large effect size. In our recent study on the German and Dutch child cohorts, we found large effect sizes, particularly for neonates for AA, but also for DHA. Three percent of DHA variation is explained by these polymorphisms. Perhaps you think that a 3% variation is not large, but if you look at the impact on outcomes, the effects are large. For example, in a European survey, we found that the people with the less common type had only half the risk of dermatitis and allergic rhinitis compared to the people with the other type.

Dr Campoy described the results of research indicating a marked effect of breastfeeding on cognitive development in children at 8 years of age. Children who were breastfed in that study had an IQ advantage over those who were not breastfed. We have seen that in many observational studies from all over the world over the years. We are not sure how to interpret this, because the choice to breastfeed is associated with socioeconomic status, education, and so forth.

I think this study is the first one that gives us confidence that breastfeeding is really causal because if you have the less common variant for fats, if you are less able to

synthesize high AA and DHA, then breastfeeding provides an additional 4.3 IQ point advantage at age 18. That is one third of the standard deviation, which supports the concept that the lipid supply with breastfeeding is causal for cognitive benefit.

My conclusion is that if we do a study on fatty acids, whether it is a cohort study or intervention study, and we have a sizable number of subjects, we must make sure to tier type the subjects.

Dr Rueda: Dr Cunningham-Rundles, you described the importance of the balance between T-helper cells 1 and 2 (Th1, Th2) and the importance of this balance during immune development on promoting an aggressive response against infection or a tolerance response. On the other hand, I believe that the placenta, and more specifically the decidual cells in the placenta, are typically Th2. That is one of the reasons why the mother does not reject the fetus during pregnancy. Is it possible to modulate the Th1 and Th2 balance on decidual cells in the placenta during pregnancy? Also if we modulate that balance, do we have any risk promoting a higher incidence of abortion in those pregnancies when we try to promote a Th1 response to decrease the risk of developing allergy (a typical Th2 response) later in life?

Dr Cunningham-Rundles: I think that is possible. Certainly anything we do that changes cytokine balance we need to do in the context of perinatal life at an appropriate point and in the postbirth period. One of the big mysteries is exactly when to do this intervention because this balance toward Th2 in vitro is clearly beneficial. It just is not the same after birth.

Dr Poston: You mentioned effects of vitamin A and folic acid, but not of vitamin D.

Dr Cunningham-Rundles: We are interested in working on this with children who are likely to have allergic airway disease, but we do not have any data yet.

Dr Godfrey: I would like to follow up on Dr Steckel's points with a question to Dr Cunningham-Rundles. If you look across the developing world, susceptibility to infection and high rates of infection are important factors. In the Gambia studies, one of the seminal papers was published on mortality following birth in hungry versus not hungry women. It showed a delayed effect on mortality—specifically on mortality from infectious diseases after the age of 15 years—pointing toward some perinatal effect on immune development. Does any evidence show what the nature is of the specific exposure or the mechanism that might train that effect?

Dr Cunningham-Rundles: I do not know of anything. However, the impact of early undernutrition on thymic development, which manifests itself in early adolescence, is perhaps relevant.

Dr Koletzko: The original hypothesis was to affect undernutrition because it was related to birth in the season when caloric supply was low. I was recently in a discussion that I found quite stimulating that proposed this might relate to a change in early microbiotic exposure because it was a different season of the year and different crops were available.

Dr Godfrey: My recollection was that the researchers looked at subsequent studies for effects on neonatal thymic involving leukocyte responses and found very little, suggesting that perhaps it was postnatal effects from the prenatal nutrition.

Dr Cunningham-Rundles: I think that is true. I am a little hesitant about that study because I have seen some attempts to reproduce it. It seems to occur in only one environment. However, it does seem likely that it is an environmental event, and I think this is a reasonable way to look at it. I only mentioned the study because I think it opens the door to the likelihood that specific areas provide exceptional challenges and that the needs of development still are not understood.

Dr Miller: I would like to build on a theme that I have heard the last couple of days. Dr Koletzko, you talked about variation of normal nutrition, and then we discussed the range of normal. I keep seeing two important areas for us here. One is the rapid change in obesity and diabetes that we are confronted with in the context of evolution during the last decade or two. When we talk about the history of "normal nutrition," most of that was undernutrition, such as nutrition among hunter/gatherers.

Do we have the tools to even understand what public health issues we will face in the next 10 to 20 years given the rapid change we are seeing in what we think is normal nutrition and normal nutritional management? What can we do on the public health side of these issues?

Dr Koletzko: Someone once wrote, it is very difficult to make predictions, especially if they relate to the future. But you are right. The change that has happened in the last 2 decades is not easily paralleled by what happened in the previous 2000 years in terms of the changing body composition of mankind. Many people have speculated and written about the key factors, the triggering factors, and we could probably discuss the prioritization of key factors for the next 2 hours.

What do we expect to happen in the future? Clearly, the consequences of obesity such as the diabetes complex remain a key challenge. As a pediatrician, I particularly worry about behavioral challenges and changes in the immune response. We have seen an epidemic of allergic manifestations with the speed that nobody would have predicted.

The increase in allergic manifestations in China is probably yet to come, but if our hypotheses on hygiene and microbiotic potential and predictors are correct, then China probably will see an explosion of allergy such as Thailand is seeing right now. I am not sure what other people predict as the key challenges for the next 2 decades.

Dr Godfrey: An article written about 30 years ago looked at the range of disorders beyond those we have today, including appendicitis, which has declined over the 20th century, and argues that some powerful forces are at play now. The article predicted that although coronary heart disease is declining in westernized communities, we may soon see a rise in the disease driven by childhood obesity.

In fact, this was a topic at the United Nations' high-level special assembly meeting on the control of noncommunicable diseases in September 2011. According to the Non-Communicable Disease (NCD) Alliance, which includes a group of organizations concerned about diabetes, heart disease, and cancer, legislation is a way to control. Another group of us disagrees with that. We have argued to various government delegations that behavioral components to noncommunicable diseases are a part of the solution. The World Health Organization head for The NCD Alliance is also clear that without a technique for the development of such a component, the chances for halting these changes are not great.

Dr Poston: We have some evidence that pregnancy is an extraordinarily good time to change behavior. Women are susceptible to health messages in pregnancy. For instance, women are susceptible to messages about reduction of alcohol intake during pregnancy in a way that is not possible in any other state. I agree with Dr Godfrey that pregnancy is the time to start.

Dr Riley: Dr Poston, do we know that women are perhaps susceptible during pregnancy and do we know that they maintain that lifestyle after the pregnancy?

Dr Poston: Those studies are in process. Intervention studies are looking carefully at whether women maintain that behavior subsequently, so 6 months postpartum, we will try to find out whether women can maintain these behavioral changes—physical activity and diet. Dr Abrams also will address the potential of behavioral

change affecting the postpartum period and weight retention. Some early evidence suggests that these interventions may have long-lasting effects.

Dr Riley: I was at a conference presented by Dr Atkinson in which a researcher from North Carolina brought up the fact that in the United States only 50% of pregnancies are planned, and that we probably have a chance to make an impact when the pregnancy is planned. If the pregnancy is unplanned, the women may not want to make behavior changes. I do not know whether you have looked at that in England or in any of your other studies.

Dr Poston: We have not.

Dr Catalano: Many issues exist relating to outcomes among women with preexisting diabetes who are planning a pregnancy. This is not something subtle that will happen in 30 years; it is about an increased risk of miscarriage or congenital anomalies. In studies, even in Scandinavian countries, we have seen an effort to improve glucose control prior to a planned pregnancy because of the increased risk of type 1 diabetes in this population. However, the ability to have women with pre-existing diabetes see a physician in order to improve glucose control has not been particularly successful. In California—Dr Abrams, you may want to comment—the Sweet Success program has not proven very successful in enrolling women prior to a planned pregnancy, even though many women are aware of the short-term risks. I believe that programs, even for those women who are planning their pregnancy and know they have a disorder that could have a disastrous outcome, are not quite successful because many women think those bad outcomes will not happen to them. The patients I see, because the risk of having a birth defect is perhaps 5%, 10%, or 15%, think they will fall in the 85% to 95% category that will have a good outcome.

Dr Abrams: I suspect that psychosocial components do exist for women who did not plan their pregnancy and have other things they are working on in their lives, those who do not want to make diet and exercise, or whatever the intervention is, their highest priority. However, I believe that most women want to have a healthy baby, regardless of whether they plan their pregnancy or not, so I think we still have a potential hook to get to them that we usually do not have.

But that said, I believe we are a little shortsighted to think that pregnancy is the time to intervene. We probably need national campaigns to get people aware of the fact that pregnancy is really important. We have to go to the schools and get to children

with the message that pregnancy is an important time, so that it becomes part of our culture and does not hit them for the first time when they become pregnant. Right now, we are using childhood obesity as a way to try to push policy related to obesity, but I tell my friends who work only on childhood obesity that they are too late. I would love to see a groundswell of support for the idea that social policy should recognize the importance of the early pregnancy period. The message has to go beyond the clinical setting.

Dr Marriage: Dr Poston has said that pregnancy is a teachable moment, but we also have looked at research on how great the impact of prepregnancy weight is on subsequent obesity. In my view, perhaps the focus should also include preconception because some women are already obese going into pregnancy. The obesity problem begins in childhood, and it would be beneficial if we could help prevent women from entering pregnancy with excess weight.

Dr Poston: Much effort has gone into trying to teach young people about nutrition and illness, and on the whole, they are not interested. Evidence indicates that if we try to teach them in school, it will not work. We have to do something fairly drastic.

Dr Godfrey has a plan to implement this outside of the school environment to try and change their attitudes.

Dr Godfrey: The science of behavior change is not a perfect science, but it is moving forward beyond what is done for diabetes. We think that an opportunity exists with school children, not just to teach them some things because they often know “five a day” and that sort of information, but to promote behavior change and change attitudes beyond knowledge.

Dr Rueda: What Dr Koletzko mentioned about projects supported by the EU, such as EARNEST or EARLY NUTRITION, is perhaps a way in which we can interact with different communities, different sectors of the population, and promote guidelines that can contribute to that goal of changing behaviors in the general population.

Dr Godfrey: We are trying to get funding for a trial with 4000 school children a year to see whether we can or cannot change what actually goes into their mouths—not just in the days to come, but in the months and years to come—and whether we can change attitudes about physical activity and future pregnancy. It is the sort of question we feel needs an answer.

Dr Abrams: I hope it works, but it is also important to mention that we can try to do this person by person or we can face the reality that we live in a tempting

environment that makes it difficult for many people to avoid obesity. We need social policy changes that go beyond education and beyond behavior change, and those are very hard to make.

I think that governments are going to have to start dealing with social policy changes that create a more healthful environment. It seems unfair that children live in this environment that encourages obesity and then we have to try to teach them to avoid temptations to control their eating. That said, we need to work at both the individual and environmental level.

At the University of California at Berkeley, we have tried to make the food supply better for students. However, although students may say they want healthier food, the pull to fatty, sugary foods does not always match what they say. For example, our students may agree in theory that the government should subsidize healthier food and that the university should not encourage them to eat fast food, but they are not so sure when they realize that this means that they might not find high-fat foods in the dining commons in the middle of the night when they are studying and craving French fries and that the cost of those French fries might increase.

Dr Poston: Case in point, we have just carried out a survey in a deprived area of London where many fast food restaurants are located near schools. That is the environment. How do we negate having fast food shops around schools? We are trying.

Conference attendee: Do these obese children have problems with metabolism, energy, and enthusiasm? When I was younger, I could go 1½ days without eating and with a high-energy level. As we get older and go out shopping, we have to stop and get a cup of coffee and a doughnut because our energy is falling and we are not handling our sugar. Do these young people have a harder time getting the energy to do things because the events that occurred in programming make it harder for them to utilize their calorie resources? Are we up against a very big hurdle? This is not just about changing behavior, and it is not just about moving aside the doughnut shops. It is really about figuring out a way to bring the physiology of these young people back.

Dr Murray: I have worked in weight management with these children for 5 years. I am not convinced that many of the really overweight children had a good conception of either hunger or satiety. I always was impressed with the unstructured nature of their day, the way that sleep times and wake times and mealtimes and snack times blurred throughout the day. We sent a number of these children for bariatric surgery. After gastric bypass surgery, they understood for the first time

what I meant by satiety because the signal was so strong. However, I do not think that many of the overweight children are low on blood sugar and energy. That is not what is driving them. It is much more the psychology of appetite driving them than it is the hunger and satiety cues.

Dr Godfrey: As Dr Koletzko alluded previously, it seems that several different routes to childhood obesity exist. For example, one route is driven by maternal obesity and neonates' demonstrably high adipose tissue at birth. Another route is typified by the original Dutch family data from 1976, which involves undernutrition of the mother in which appetite stimulation in the child occurs through a mismatch route in which the developing fetus is triggered to think that it needs to eat whenever food is available. Such infants are thin at birth but then progressively become fat during childhood. I think this is a complex area, which has a number of metabolic routes to get to a common area.

Dr Poston: In fact, there are studies that show that the offspring of women who get obese in pregnancy grow up with a strange attitude toward satiety. They eat more. We and others have shown that the hypothalamus is structurally changed in the appetite regulator and pathways. In a follow-up observational study that Dr Godfrey and I are involved with, we are looking at satiety in children and relating it to associations with maternal factors such as maternal body mass index. We will do the same with children, because I think extraordinarily good evidence exists for early programming of appetite.

Lifestyle Intervention Trials During Pregnancy

Barbara Abrams, DrPH, RD

Recent data from the US Centers for Disease Control indicate that the prevalence of excessive gestational weight gain (Table) approaches 40% among women with a normal prepregnancy body mass index (BMI), and is even higher among women who are overweight.[1] Excessive maternal gestational weight gain is an established risk factor for increased risk of cesarean delivery, fetal macrosomia, and maternal weight retention in the immediate postpartum period and is possibly related to a wider array of other adverse pregnancy outcomes.[1]

Table. Cutoff Values for Excessive Gestational Weight Gain Based on 2009 Institute of Medicine Recommendations[1]

Pregravid BMI Category	Total Weight Gain (lb)	Rate/Week (lb)*
Underweight (<18.5 kg/m^2)	>40	>1.3
Normal weight (18.5–24.9 kg/m^2)	>35	>1.0
Overweight (25.0–29.9 kg/m^2)	>25	>0.7
Obese (≥30.0 kg/m^2)	>20	>0.6

*2nd and 3rd trimester
Source: Adapted from the National Academy of Sciences, 2009.

Growing evidence also exists to show that high maternal gestational weight gain increases the risk of child and adolescent obesity.[2,3] To address the health risks associated with excessive gestational weight, the 2009 Institute of Medicine Report, *Weight Gain During Pregnancy: Reexamining the Guidelines,* recommended that "those who provide prenatal care to women should offer counseling, such as guidance on dietary intake and physical activity, that is tailored to their life circumstances."[1]

In theory, this is an excellent idea. Unlike preexisting obesity, gestational weight gain is potentially modifiable during the course of pregnancy, with the potential to reduce negative in utero influences on the fetus that could persist over the child's life.[4] Pregnant women are concerned about having healthy babies and are perhaps more motivated to change their behavior during pregnancy than at other points of time.[5] It is possible that intervention during pregnancy could correct or improve

habits related to weight management that could translate into better health for women and their families over the long term.

The consistent contact with the medical care system through prenatal care could provide an ideal vehicle for delivery of behavioral interventions to women. However, current prenatal care systems are not set up to provide these services. In order to change the medical care system, research-based evidence is required to determine effective intervention strategies to promote healthy gestational weight gain in the clinical setting.

Interventions to Improve Maternal Diet and Physical Activity

In the past year, six different groups have published critical literature reviews comprised of approximately 12 trials investigating how behavioral interventions to improve maternal dietary intake and/or physical activity reduce excess gestational weight gain. One review focuses only on women who began pregnancy overweight or obese,[6] while five reviews include a wider range of prepregnancy BMI.[7-11] Three groups conducted meta-analysis.[7,8,12] and three did systematic reviews. Only controlled studies were included, and most were randomized controlled trials. Conducted in Australia, Belgium, Denmark, Norway, Sweden, and the United States, the sample size of these trials ranged from 41–560.

Researchers used a variety of intervention strategies, including counseling and education about weight gain, healthy eating, and physical activity and/or monitoring of weight gain, with or without feedback. The intensity and frequency of interventions and the number and combination of different components are variable. Even though all reviews used high-quality methodology to assess the evidence and all looked at the same accumulation of data, the conclusions vary. Three of the reviews conclude that interventions can effectively reduce gestational weight gain, although results are inconsistent,[8,10,11] two conclude that interventions are ineffective,[6,7] and one concludes that the quality of the studies is too weak to consider their findings for evidence-based guidelines.[9] Overall, these reviews demonstrate a clear need for more definitive research on which to base clinical practice.

The Fit for Delivery Study, conducted by Dr Suzanne Phelan and a multidisciplinary team and published in April 2011, adds to the knowledge summarized by these

reviews.[13] Informed by Social Learning Theory, this randomized, assessor-blinded, controlled trial tested an intervention that included the following:

- Nutritional counseling provided by a dietitian at enrollment, with a diet prescription of 20 kcal/kg
- A book to aid women in reducing fat and calorie intake
- Three telephone calls with the dietitian during the course of the study to assess progress
- Encouragement to moderately exercise, supported by a supplied pedometer with the ultimate goal of walking 10,000 steps/day, and the request that women keep a record of their progress
- Provision of a gestational weight-gain goal for each woman, based on the 1990 Institute of Medicine Recommendations,[14] as well as a scale and directions to monitor and record weight gain
- Feedback on weight-gain progress by postcard after each prenatal visit
- A "stepped-up care" approach for women who gain too much weight, with more frequent contacts and structured meal plans and behavioral goals
- Regularly mailed educational materials and "challenge" cards to strengthen motivation to healthfully control weight

Controls received one 15-minute meeting with the study dietitian to discuss general principles of diet and physical activity during pregnancy, as well as newsletters throughout the study that covered aspects of pregnancy unrelated to weight management. Both groups received standard prenatal care and pamphlets from the American College of Obstetricians and Gynecologists and the March of Dimes. The study randomized 401 women—200 to standard care and 201 to the intervention. Half of each group had a normal and half an overweight/obese BMI. Fig 1 shows that the intervention significantly reduced excessive gestational weight gain among those with normal BMI, but not those who began the study overweight or obese.

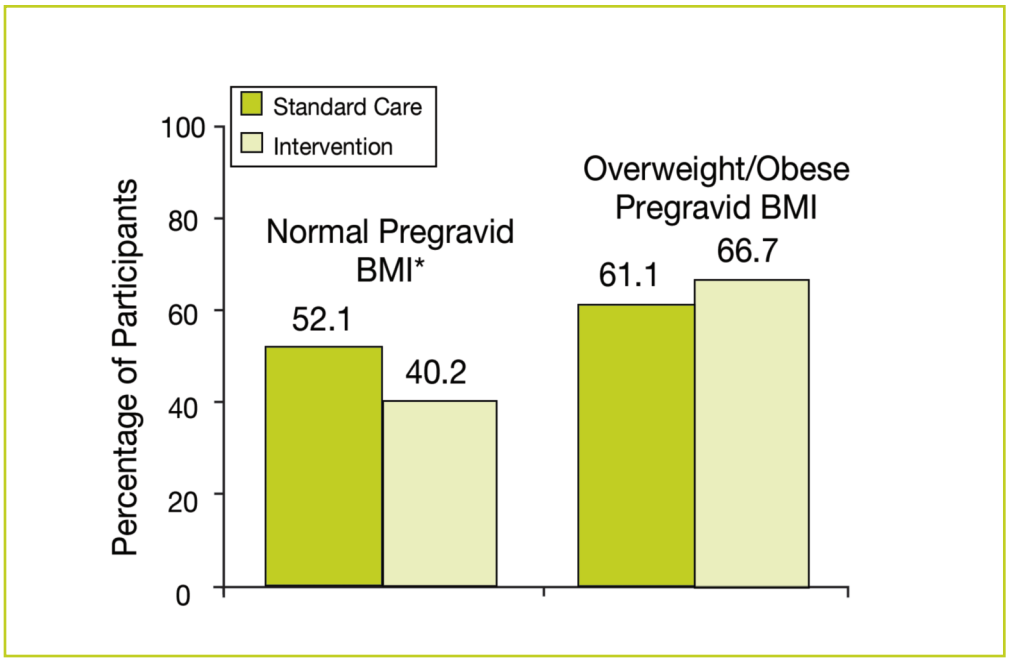

Fig 1. Excessive weight gain† in the Fit for Delivery Study women.[13] Results were based on an intention-to-treat analysis.

**P*<0.05
†Total pregnancy weight gain >35 lb for normal weight women and >25 lb for overweight/obese women

Source: Phelan S et al. Randomized trial of a behavioral intervention to prevent excessive gestational weight gain: the Fit for Delivery Study. *Am J Clin Nutr*. 2011;93:772-779. Reprinted with permission of the American Society for Nutrition.

However, at 6 months after delivery, the prenatal intervention increased the proportion of women who returned to their prepregnancy weight (defined as retaining <1 kg) in both groups (Fig 2), despite the fact that no additional intervention was provided after delivery.

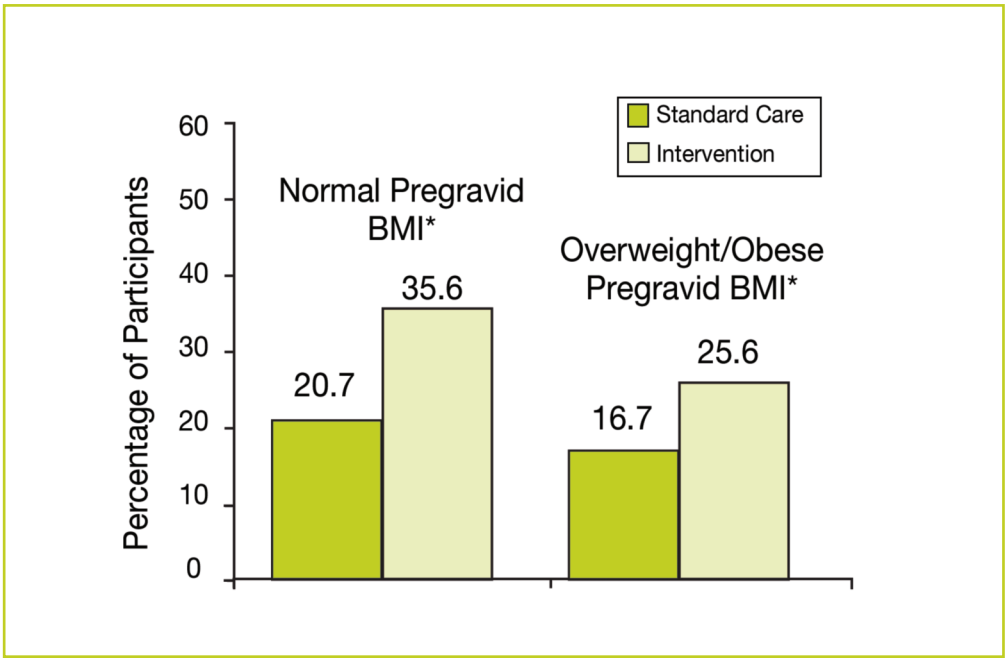

Fig 2. Postpartum weight status† 6 months after birth in Fit for Delivery Study women.[13]
Results based on an intention to treat analysis.

*$P<0.05$
†±0.9 kg or below prepregnancy weight

Source: Phelan S et al. Randomized trial of a behavioral intervention to prevent excessive gestational weight gain: the Fit for Delivery Study. *Am J Clin Nutr.* 2011;93:772-779. Reprinted with permission of the American Society for Nutrition.

These results, in addition to those from other trials, suggest that it is possible to moderate gestational weight gain through methods that are implemented in the clinical setting. Echoing previous results, Fit for Delivery intervention did not reduce excessive gestational weight gain in overweight/obese women, the group at highest risk for poor pregnancy outcomes. However, our finding of some effect on weight in this group postpartum is encouraging, suggesting that the prenatal intervention may have had longer term impact. Although a step in the right direction, the finding that 40% of normal-weight women in the intervention group still gained weight excessively indicates that even in normal-weight women, more effective strategies are needed.

Future Research and Improved Behavioral Interventions

Reviewers suggest a number of important ways to improve the study of behavioral interventions for pregnant women.[7-10] In addition to strengthening and standardizing study methodologies and reports to allow comparability, a fresh look at the interventions themselves is needed. Virtually all studies to date focus solely on changing each individual woman's diet and physical activity, but pregnant women do not live in a vacuum. It is likely that excessive gestational weight gain is a function of the same neighborhood and environmental factors that cause obesity in children and other adults, thus multilevel interventions are needed.[15] Still, we need to better understand the physical, psychological, social, cultural, and financial barriers that women face in addressing weight control during pregnancy and design interventions responsive to women's experiences and concerns. Evidence also shows that women receive contradictory messages from family, friends, the media, and clinicians about weight management during pregnancy. Use of a mixed-method research design, which combines qualitative and quantitative approaches, could help inform new strategies based on established behavioral theories.

Finally, studies in nonpregnant populations suggest that successful weight control is possible through more intensive lifestyle treatments than those that are typically used in pregnancy, such as a calorie prescription supported by structured meal plans and meal replacements, high levels of physical activity (60–90 minutes/day), daily monitoring of weight and food intake, behavior therapy, and continued patient-provider contact.[16] The safety, acceptability, and efficacy of these approaches in pregnancy, as well as application of these methods before and after pregnancy, deserve serious study.

As researchers augment current knowledge with new perspectives, there is every reason to believe that future research will yield the keys to utilizing the "teachable moments" of pregnancy to promote health in mothers and their children.

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Q & A

Q: Perhaps I missed it, but how did you determine if an obese person gained excessive weight given that the 1990 Institute of Medicine (IOM) recommendations gave no upper limits for women who were classified as obese?

Dr Abrams: We used the 1990 IOM upper limit for overweight women, which is 25 lb. When the 1990 committee wrote its report, there was concern that because gestational weight gain was severely restricted in the previous generation, a recommended upper limit for obese women would encourage clinicians to become overzealous in limiting maternal diet for obese women.

Because the committee did not have data to support the upper limit, it recommended that obese women should gain at least 15 lb, which is the estimated weight of the products of conception (fetus, placenta, amniotic fluid, increased maternal body water, blood volume, and fat). The 2009 IOM committee did put an upper limit for obese women, and called for research to fine-tune the amount that is consistent with the best maternal and child health outcomes.

Q: I have just a quick comment in relation to these interventions. Two very important things to consider are whether the ladies actually change their behavior and whether the intervention adopted is effective. These are two different things. Do you have any idea whether either your normal weight or overweight groups actually did change their behaviors?

Dr Abrams: I can speak very preliminarily. We actually are working on a paper right now, but I have not seen all the data. My understanding is that the normal weight group significantly reduced their fat intake, which was one of our targets. The overweight group reported increased physical activity.

Q: In that regard, did you look at the relationship between those women who gained less weight and birth weight?

Dr Abrams: No significant differences existed between the intervention and control groups for mean birth weight, low birth weight, or macrosomia. We did not compare birth weights by maternal weight gain, regardless of the intervention.

Undernutrition and Overnutrition During Pregnancy in India: Dual Teratogenesis

C. S. Yajnik, MD, FRCP

India is one of the diabetes capitals of the world and at the same time the capital for low birth weight (LBW) and perinatal mortality. This paradoxical situation should have suggested a link between the two. This was recognized only recently after the pioneering studies by David Barker and his group in the United Kingdom, where they showed that LBW is a risk factor for future diabetes.[1] This is explained by the concept of fetal programming (ie, an irreversible effect on structure or function with long-term consequences). Fetal growth and development are influenced by an interaction between genetic factors and the intrauterine environment.

Fetal programming can manifest in various ways. It might affect size, body composition, and structure and function of systems, organs, and cells. Sometimes it may affect physiology without affecting size. It is increasingly appreciated that epigenetic changes are at the center of programming. These changes are mediated by methylation of DNA and acetylation of histones and through the role of microRNAs, all of which modify gene expression.

The Diabetes Unit at King Edward Memorial Hospital, Pune, India, has made important contributions to programming research. The original observation was that diabetes occurred in Indians at a much lower body mass index (BMI) compared to Europeans, which was possibly because of their higher central obesity and higher body fat percentage (adiposity). This led to the “thin-fat” Indian concept (Fig 1).[2,3] It also was noticed that Indians get diabetes at a much younger age.[4]

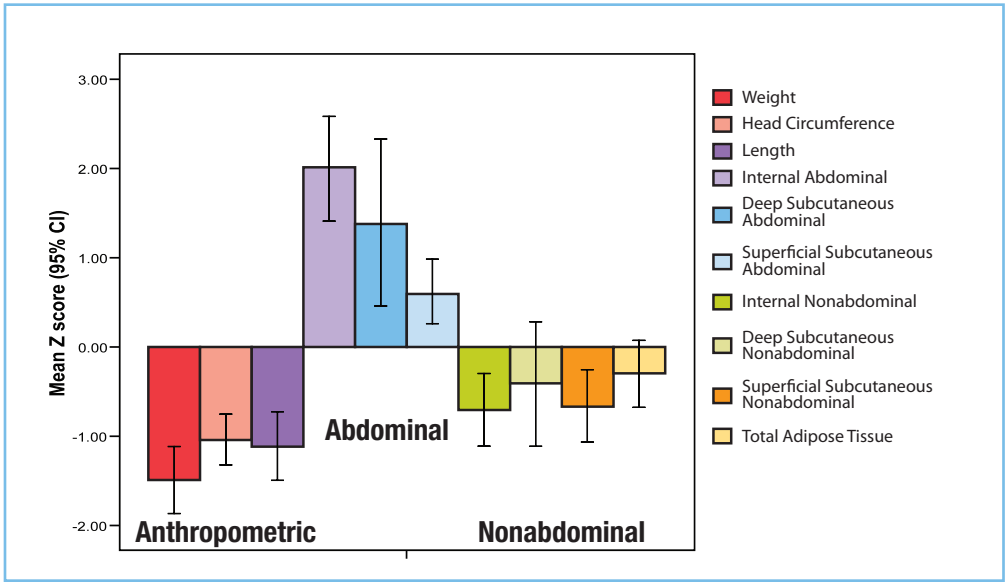

Fig 1. The 'thin-fat' Indian.[3] This figure shows the amount of body fat in Indian babies relative to that of English babies (standard deviation diagram). Indian babies are lighter, shorter, and thinner. They have a similar or smaller amount of nonabdominal fat, but higher amounts of both intra-abdominal and subcutaneous abdominal fat.

Fetal Growth and Programming

In 1991, researchers from King Edward Memorial Hospital joined Barker and Caroline Fall in the "fetal origins" research. The first collaborative research Pune Children Study confirmed that lower birth weight was associated with higher insulin resistance, as early as 4 years of age.[5] Children who were born small but grew big had the highest level of risk factors for diabetes and cardiovascular disease.[6] These findings suggested that intrauterine nutrition was possibly an important contributor to the risk of adult disease in Indians.

At the same time, it was known that fetal overnutrition, as in maternal diabetes, also increases the risk of obesity and diabetes in the child. A stage was set to investigate the factors influencing fetal growth and programming. This was the birth of the Pune Maternal Nutrition Study (PMNS), which was established in six villages near Pune in 1993. More than 800 pregnancies were studied. The children are visited every 6 months for anthropometric measurements, and parents and children are investigated every 6 years for a detailed assessment of body composition, cardiometabolic risk factors, and neurocognitive development.

The average mother in the PMNS was short (152 cm) and thin (BMI 18.1 kg/m^2), and gave birth to a light and thin baby (birth weight 2.7 kg, ponderal index 24.1). Interestingly, it was found that Indian babies were thin but fat (more adipose) compared to European babies, and that maternal intake of micronutrient-rich foods was a strong determinant of fetal size.[7] Paternal size predominantly influenced skeletal measurements, while baby's adiposity was predominantly determined by maternal factors. Short and fat mothers gave birth to the most adipose babies, suggesting an intergenerational influence of maternal early life "growth retardation" and her subsequent weight gain on body composition of the growing fetus.[8]

Follow-up of these children revealed that higher maternal folate in pregnancy predicted higher adiposity and insulin resistance at 6 years of age. The most insulin-resistant children were born to mothers who were vitamin B_{12} deficient and had high folate concentrations.[9] In addition, it was found that maternal vitamin B_{12} and folate predicted the child's neurocognitive function.[10] The Parthenon study in Mysore found that maternal vitamin B_{12} deficiency is associated with adiposity and gestational diabetes mellitus.[11] Thus, results in Indian studies point toward an important role for maternal one-carbon (1C) metabolism in fetal growth and programming. This is possibly because of the role of 1C metabolism in synthesis of nucleic acids, genomic stability, and the epigenetic regulation of gene function.

Noncommunicable Diseases

One of the challenges in the epidemiology of noncommunicable diseases (NCDs) in India is to reconcile the fact of rapid spread of the epidemic to the poor and the deprived, along with increasing affliction of the affluent. The finding of a disturbance in 1C metabolism in the undernourished (vitamin B_{12} and protein deficiency), as well as in the urban glucose-intolerant mothers (vitamin B_{12} deficiency associated with obesity and hyperglycemia), provides a unique explanation and an opportunity to tackle both fetal growth restriction (nutrient-mediated teratogenesis) and fetal macrosomia (fuel-mediated teratogenesis), which is referred to as a dual teratogenesis[12] (Fig 2).

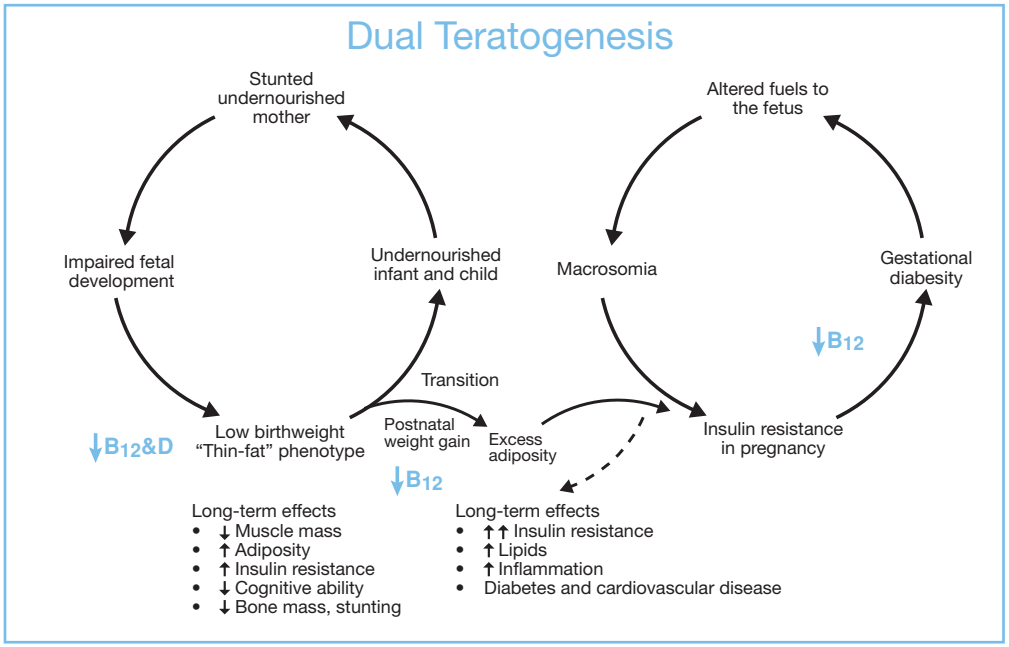

Fig 2. Dual teratogenesis.[12] This figure is a diagrammatic representation of the life cycles of babies born to undernourished and overnourished mothers (Indian and from other developing countries). Babies of undernourished mothers suffer intrauterine undernourishment and are born thin-fat. They are more insulin resistant. If postnatal nutrition levels are low, they remain thin-fat but "normal" adults and propagate the cycle. If these babies are overfed (on migration to cities or because of rapid economic transition), they become more adipose. Such mothers can develop gestational diabetes, which causes fetal macrosomia. Such babies are at higher risk of diabetes later in life. Research has shown an association of low circulating vitamin B_{12} with both the cycles, and vitamin D deficiency may play a role.

Thus, it is clear that a substantial proportion of adult health is programmed in utero. Health and nutrition of young girls is of paramount importance and is a major influence on the health of the next generation. The current idea of preventing NCDs in the middle-aged and elderly by difficult-to-do lifestyle adjustments is a very ineffective model. The next logical step in India is to improve 1C metabolism in adolescents for intergenerational prevention of adiposity, diabetes, and other related conditions. Improving the early life environment is perhaps more cost effective in preventing an NCD epidemic than controlling only the lifestyle factors in adults.

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Q & A

Q: Thank you for a fascinating presentation and amazing data. I am just puzzled about the observation that the effects of folate and B_{12} go in opposite directions, when my simple memory of the metabolic pathways is that there are synergistic effects in the homocysteine pathway.

I am just wondering whether you might see a contribution of folate and B_{12} that reflects certain dietary lifestyle patterns with people who are on a stricter vegan pattern with lower B_{12}, but with a high vegetable and folate intake. Is that a possible explanation? Are certain lifestyle factors and other dietary components or lifestyle components associated with this, perhaps causally, rather than folate and B_{12}?

Dr Yajnik: That is an excellent question. We have considered the possibility that our results are confounded by lifestyle factors, basically the protein intake, because protein and B_{12} go together. The Mendelian randomization results suggest that our nutritional associations are likely causal, but I am very willing to accept that there is still some confounded by lifestyle factors. This is the reason why in our planned intervention we have included a multi-micronutrients and milk arm to take care of other nutrients that might influence these associations.

Q: Indeed in the Mendelian randomization, I was not aware of the snips that you looked at for B_{12}. Is it also going in controversial directions then?

Dr Yajnik: They are going in the right direction. Holotranscobalamin levels represent the active form of B_{12}. As I showed, lower holotranscobalamin was associated with lower birth weight, indicating that B_{12} was contributing to fetal growth, and fucosyltransferase 2 *[FUT2]*, which is a marker for circulating B_{12}, again goes in the right direction on the prediction of insulin resistance in the child.

Just now, we are comfortable enough with what we have found to act on it. I am sure the story is more complex. There are a number of observations in the United States after folic acid fortification of flour—the imbalance between B_{12} and folate levels has widened. Normally in B_{12}-sufficient individuals, higher folate concentrations are associated with lower homocysteine concentrations. However, in vitamin B_{12}-deficient individuals, higher folate is associated with higher homocysteine and higher methylmalonic acid. Many papers in the United States have raised concerns about fortification of folic acid and the possible harmful effects it might have on a number of conditions, including anemia, neurocognitive decline, and some forms of cancer.

Nutritional Status of Pregnant Women in China

Chunming Chen, Professor

This overview presents recent information on the nutritional status of pregnant women in China. Data from 2000, 2002, 2006, and 2010 showed that iron-deficiency anemia (IDA) prevalence was 19.1%, 30.3%, 40.7%, and 36.6%, respectively. The difference is possibly because the sampling since the 2000 data on iron deficiency (ID) was 42.6% by a survey conducted by the collaborating team on children, pregnant women, and childbearing-age women, and the data from the United Nations International Children's Emergency Fund (UNICEF) in 2010 was in three poor counties in China. IDA prevalence in pregnant women in the 1st, 2nd, and 3rd trimesters was 9.6%, 19.8%, and 33.8%, respectively, but the ID percentage for all trimesters was approximately 40% (2000 data).[1,2]

Little difference was observed in deficiencies of other micronutrients, such as vitamin C, vitamin A, vitamin B_{12} , vitamin B_2, and folate between anemic and nonanemic pregnant women in the 3rd trimester in four provinces during 1999–2001.

Nutrient supplementation is not popular during pregnancy. One third of pregnant women never used supplementation, and 40%–50% used iron or folic acid supplements. Since 2010, free supplementation of folic acid pills for childbearing women became one of the components of the basic public health service of reformed medical care nationwide, but this still is not well implemented because of shortcomings in the delivery mechanism.

The information on the nutritional status of pregnant women 1 year after the Wenchuan earthquake in the affected area indicated that when comparing with the data from 2002, the dietary intake of fruits, soybeans, and dairy products increased, but decreased in animal foods and vegetables. Iron nutrition still was worse than in the rural groups in 2002. Vitamin deficiency prevalence was about 70%, and zinc deficiency was 61.6%. Even the relief from such a serious natural disaster was surprisingly successful in terms of dietary improvement, which was attributed to the support of people of the whole country and later by 18 specified provinces. But more attention is needed.

Nutrition is very important during pregnancy for child growth. In 2002, data from 1380 pairs of mother and child illustrated that 45% of the stunted children under 2 years of age were from mothers whose height was below 140 cm. Children were significantly shorter than the children whose mother's height was more than 155 cm. Girls born in the disaster years in China (1959–1961) exhibited a 20%–60% increase in overweight and obesity risk in their adult life (Table).

Table. Maternal Nutrition and Obesity Risk During Adult Life[3]

Year of Birth		n	Overweight		Obesity	
			n	%	n	%
Girls born in disaster years	1959	822	253	30.78*	87	10.58*
	1960	864	280	32.41*	89	10.30*
	1961	680	220	32.35*	61	8.97
			Odds ratio =1.289—1.372		Odds ratio =1.243—1.465	
Girls born after disaster years	1964	1473	390	26.48	108	7.33

Prevalence of girls born in 1964 as the reference
*$P<0.05$

Multinutrient supplementation is of benefit in reducing the low-birth-weight (LBW) rate of infants. This was proven by observing women during pregnancy in a western county who were provided with multinutrient pills with 10-mg iron supplementation. The LBW percentage declined from 7.31% to 3.21% in 1 year. As estimated by the World Bank, moving one LBW infant to the non-LBW category could save $510 (US dollars)/infant in a low-income context. The outcome of improved nutrition for pregnant mothers deserves further investigation and in more detail.

In 2006, the fetal macrosomia incidence in 14 provinces was 7.5% in urban areas and 6.3% in the rural areas, and it was higher in the eastern provinces, with incidence of 8.2%. A survey of rural areas in six counties in 2008 showed much higher figures, which were up to 9.91% with big differences between the western province Sichuan and the eastern province Anhui, 4.49% vs 13.14%. Apparently, surveys on more representative samples are required for strategic planning.

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Q & A

Q: Thank you for that very interesting presentation. I was wondering, do you have recommendations in China for how much weight women should gain during pregnancy?

Dr Chen: We do not have that right now. People always ask that. We do not have many studies on that. We cannot say what the Chinese recommendation is, so they follow the IOM (Institute of Medicine) recommendation.

Q: Just to follow up with that. I was talking with an obstetrician in Shanghai a few weeks ago, where a series of what they call obstetrics and gynecology hospitals are established across the area. He was telling me that 98% of the women who receive care in these hospitals follow IOM guidelines. This is a completely different obstetric culture. These mothers want to have an appropriate weight gain.

Dr Chen: The nutrition for adolescents in China is a problem, because obesity is not of very much concern. Even in the rural areas, the obesity or overweight prevalence among girls is lower than among boys.

The mothers and the girls always are concerned about their weight. For the boys, it is not that much of a concern right now. The girls control their diet, so many of those young women in China are smaller and shorter. This should become a greater concern for the next generation. We still need some research on that.

Challenges of Addressing Overnutrition and Undernutrition During Pregnancy in Chile/Latin America

Francisco Mardones, MD

Future perspectives and actions are needed to address issues and challenges related to both overnutrition and undernutrition in pregnant women in the Chile/Latin American and Caribbean (LAC) region, specifically development of an agreement on maternal anthropometric classification of nutritional status and weight gain guidelines in the LAC region, the need for further study of diet quality and multi-micronutrient (MMN) supplementation in the LAC region, and proposal of new newborn indicators to assess the medium- and long-term health risks associated with early experiences, especially during pregnancy.

Maternal Anthropometric Classification of Nutritional Status and Weight Gain Guidelines

International recommendations during the 1970s and 1980s established 12.5 kg as the optimal weight gain for pregnant women regardless of pregestational weight and height.[1,2] A standard developed in Uruguay served this purpose in the LAC region during the 1980s.[3] In the 1980s, studies on healthy Chilean women with term deliveries of 39–41 weeks, singleton pregnancies, and healthy newborns considered the influence of maternal nutritional status at the beginning of pregnancy and height.

The Rosso and Mardones (RM) Chart was designed to define the maternal "critical body mass" normal weight/height area, which was associated to an "optimal" birth weight (ie, the mean ±1 SD of this healthy population) (Figure).[4,5] This approach was in agreement with a recent World Health Organization (WHO) proposal to establish optimal birth weight.[6] Between 1987 and 2005, the Chilean Ministry of Health used the RM Chart to diagnose adequate or normal maternal body mass index (BMI) and consequently recommend weight gain during pregnancy. This chart also was incorporated in other LAC countries at the national level—Argentina, Brazil, Colombia, Ecuador, Paraguay, Panama, Uruguay, and Brazil. However, Brazil and Uruguay are not currently using it.

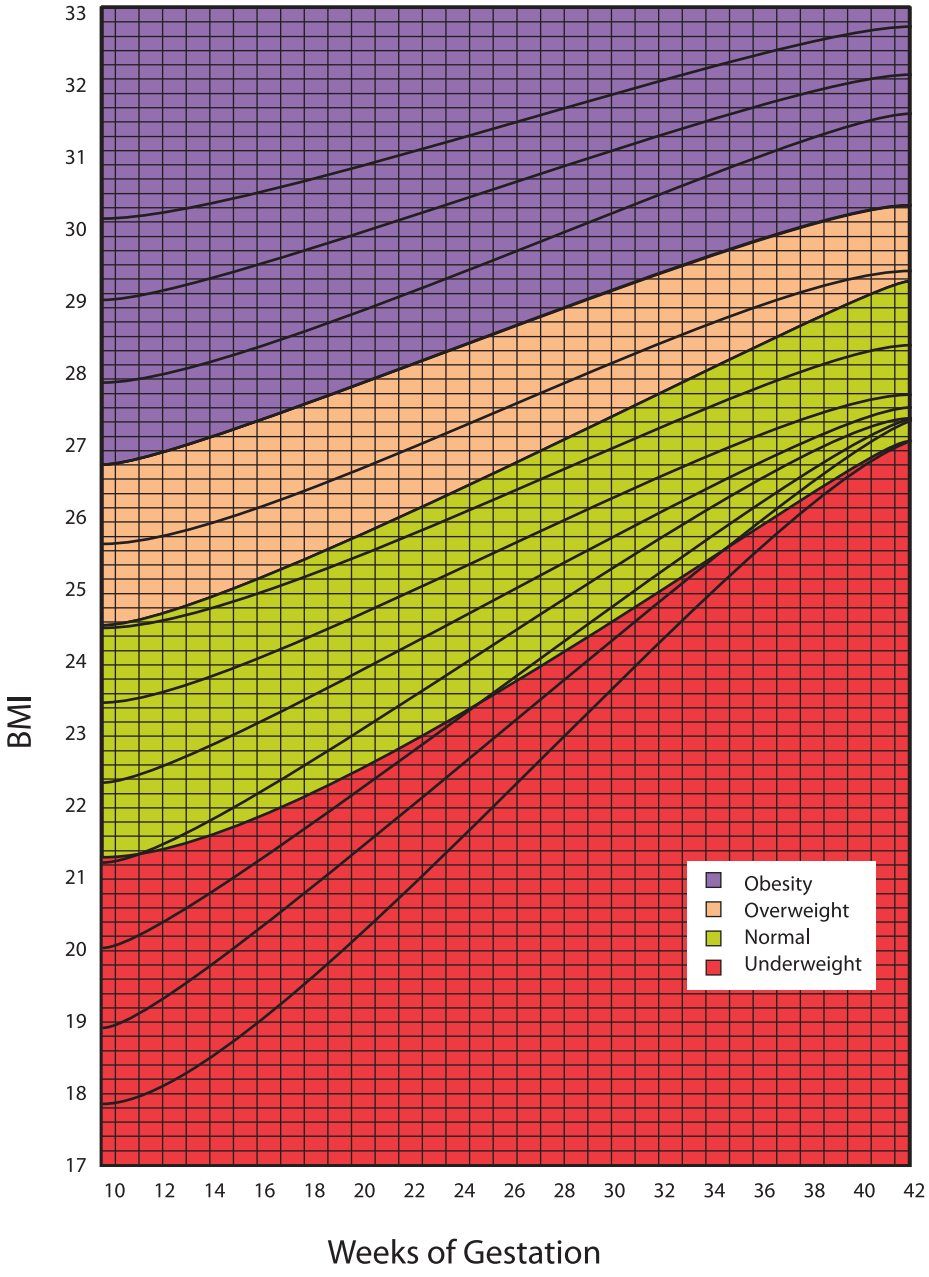

Figure. Rosso and Mardones Chart for guiding weight gain during pregnancy.[4,5] Weight for height is expressed as BMI.

Source: Mardones F, Rosso P. Design of a weight gain chart for pregnant women [article in Spanish]. *Rev Med Chil.* 1997;125:1437-1448. Translated by permission of Sociedad Medica de Santiago.

During 2005, Chile adopted the Atalah et al (AeA) proposal and changed the chart's lower and upper cutoffs, widening the normal BMI range.[7] Brazil adopted the same proposal in the last few years. Mexico recently adopted the pregnancy weight-gain guidelines issued by the Institute of Medicine (IOM), United States.[8] Central American countries have not decided which norm to use, and other countries, such as Peru, Uruguay, and Venezuela, are using the four previously mentioned norms, but not unanimously in their different provinces.

RM and AeA charts differ from the norm in the United States, mainly because the latter weight-gain guidelines were not determined proportionally for the individual height of each woman. That design inhibits its use in the LAC region, because women from our countries show an important variability in height—1 SD of mean height is about 6 cm. When using the RM and AeA charts, short and tall women are recommended to gain proportionately more and less weight than average height women respectively.

In the last 15 years, the LAC region underwent important changes in the nutritional status of pregnant women, and this nutritional transition has reached many countries. Chile reduced the prevalence of underweight pregnant women from 25.7% in 1987 to 13.3% in 2001, increased maternal overweight from 18.8% in 1987 to 21.8% in 2001, and increased obesity from 12.9% in 1987 to 32.6% in 2001.[3]

An adequate weight gain represents an important goal in prenatal care because of its influence on fetal growth and maternal health. Thus, health care providers should have at hand easy-to-use instruments for setting desirable weight gain goals for each individual mother and for monitoring weight gain during the course of pregnancy.

New research on the different outcomes associated with maternal preconceptional nutritional status and weight gain guidelines is needed. Unfortunately, implementation of experimental trials in the LAC region on this regard is currently not taking place. However, reports of observational studies are occurring. For example, a recent study in Chile used different norms in relation to these perinatal outcomes—risky birth weight (ie, <3000 g and ≥4000 g) and cesarean delivery. The study concluded that an independent and combined influence of preconception nutritional status and gestational weight gain on perinatal outcomes occurred when using standards to classify those parameters developed in the United States (IOM) and Denmark respectively.[9] The next study should look at the RM and AeA charts.

Diet Quality and MMN Supplementation in the LAC Region

Recent studies have shown that low-height, overweight pregnant women who attend the Chilean public health system have poor-quality hypercaloric intake.[10] Maternal poor-quality nutrition causes fetal growth restriction or macrosomia.[11] Balanced energy/protein supplementation of pregnant women is shown to increase birth weight, but the effects are, in most cases, only modest. Potential benefits of providing micronutrients to pregnant women, either through tablets or fortified food products, are now under investigation.

A supplement containing the recommended dietary allowances (RDA) of 15 different micronutrients was proposed,[12] supported by the findings of randomized controlled trials with a positive difference in mean birth weight of about 60 g in healthy pregnant women. During the last decade, Chile supplied two micronutrients, folic acid and iron, to the general population of pregnant women through white-flour fortification and delivery of ferrous sulphate tablets, respectively. Other countries in the LAC region are doing so, but recent information is not available. Therefore, when public programs already provide folic acid and iron, the possible impact of a combination of MMN supplementation deserves further exploration.

The previously mentioned birth-weight improvements are similar to those achieved in an earlier randomized controlled trial of an MMN-fortified milk-based supplement. A 73-g birth-weight increase in the experimental group was observed at a time when delivery of iron tablets and folic acid fortification were not public programs.[13] On the other hand, several studies have shown that either omega-3 fatty acids or fish consumption increases birth weight and prolongs gestation.[14,15]

In a recent trial, powdered milk was fortified with α-linolenic omega-3 fatty acids, plus a similar mix of vitamins and minerals.[16]

Treatment analysis showed that:

- Mean birth weight was higher in the intervention group than in the control with a difference of 118 g (95% CI, 47–190 g)
- Birth length had a difference of 0.57 cm (95% CI, 0.19–0.96 cm)
- The combination of MMN supplementation and omega-3 fatty acids still can have an important impact on fetal growth, higher than the previous 60-g difference in birth weight, when folic acid and iron are universally provided

In the intention-to-treat analysis, the birth-weight difference was significant, but with just a difference of 65.4 g (95% CI, 5–126 g). An influence in gestational age also was noted with an incidence of very preterm births (<34 weeks), lower (0.4% vs 2.1%) in the experimental group. Those findings were affected by an important amount of women who did not consume powdered milk because of a product failure, reducing the on-treatment groups from slightly more than 450 women each to about 350 women each. A new study is needed in this regard and also in light of new research supporting omega-3 supplementation during pregnancy.

Two other studies performed in Mexico did not produce favorable results in the LAC region.[17,18] It seems, on average, that the areas where the trials were performed did not lack supplemented micronutrients, although primigravidae supplemented with omega-3s had heavier infants with also larger head circumferences than controls.[17] It was concluded that prenatal docosahexaenoic acid (DHA) supplementation of primigravid women may result in increased infant birth size in a population where dietary DHA intakes are very low, and benefits of the intervention on infant health and neurodevelopment are under study.[17] Long-term possible results also should receive consideration. Maternal calcium supplementation trials done in Argentina have reported favorable long-term results in children on incidence of hypertension and caries.[19]

Newborn Indicators to Assess Health Risks Associated With Early Experiences, Especially During Pregnancy

New gold standards for fetal growth are needed, because birth weight is not always associated with medium- or long-term health outcomes.[20] Body composition at birth is associated with insulin resistance in adulthood, and new epigenetic measurements are under consideration as possible risk factors for later adiposity of children.[21,22]

The importance of birth length in this regard also is recognized in many countries throughout the world. For example, the University of Pelotas group in Brazil showed that birth length strongly predicts adolescent height and that birth weight's effect disappears when adjusting for birth length.[23] Adult height also is highly associated to general mortality in Chile and elsewhere, indicating the public health importance of birth length.[24] Different studies conducted in developed countries demonstrated that cognitive capacity was affected. Recent studies in school-age children in the LAC region, specifically in Guatemala and Chile, show similar results, including a higher effect of birth length when compared to birth weight[25,26]; in Chile birth length at <50 cm had the worst results.[26]

Obesity rates in school-age children are inversely associated with adjusted birth length in Chile[27]; the highest rate was reported in children with birth length <50 cm. Uauy et al have commented that children societies undergoing nutritional transition are presenting excessive weight gains but low growth in height.[28] Both combined phenomena increase BMI rates, so it becomes indispensable to enhance birth length to address obesity.

Another Chilean study of school-age children showed significant inverse associations between three perinatal factors—birth weight, birth length, and gestational age—and metabolic syndrome factors. For example, hypertension had an odds ratio for birth length <50 cm of 1.46 (95% CI, 1.13–1.88).[29]

The idea of considering birth length <50 cm as a possible new indicator of fetal growth would need further studies to show its possible association with health outcomes in the middle or long term. The proportion at the local or national level of this indicator in Argentina, Chile, and Uruguay has reached 48%, 44.5%, and 62.7%, respectively[26]; the latter figure is possibly associated with a higher prevalence of maternal smoking. Other countries in the LAC region and different populations of Australia have reached the following proportions (Table).[26]

Table. Proportion of Birth Length <50 cm

Area	Year(s)	Percentage
Argentina (Sardá Maternity Hospital, Buenos Aires)	1988–1999	48%
Brazil (city of Pelotas)	2004	76.5%
Chile (national figures)	2000–2002	44.5%
Colombia (department of Caldas)	2003–2009	54.5%
Colombia (city of Medellín)	2009	67.6%
Eastern Australia (indigenous mothers, aboriginal or Torres Strait Islander)	2002–2008	50.8%
Eastern Australia (nonindigenous mothers—Caucasian, Asian, Indian, African, Maori, and other ethnicities)	2002–2008	37.6%
Eastern Australia (Caucasian mothers)	2002–2008	36.6%
Uruguay (national figures)	2009	62.7%

Sources:

Argentina, Chile, and Uruguay: Mardones F, Grandi C, Moratorio X. Fetal life and healthy growth. In: Uauy R, Carmuega S, eds. *Healthy Growth in the South Cone of Latin America.* Buenos Aires, Argentina: Danone. In press.

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Guatemalan early nutritional interventions have demonstrated that higher birth length and consequently improved adult height result in substantial gains in human capital and economic productivity.[25] International experiences also have shown that in order to enhance birth weight and height, it is necessary to overcome maternal restriction because of short maternal stature.[30]

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Q & A

Q: What do we know about the long-term effects on the child of maternal stunting? You have talked about the weight the mother brings to the pregnancy, but you have opened up a whole different way of looking at the problem, and I assume also the one from India.

I heard one talk where somebody was talking about the granddaughters, their pregnancies expressing what happened in the grandmother. It interested me because in the 1950s weight gain was restricted in pregnancy. The obesity epidemic is kind of happening in the granddaughters of those people. Do you think

that maternal stunting gets passed along as well, and that it is not just a question of what happens in that fetal environment in pregnancy, but also what happened in that mother's mother's pregnancy?

Dr Mardones: What I observed in Chile is that in the last 20 years, maternal height has not improved or moved, probably just 1 or 2 cm, but quite slowly. This is a sign of inequality in some way that we need to improve the maternal diet or the quality of the diet in the general population and that is probably not well considered. We saw information from the Southampton survey that problems also exist in developed countries.

The maternal nutritional status is not improving around the world in the speed that it should. That is my impression. In this audience, most probably, we have more height than our parents, but this is not the case in many low-income populations in Latin America.

Discussion

Richard Steckel, PhD

Dr Godfrey: Postnatal nutrition was not the main focus of this conference, but we have some consensus that nutrition has its greatest impact during the first 1000 days. I wonder whether the slave heights that were measured reflected recent undernutrition of those individuals that depressed their growth rate. I think it was suggested that the slaves did not experience prenatal or perinatal undernutrition, but rather postnatal undernutrition, so that they were more amenable to catch-up growth when they went to the plantations. Presumably, they came on a trans-Atlantic voyage.

Dr Steckel: No, these were all American-born slaves. As early as 1750, a majority of the African-American population was native born. Some of the adults measured in 1819 or 1820 were born in Africa, but most of the measurements I described were made after 1819. By 1830 or 1840, most of the African-born slaves would have died. We did not have a big crop like sugar to export. Before 1807, we grew crops like tobacco, indigo, and rice. The demand for those products grew slowly, so we did not need to import many slaves. However, places like Jamaica that grew sugar kept importing slaves at high rates, such that 80% to 90% of the population at any one time was from Africa.

Dr Riley: Dr Abrams, you believe that pregnancy is a teachable moment. We brought this up previously. Do you use any tool to determine whether the people joining your study are ready to change their behavior, and do they reflect what you think the average obstetrician is seeing in his or her practice?

Dr Abrams: I think they do reflect what the obstetrician is seeing, but not completely, of course, because women who participate in research trials are likely to differ from the general population of all women. We had a good representation of low-income women, but we did not have many African-American women, who are at the highest risk for perinatal-related obesity in the United States.

How do you know if somebody is ready to change? I do not know. I think Dr Poston might have something to say about that.

Dr Poston: We do many structured interviews with women to determine the barriers to behavioral change, and I think that is important. Our intervention has an educational component, and we see a change in attitudes to behavioral change throughout the course of the intervention. I think it is important that women change their perception of perceived barriers to change in health behaviors because of the intervention. Our intervention is intense. The women are seen eight times during their pregnancy. Sessions are based on cognitive and control theory derived from work with nonpregnant, obese populations. Repeated contact points with health care professionals usually are effective.

Dr Abrams: This field is in its infancy, like toddlers or preschoolers now. I think what we will learn from ongoing studies in Australia and the United States will help answer your question. However, putting on my clinician hat, not my research hat, I would say that we should not stop now. We still should get the message out. Every baby that is conceived and born in an obesity-promoting environment or in an undernutrition, micronutrient-deficient environment is a life. This is an emergency. I do not want people to feel like they are off the hook until we get the results of the UPBEAT Program. We have enough evidence that we could take baby steps right now in clinical care. We could make big changes if our health care system put energy toward preventing problems, as opposed to treating them after they happen.

I just want to make sure nobody walks out of here thinking that, based on my presentations or any of the other presentations, we do not know enough. We do not know enough to make formal policy. But I believe that we do know enough to encourage every obstetrician and every front-line provider to talk to their patients about the importance of a healthy lifestyle during pregnancy, and to encourage women to eat healthy food in moderation and to walk every day. I met some of the midwives involved in Dr Poston's project, and if I were a pregnant woman in that project, they would motivate me to make healthy behavior changes. Their intervention is related to insulin levels through eating a low-glycemic diet. I believe we need intensive interventions, with lots of contact, not just pamphlets.

Dr Poston: I agree. The guidelines for weight management in the United Kingdom have changed quite a bit. One recommendation is that health care professionals should talk to women about the risks of obesity, the importance of a healthy diet of five portions a day of fruit and vegetables, and portion control. At the moment in the United Kingdom, that message is not getting across to obese women. Midwives have little time to talk to the women at the initial antenatal visit, and they generally skirt the topic of obesity.

Dr Abrams: In 1992, the Institute of Medicine followed its 1990 Institute of Medicine report on nutrition in pregnancy with a little booklet called *The Implementation Guide*. That booklet laid out a plan for nutritional care preconceptually, during pregnancy and postpartum, and it is not rocket science. Its philosophy is that the front-line provider—eg, physician or midwife—should set the tone, give some basic nutrition information and counseling, and keep following the women. Perinatal nutritionists with the time and expertise would be available for consultations, in order to work more intensively to help women who need more help to change. I worked on a team like that, and I can tell you it is possible to have an integrated team that can help women, and the impact would go beyond pregnancy, because women tend to serve as the nutritional gatekeepers for the entire family.

I do not know whether the obesity epidemic is generating more interest in nutrition among physicians. I used to try to teach the OB-GYN residents, and although they were always very nice to me, I know that compared to a topic such as hemorrhage, my topic was not very interesting.

Dr Catalano: What the operating room residents hear about is much more interesting than nutrition at that stage of their career.

Dr Abrams: When I was at the University of California at San Francisco, I worked as a clinical nutritionist and had a faculty appointment. When the medical students came to do their clerkship, they had a half day of lectures. The first lecture was on how to wash their hands before going into the operating or delivery room, and the second lecture was on maternal nutrition. I know that the symbolism of including a lecture on nutrition right from the start caught the medical student's attention. Encouraging lifestyle changes related to childbearing will require all health professionals to agree that this is important and possible, with evidence-based methods for delivering relevant, holistic, and effective interventions.

Dr Poston: I would like to add that we are concerned about the mental health of obese people, a topic that is neglected. We are getting depression and anxiety scores for our women. Many of them are depressed, and an association between depression and obesity among pregnant women exists. Quite a few people say that unless we pay attention to this and refer those women to appropriate care, we are not going to get anywhere with this issue. We are about to start a study to look into this in more detail.

Discussion

Dr Campoy: We have established groups of adolescents in a program that is focused on changing behavior and lifestyle (EVASYON). Most of them were depressed, or they did not believe in the program because they had seen another doctor or participated in another program and they had not lost weight. However, as we organized the adolescents in groups of 10 to improve their health lifestyle, it worked very well, because it was only necessary that one or two participants believed in the program and were ready to change. It was these adolescents who pushed the others to accept the changes.

In the case of pregnant women, I think most of the obese women are interested in these changes of habits as well, because they are afraid of how obesity will affect their delivery, their offspring, and their retention of weight. They need help and not just to hear “do not gain excess weight,” “follow this diet,” or “do that physical activity,” because some of them cannot do physical activity and all of them need psychological support as well.

Conference Overview and Debate on the Impact of Prepregnancy Nutrition

Robert Murray, MD

I am a pediatrician who is interested in children's weight. My perspective is different from that of some of the other conference participants because my area of research is in schools. So I tend to look at children beyond their fetal and early childhood years.

One observation I am taking away from this meeting is the fact that parenthood is changing, pushed back from birth to conception, and to some extent, to preconception. In this sense, everything the pregnant woman does currently seems wrong. I talked about fetal origins to a group of school dietitians a while back, and one of them came up to me afterward and said, "Oh, good, now I have another thing to blame my mother for." She was thrilled.

I worry a bit about the conversation we are having in this country about obesity and child neglect. I wonder whether we put a great deal of pressure on the mother when we look retrospectively at the child who develops cognitive problems or bone problems or health problems. This programming places the burden of the child's future on the mother.

I will give you an example of what I have in mind. I was talking to someone recently who seriously thought that we should force all women to breastfeed unless they have an excuse from their doctor. I think this perspective is extreme, but this is not a maternal free-speech issue—the child pays the consequences.

I have worked in inner-city schools in Columbus, Ohio, that were urban and poor with a mixed population of whites, Hispanics, and African Americans. We screened students in kindergarten at age 5 years, in third grade at age 8 years, and again in fifth grade at age 10 years. We looked at body mass index (BMI) and blood pressure, and we looked for acanthosis nigricans, the darkening of the skin that signals early insulin resistance.

We found that about 30% of 5-year-olds were overweight. Their blood pressure was fine, and they did not show signs of acanthosis. By 8 years of age, 40% were overweight or obese, and of that group, 30% had prehypertension or hypertension and nearly 20% showed insulin changes, at least in terms of acanthosis nigricans.

By age 10 years, 50% were overweight or obese with the same mix of adverse metabolic findings.

I want you to think about the children you see. Think about an 8-year-old child who already has hypertension and who already has taken the first steps toward insulin resistance and, possibly, diabetes. In addition, extremely obese 8-year-old children are ostracized by their peers and often by their family. When we look at their quality of life as they get older, we see that this isolation does not go away. Here is a child who is 8 years old and has done nothing wrong, has made none of the important decisions, but is now hypertensive, prediabetic, and isolated by peers. The question is, is that the result of neglect? Are we putting a great deal of responsibility on parents for how that child lives the rest of his or her life?

What do the conference participants think? Is this a neglect issue if parents do not follow our direction? Should we, as David Ludwig at Harvard suggests, pull in children services to deal with people who do not take care of their children or with mothers who do not take care of themselves in pregnancy? Are we implying that what mothers do or have done to themselves represents serious, potentially life-long events that merit greater scrutiny?

Dr Abrams: I would say that this is the biggest argument for changing the environment, because you cannot blame the individual mother and you cannot blame the individual child. We are swimming in this environment that just invites us to eat, and sometimes the climate makes it difficult to get exercise. What pregnant woman is going to go out to exercise in summer heat or in ice and snow?

This leads me back to my passion about education. Can we find messages that help physicians and the women in our society understand that this is an emergency—messages that say that eating this bag of potato chips is not a good idea and that repeated inappropriate eating and lack of exercise may program one's child for the kind of suffering we have described? In my view, this needs to occur in the long run at a societal level, and in the short run, through the kind of trials we are doing and the kind of messages we are trying to get out.

Dr Godfrey: I would say this is not so much neglect as it is the level of science and health literacy of the parents. Quite strong literature about the importance of health and science literacy exists. In the school programs we are evaluating, the primary goal arguably is to increase science and health literacy in children and to make the information accessible in a way that they can act on it.

Dr Murray: I have thought about the statement about requiring women to breastfeed and how ironic it is. The man who made this statement is a Texas

conservative who generally would want the government off his back, but in this instance he wanted the government or society to insist that women breastfeed.

We have done a good job of educating pregnant women about fetal alcohol syndrome. Consequences of conditions such as that are a little more in your face than the consequences of obesity. If they drink, take cocaine, do heroine, or smoke, we know they will harm their child and they know they will harm the child. If a woman drinks alcohol to excess and causes harm to her baby knowingly, is that neglect?

We have spent 40 years on tobacco education, but 3000 teens take up smoking every day. It is not because they do not know what we have told them, it is because they do not care.

Dr Godfrey: That is the societal norm.

Dr Shamir: Not breastfeeding is not neglect. I think now we are moving this discussion to a different place, where I am not sure it should go. We are not talking about smoking, which is lethal. We want to improve our children's health, and it is our responsibility to provide the best nutrition that will give fetuses, and later newborns, the best start in life that we can. However, if we fail, the result is not lethal. We have to put this in perspective, so I think that a slightly lower level of passion about breastfeeding—the flame—is needed.

Dr Murray: We are having this conversation about soda taxes, too. Doing public policy with this sledgehammer approach is complicated.

Dr Sherry: Out of all the data that we have seen at this conference showing that if we put a black box over the "why," would we still look at the negative outcomes and how they coincide with health quality and mortality? We are doing that to our children. When we see fetal alcohol syndrome and undernutrition in children, we call in social services. In extreme cases, we have interventions for parents who put their children in this situation, regardless of why or how. In extreme cases in which parents do not know or care about nutrition and their 8-year-old children are overweight, the children did not make any choices; their parents chose for them.

Dr Murray: I always felt uncomfortable with the child-neglect issue when I worked in the hospital weight management center, because I felt that we physicians—and society in general—have not always done what we were supposed to do. The parents alone are not to blame. They live in this cornucopia of other factors that drive obesity.

Conference attendee: As a nutrition company, what can Abbott do to help you? At what life stage can we help break this cycle? Most of you would recommend intervening during pregnancy, but would you recommend the early trimester or later trimester?

Dr Murray: At what points do you think we could intervene? We are at Abbott Nutrition, one of the great nutrition think tanks in the world. What can Abbott do, and what is the capacity of industry for intervening to change the outcome?

Dr Marriage: This conference has focused on pregnancy, but I feel strongly that we need to look at childhood nutrition. I think as with public policy, as a nutrition company, we need to look at the entire lifecycle.

Dr Murray: That is where the opportunity is.

Dr Rueda: I think it is not a question of one or the other. I think it is a question of working together. We need to figure out what to do in the future together to accomplish some goals.

Dr Murray: For industry to work with public health.

Dr Rueda: Yes. Industry should work with key opinion leaders, policy makers, regulatory bodies, government, and education systems.

Conference attendee: A number of you have said that you would love to do research in some additional lifecycle stages, but resources are limited. From a scientific perspective, as you look across the continuum of life, where is the best opportunity to make the greatest impact on overall health outcomes?

Dr Campoy: I strongly support the idea presented earlier that we need to begin with the nutritional and lifestyle education of pregnant women. Why? Not only because this is the beginning of the offspring life, but also because when we educate pregnant women, we have the opportunity at the same time to educate the family and then surely the children. If pregnant women are educated in nutrition and lifestyle at that special moment, and they understand the importance of these habits to become the healthiest they can, they will pass these ideas on to their children. In my country, we have programs to improve childhood nutrition, but we do not have nutrition education for pregnant women. I think that this is a very good opportunity for change.

Dr Koletzko: I agree, but I would add that we should not focus only on the mother. In the study we did on breastfeeding success in Bavaria, the strongest predictor of

breastfeeding success was the father's support. An unsupportive father increased the risk of not breastfeeding. I think we should follow the lifecycle approach and push on early life as a window of opportunity to intervene, from before pregnancy through early childhood, addressing the whole family and not just the woman.

Dr Murray: Here is a question I grappled with yesterday and particularly this morning as we talked about India, South America, and China—should we focus on weight or diet? I ask that because weight is difficult to change, but you can improve diet, and it can improve incrementally. You also can improve activity incrementally. As long as we do not call activity "sports" or "exercise," we can get people to change. We are very weight conscious here in the United States, and maybe we should move to the United Kingdom model in which the focus is on nutrition intake, daily activity, and behavioral changes.

Dr Poston: Weight is obviously important because it is associated with increased morbidities and mortality, but I think if we focus on diet, weight will follow. Too much emphasis is placed on weight and dieting. Pregnant women we talk to say they have tried all of the diets, so they do not want us to talk about diet. We then just suggest that they swap one food for another to influence their glycemic load. Ultimately, these substitutions will lower calories, and less weight gain may occur.

I heard that a high-fat diet alone can have deleterious effects in programming. Several animal studies have suggested an impact of a high-fat diet as opposed to the obesity. Both are probably detrimental, but diet itself and switching from a high-fat to low-fat diet can have effects that are independent of weight. I agree that we should switch the emphasis a bit.

Dr Murray: I was thinking of resistance training. Exercise can have an impact on insulin resistance without necessarily changing weight much.

Dr Abrams: I agree. I learned earlier in my career from my epidemiological research that weight gain in pregnancy is enormously variable. I think that is one reason that health care providers in the United Kingdom decided not to focus on it. Someone wrote a paper that argued that we could not use weight in pregnancy as a screening tool for low birth weight or preeclampsia, and that is true. The sensitivity, specificity, and predictive value of weight are terrible. Apparently, human beings have developed a wide variety of responses to pregnancy.

One benefit of weight is that it is easy to measure, and it is a starting point. However, just measuring weight does nothing. We must sit down with the patients, do an assessment, and find out what is going on. I have worked with women who

gained 60 pounds in pregnancy but had an excellent diet. They were incredulous this was happening to them, but I left them alone because they were doing OK. Without fail, those women had normal, healthy babies and did not have long-term obesity problem after birth. Some women also do not gain much weight during pregnancy, but their diet is excellent, so I agree that we are weight-obsessed in this country and that it is not productive unless we do something with it.

Dr Poston: I always have had a problem with variability in weight gain in pregnancy. The weight of the fetus is variable. The weight of the placenta is variable, and the fat mass is variable, so when we measure weight in pregnancy we are not necessarily measuring fat. This is a plain physiological fact that most people do not appreciate. Gestational weight gain is associated with weight increase, with fat-mass increase, but it also is associated with variability, and that is why the Institute of Medicine guideline ranges are so wide.

Dr Abrams: I would suggest wider guidelines.

Dr Poston: But what weight range do you recommend to a woman when differences are in kilograms? It does not help them.

Dr Chen: I think industry can do something for the nutrition of pregnant women in a developing country like China. In China, a difference is seen in nutrition between rural and urban areas, but it is not easy to educate pregnant women about a good diet anywhere. In urban areas, families have paid too much concern to the diet of pregnant women, and it usually makes the diet unbalanced. In rural areas, a cultural barrier to improving a pregnant woman's diet exists as well. In remote areas, culturally a pregnant woman cannot eat better food than her mother-in-law. When I asked a woman whether she has better nutrition during pregnancy, she said "No, I do not, because I have a mother-in-law, and I cannot eat better than she does."

Beyond education, we can provide food. In rural areas, it is not easy to educate people to give good complementary food to young children because of their poor economic situation. So, we provide a daily nutrient-dense supplement food for complementary feeding of young children between the ages of 6 and 24 months, which has proven effective in reducing undernutrition and anemia prevalence and providing higher intelligence potential for children until 6 years of age.

Dr Murray: This is where industry can help.

Dr Chen: Simple food. It may help to have a special supplement food for pregnant women in the rural areas, but first, we should solve the problem of micronutrient deficiency to have healthier babies.

Dr Murray: Maybe programming represents a positive cost-to-benefit.

I am intrigued by the concept of preconception, because preconception actually may extend into the 1st trimester of pregnancy if a woman then discovers that she is pregnant. Healthy behaviors are a lifecycle issue, as Dr Marriage suggested. The behaviors that we know may lead to trouble in pregnancy begin around 9 or 10 years of age among girls in this country. Even though they are only 9 or 10 years old, in my opinion, they are preconceptional. At that age, girls begin to become deficient in folic acid, vitamin D, vitamin A, vitamin C, and iron—deficiencies that then appear in adolescence.

Can we do an intervention at that point to keep those young girls healthy, so that later in life when they get pregnant, they will have good outcomes?

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