Examining a Developmental Approach to Childhood Obesity: The Fetal and Early Childhood Years: Workshop Summary

DETAILS

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Examining a Developmental Approach to Childhood Obesity

The Fetal and Early Childhood Years

Workshop Summary

Leslie A. Pray, *Rapporteur*

Food and Nutrition Board

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"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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PLANNING COMMITTEE ON UNDERSTANDING THE DYNAMIC RELATIONSHIP BETWEEN BIOLOGY, ENVIRONMENT, AND EARLY CHILDHOOD DEVELOPMENT ON RISK OF OBESITY[1]

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[1] Institute of Medicine planning committees are solely responsible for organizing the workshop, identifying topics, and choosing speakers. The responsibility for the published workshop summary rests with the workshop rapporteur and the institution.

Reviewers

This workshop summary has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published workshop summary as sound as possible and to ensure that the workshop summary meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this workshop summary:

Rachel Ballard-Barbash, National Institutes of Health, National Cancer Institute
Sandra Hassink, Institute for Healthy Childhood Weight, American Academy of Pediatrics
James Ntambi, University of Wisconsin–Madison
Catherine Spong, National Institutes of Health, National Institute of Child Health and Human Development

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this workshop summary was overseen by **Joanne Lupton,** Texas A&M University. Appointed by the National Research Council and the Institute of Medicine, she was respon-

sible for making certain that an independent examination of this workshop summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this workshop summary rests entirely with the rapporteur and the institution.

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1

Introduction[1]

Recent scientific evidence points to the origins of childhood obesity as an outcome of the dynamic interplay of genetic, behavioral, and environmental factors throughout early development, with a compelling body of evidence suggesting that both maternal and paternal nutritional and other exposures affect a child's risk of later obesity. The burgeoning field of epigenetics has led researchers to speculate that many of the observed associations between early developmental exposures and later risk of childhood obesity are mediated, at least in part, through epigenetic mechanisms (see Box 1-1). On February 26–27, 2015, the Institute of Medicine (IOM) Food and Nutrition Board and the IOM and the National Research Council Board on Children, Youth, and Families convened a workshop in Washington, DC, to explore the body of evolving science that examines the nexus of biology, environment, and developmental stage on risk of childhood obesity (see Box 1-2). The workshop focused on the prenatal period, infancy, and early childhood and addressed evidence from both animal and human studies. Workshop objectives developed by the planning committee were to (1) identify epigenetic-mediated relationships between exposure to risk factors during sensitive periods of development (gestation through age 3) and subsequent obesity-related outcomes; (2) explore the science around periods

[1] The planning committee's role was limited to planning the workshop, and this summary has been prepared by the workshop rapporteur as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants and are not necessarily endorsed or verified by the IOM, nor should they be construed as reflecting any group consensus.

BOX 1-1
What Is Epigenetics?

Shari Barkin defined epigenetics as "changes in gene expression via changes in posttranslational and posttranscriptional modifications." Robert Waterland defined it as "the study of mitotically heritable stable alterations in gene expression potential that are not caused by changes in DNA sequence." However, many workshop participants used "epigenetics" in its broadest sense—that is, in reference to biological phenomena that can be attributed to more than just what genes are present but also whether and how those genes actually function. Derived from the Greek prefix "epi-," epigenetics literally means "above genetics."

BOX 1-2
Statement of Task

An ad hoc committee will plan a 2-day public workshop exploring the body of evolving science that examines the nexus of biology, interaction between biology and environment, and developmental stage on risk for childhood obesity. The workshop will include attention to the prenatal period, infancy, and early childhood and will include evidence from animal and human studies. The committee will define the specific topics to be addressed, develop the agenda, and select and invite speakers and other participants. A summary of the workshop will subsequently be prepared by a rapporteur with the assistance of staff and then undergo the National Academies report review process prior to release.

of plasticity and potential reversibility of obesity risk in the context of early childhood development; and (3) examine the translation of epigenetic science to guide early childhood obesity prevention and intervention to reduce obesity risk (see Box 1-3). This report summarizes the information presented and discussed at the workshop and is not intended to serve as a comprehensive overview of the topic. The information and suggestions for future action included here reflect the knowledge and opinions of individual workshop participants and should not be construed as a consensus.

BOX 1-3
Workshop Objectives

The workshop objectives developed by the planning committee were to

- Identify epigenetic-mediated relationships between exposure to risk factors during sensitive periods of development (gestation through age 3) and subsequent obesity-related outcomes.
- Explore the science around periods of plasticity and potential reversibility of obesity risk in the context of early childhood development.
- Examine the translation of epigenetic science to guide early childhood obesity prevention and intervention to reduce obesity risk.

OPENING REMARKS

In her welcoming remarks, workshop planning committee chair Shari Barkin described epigenetics as an emerging field of study, one aimed at understanding the phenotypic changes caused not only by changes in DNA, but also by changes in gene expression. For example, when researchers transported larvae from docile European bee hives to a killer bee hive and foster-raised the normally docile bees with the killer bees, they found that not only did the European bees develop an aggressive killer bee phenotype, but their actual gene expression also changed. DNA is a code requiring context. Genes cannot change, but gene expression can. "We are built to be 'permeable fluid beings,' rather than 'solitary unitary isolates,'" Barkin said, referring to science writer David Dobbs's concept of the recursive reconstruction of the self (Dobbs, 2013). (See Dobbs, 2013, also for a description of the bee-fostering research.) "We're constantly remaking ourselves and turning ourselves over."

In humans, good data exist to show that rapid infant weight gain in the first 6 months of life is strongly associated with obesity, insulin resistance, and metabolic dysfunction in early adulthood (Leunissen et al., 2009). The infant weight gain observed in that study represented an increase of just 1 centile, Barkin noted. As a clinician, she regularly observes infants crossing many centiles. She emphasized the dynamic nature of the multiple external and internal factors that interact during this early period and contribute to later childhood obesity, and encouraged workshop participants to consider how scientists' growing knowledge of this dynamic, including the role of epigenetics, can shed light on where to target childhood obesity prevention efforts (see Figure 1-1).

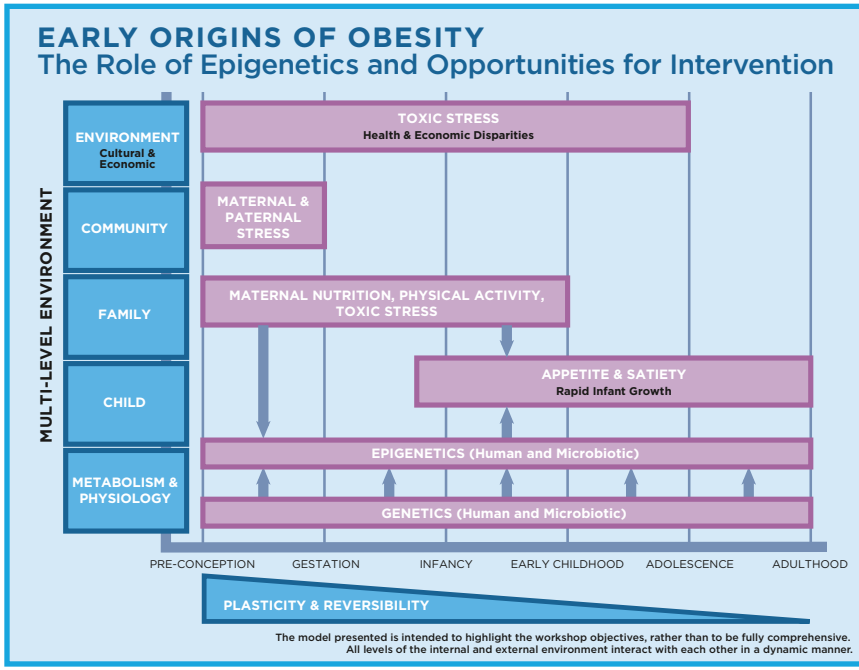

FIGURE 1-1 Early origins of obesity: Multiple external and internal factors interact to cause childhood obesity.
SOURCE: Distributed by the workshop planning committee on February 26–27, 2015.

David Klurfeld of the U.S. Department of Agriculture's Agricultural Research Service briefly touched on the history of the origins of epigenetics in his opening remarks. Although C. H. Waddington is credited with coining the term "epigenetics" in 1942 to describe how genes interact with their surroundings to produce a phenotype, Klurfeld referred workshop participants to an article in the March–April 2015 issue of *American Scientist* on Ernest E. Just, an embryologist on the faculty of Howard University in Washington, DC (Byrnes, 2014). While Thomas Hunt Morgan of Columbia University proposed that genes control all cellular events throughout development, Just proposed what he called the theory of genetic restriction, that is, that cytoplasmic factors interact with nuclear elements to induce cell differentiation. The truth, Klurfeld stated, appears to be somewhere between these two extremes. Although the concept, or field, of epigenetics can be traced back at least as far as Just, for Klurfeld epigenetics is new. He described it as a "gee whiz" field, with a lot of fascinating numbers associ-

ated with it, such as the fact that DNA in a single cell is 2 meters long but folds into a nucleus 6 microns across.

For Sandra Hassink, on the other hand, epigenetics has been on the American Academy of Pediatrics's strategic plan for the past 5 years, representing what she described as a deep desire among clinicians to understand the underpinnings of child health. Hassink showed graphs from the Centers for Disease Control and Prevention of the prevalence of obesity across the United States and stated that two things always strike her when she looks at the graphs. The first is the geographic variation, which leads her to ask what is happening in those areas where prevalence is so high, and the second is that the country is still dealing with massive health disparities. Hassink started her obesity practice in 1988, when, she said, obesity was not on people's radar screens. Initially, as she watched obesity move through the adult community, she and others thought that children would somehow be spared. But they are not spared. They are suffering from obesity in high numbers at very early ages and with a dramatic impact on their health. Today, according to Hassink, 17 percent of children are obese and 30 percent are overweight or obese. These children, she observed, look in many ways like middle-aged adults in terms of their health profiles. She remarked that while she did not enter pediatrics expecting to see this, in fact that is what pediatricians are seeing and dealing with every day. Moreover, they are operating in what Hassink described as a "very complex socioecological milieu," and they have only "very blunt instruments," namely, food and exercise.

Clinicians know that they need to focus on very young children in particular because, Hassink said, once a child develops obesity or an adolescent develops morbid obesity, "there's no going back," even with extraordinary effort. But they don't have ways to intervene in any practical sense. Additionally, clinicians know that what happens to the mother happens to the fetus and infant, with environmental influences having profound effects on the physiology and behavior of energy intake and expenditure. But again, she said, "We don't know exactly where to break this cycle or how to break this cycle." Yet, there are many opportunities during early childhood to intervene. Not only is it an important period of time when lifelong nutrition and activity behaviors are being established, but, moreover, the first 20 of a family's (on average) 31 well-child visits are concentrated in the first 5 years of life, creating a window of opportunity for clinicians to partner with families and to teach parenting skills. The American Academy of Pediatrics is ready, Hassink said, to serve as an engine for translating pediatric obesity research findings into action.

Jamie Bussel of the Robert Wood Johnson Foundation began her welcome remarks by noting that although epigenetics is not on the foundation's

strategic plan, early childhood is. More than 8 years ago, the foundation announced its $500 million commitment to preventing childhood obesity, which Bussel said was the largest prospective commitment that the foundation had ever made in a single health issue. They set what she described as a "pretty audacious" goal to decrease the prevalence of childhood obesity by 2015. The evidence of progress is "strong and absolutely exciting," she said. However, while the foundation believes that it has turned a corner on childhood obesity and is on the right track, it has not gone far enough. Despite the gains, progress has been shared unequally, Bussel stated, with white children and children in higher income areas showing greater decreases in obesity rates. Today, more than 25 million children remain at risk for cardiovascular disease and type 2 diabetes. Unless this is addressed, Bussel predicted that this generation of children will be the first to live sicker and die younger than their parents.

The Robert Wood Johnson Foundation announced in February 2015 that it would be contributing an additional $500 million over the next decade to continue and expand its work in reducing childhood obesity. An important theme that will be driving the next decade of effort, not just in terms of building the evidence base, but also supporting action and advocacy, will be an intensified focus on reducing disparities. The foundation's vision is for all children in the United States to be at a healthy weight no matter who they are or where they live. This is part of a broader effort focused on the healthy development of children—a critical element of the foundation's mission to build a Culture of Health in the nation.

Bussel emphasized, as Hassink had and as many other participants would over the course of the workshop, the importance of starting early. According to Hassink, evidence indicates that children who enter school at a healthy weight are more likely to maintain a healthy weight through adolescence and adulthood. The first 1,000 days following conception is a crucial period for development and for the prevention of obesity and its consequences. Of the foundation's five "big bets," that is, five key strategies where it plans to concentrate its obesity prevention efforts, Bussel identified as the most relevant to this IOM workshop the effort to ensure that all children enter kindergarten at a healthy weight. She stressed the importance of working with parents even before their infants are born, linking families with the support and resources they need beyond the clinic walls to help their children achieve and maintain healthy weights, and the importance of gaining a better understanding of how to change what needs to be changed not just at the individual level but at the family and systems levels as well.

ORGANIZATION OF THE REPORT

Much of the workshop discussion revolved around the emerging nature of the evidence for epigenetics as a key component of the "Early Origins of Obesity" model (featured in the workshop infographic) and whether experimental findings indicate causal versus correlational or confounding associations. Session 1, moderated by Matthew Gillman of the Harvard School of Public Health, set the conceptual stage for this discussion. Chapter 2 summarizes the Session 1 presentations and discussion.

Following a conceptual overview by Robert Waterland of the Baylor College of Medicine and Andrea Baccarelli of the Harvard School of Public Health, workshop participants in Session 2, moderated by Karen Lillycrop of Southampton University, considered how the risk of childhood obesity can be affected by (1) maternal and paternal nutrition and other exposures before conception, (2) maternal and placental nutrition and health during pregnancy, and (3) postnatal maternal and infant nutrition and health. Chapter 3 summarizes the Session 2 presentations and discussion.

Moderated by Leann Birch of the University of Georgia, Session 3 speakers discussed potential opportunities for intervention and prevention based on rapidly advancing knowledge of the role of epigenetics and other factors in the early origins of obesity. Chapter 4 summarizes the Session 3 presentations and discussion.

Given the dynamism, complexity, and context-dependency of childhood obesity etiology, it is difficult to translate research results, especially findings from animal experiments, into real-world application. Moderated by Debra Haire-Joshu of Washington University in St. Louis, the session explored in detail some of the challenges as well as opportunities for real-world application. Chapter 5 summarizes the Session 4 presentations and discussion.

To conclude the workshop, Esa Davis of the University of Pittsburgh Medical Center moderated a discussion with workshop speakers on possible future research directions, with an emphasis on expanding what scientists know about epigenetic-mediated associations between early developmental exposures and subsequent obesity-related health outcomes. Judith Hall of the University of British Columbia facilitated a second discussion open to all workshop participants that focused on opportunities and challenges in epigenetic research. Chapter 6 summarizes both discussions together. The chapter ends with a summary of the workshop's major overarching themes as described by Shari Barkin.

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Conceptual Overview of the Role of Epigenetics in Childhood Obesity

OVERVIEW

A major overarching theme of the workshop was the emerging nature of the evidence for epigenetics as a key component of the "Early Origins of Obesity" model, which was represented by the workshop infographic shown in Figure 2-1, and whether experimental findings indicate causal versus correlational or confounding associations between observed epigenetic changes and either exposures or outcomes. Session 1, moderated by Matthew Gillman of the Harvard School of Public Health, set the conceptual stage for this discussion. This chapter summarizes the Session 1 presentations and discussion.

Robert Waterland of the Baylor College of Medicine described what is arguably the clearest example of the causal role of epigenetic dsyregulation in obesity in an animal model: genetically identical agouti mice developing into either lean (brown) or obese (yellow) mice, depending on the degree of DNA methylation (one of several types of epigenetic marker) at the agouti locus. Waterland cautioned, however, that there are a multitude of obstacles to understanding how epigenetic dysregulation might similarly cause obesity in humans. He called for more prospective studies in humans to help infer causality. He also identified the need to assess epigenetic variation within the context of genetic variation and to focus on tissue-specific epigenetic patterning.

Andrea Baccarelli of the Harvard School of Public Health elaborated on the challenge of differentiating causality from correlation. He described a recent study in which the authors associated methylation of a gene in fat

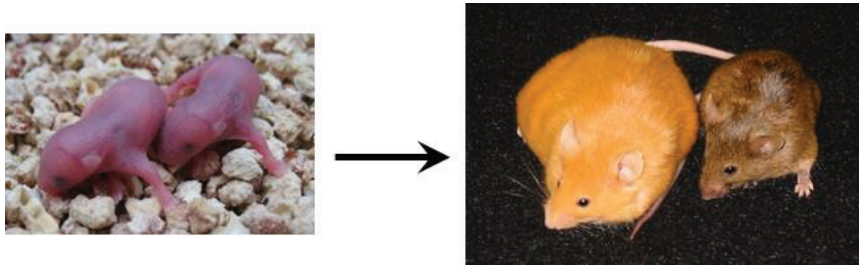

FIGURE 2-1 Genetically identical agouti mice, which were phenotypically indistinguishable at birth, grew up into very different phenotypes depending on the level of DNA methylation at the *Avy* locus.
SOURCE: Presented by Robert Waterland on February 26, 2015.

tissue with body mass index (BMI). They concluded that methylation did not determine BMI; rather, BMI determined methylation (Dick et al., 2014). Regardless of causality, Baccarelli expressed hope that in the future epigenetic markers at birth can be used to identify newborns at increased risk of childhood obesity. But again, as Waterland cautioned, many obstacles will need to be overcome before reaching that clinical point. Baccarelli speculated that epigenetics might be experiencing the same "winner's curse" that befell the field of genetics when geneticists started reporting genome-wide associations between genomic patterning and disease. Initially, many reported effect sizes were overestimated.

FUNDAMENTALS OF EPIGENETICS[1]

Much of what Robert Waterland discussed was built on ideas put forth in Waterland and Michels's (2007) review of the epigenetic epidemiology of the developmental origins hypothesis and Waterland's (2014) update of that review with a focus on obesity.

The developmental origins hypothesis proposes that during critical periods of development, transient environmental stimuli can have a persistent, even lifelong, impact on gene expression, metabolism, and risk of disease. Lucas (1991) put forth the concept of "biological programming" to describe these effects. Waterland and Garza (1999) built on Lucas's idea of biological programming and devised a mechanistic construct that they called "metabolic imprinting."

[1] This section summarizes information and opinions presented by Robert Waterland, Ph.D., Baylor College of Medicine, Houston, Texas.

Metabolic imprinting refers to adaptive responses to early nutrition that are characterized by (1) a limited period of susceptibility (i.e., a critical window effect), (2) a persistent effect that last through adulthood, (3) a specific and measurable outcome, and (4) a quantitative relationship between exposure and outcome. Waterland pointed out that the first two characteristics are very similar to the concept of ethological imprinting proposed by Konrad Lorenz in the 1970s. The latter two characteristics were intended to guide mechanistic studies of metabolic imprinting. Waterland and Garza (1999) proposed four different potential mechanisms of metabolic imprinting, namely, alterations in organ structure, alterations in cell number or ploidy, clonal selection, and epigenetic mechanisms.

Epigenetics and Epigenetic Mechanisms

Waterland stressed that today, many people talk about developmental programming as if it is "all about epigenetics," or all about epigenetic mechanisms, when in actuality it is very likely that epigenetics is just one of several different mechanisms interacting and that researchers should be studying all mechanisms in an integrative fashion. That said, the focus of his presentation was on epigenetics, which he defined as the study of "mitotically heritable and stable alterations in gene expression potential that are not caused by changes in DNA sequence." The key to this definition, Waterland stated, is the stability of the alterations. While there are many ways to alter gene expression in a cell, epigenetic alterations are long-term stable alterations.

The best way to think about the stable nature of epigenetic alterations in gene expression, in Waterland's opinion, is to think about the many different tissues and cell types in the human body, all with the exact same complement of DNA (i.e., the entire human genome), but each expressing very different subsets of that DNA. Cell type–specific expression is established early during development, and it is persistent. Even as many cells are replaced over time by progenitor cells, their progenitor cells "remember" to generate the same epigenetic markers.

Another way to think about epigenetics is to remember that the word "epigenetics" literally means "above genetics." Epigenetic mechanisms are gene regulatory mechanisms layered on top of the DNA sequence information.

There are several epigenetic mechanisms, Waterland said. Methylation of cytosine within CpG dinucleotides is clearly an important one, and one that he would be discussing in detail.

Histone modifications are another potential epigenetic mechanism. Various modifications to the amino terminal tails of the histone proteins that package DNA in the nucleus of each cell are known to be highly correlated

with transcriptional activity and chromatin structure and therefore clearly play a role in regulating gene expression potential. However, as pointed out by Henikoff and Shilatifard (2011) and others, it remains unclear whether histone modifications have the definitive epigenetic characteristics of mitotic heritability, that is, whether specific established histone modifications can convey information over mitosis.

On the other hand, autoregulatory transcription factors have been recognized for decades as being able to function epigenetically (Riggs and Porter, 1996), yet they receive very little attention these days, Waterland observed. For example, the MyoD transcription factor, which plays an important role in muscle development in mammals, binds to and regulates its own transcription. During cell division, once MyoD is turned on, the MyoD protein, which is in the nucleus, is partitioned to both daughter nuclei, perpetuating its feed-forward auto-regulation. Many other transcription factors work in the same fashion.

Finally, noncoding RNA, another epigenetic mechanism, works in a similar way in terms of being partitioned in the nuclei during mitosis and being delivered to both daughter cells.

Waterland emphasized that all of these mechanisms and potentially others as well work in a synergistic fashion to maintain different regions of the chromatin in either a more transcriptionally silent or more transcriptionally active state.

Why Focus on DNA Methylation?

Of all the various potential epigenetic mechanisms, Waterland observed that most of the presentations at the workshop would focus on DNA methylation. He asked, why? First, DNA methylation is the most stable epigenetic mark, making it a very good candidate for conveying the type of long-term memory effects of relevance within the context of the developmental origins paradigm. Additionally, researchers understand its mechanism of mitotic heritability, knowledge of which makes it a bona fide epigenetic mark, in Waterland's opinion. Also, DNA methylation can be measured in minute quantities of DNA. Moreover, it can be measured in a molecule-specific fashion, allowing for precise quantitation of the genetic influences on epigenetic outcomes.

To provide some background on DNA methylation, Waterland explained that, first, most cytosines within CpG dinucleotides are methylated at the number 5 position, converting cytosine to methyl-cytosine, a covalent modification that affects gene expression by regulating the affinity of methylation-sensitive DNA-binding proteins.

Another feature of DNA methylation to keep in mind is that tissue-specific patterns of CpG methylation are established during development.

Shortly after fertilization, the vast majority of methylation in both the sperm and egg genome is erased. Then, at about the time of the early embryo's implantation, methylation patterns are reestablished in a lineage-specific manner as part of the differentiation process. The reestablishment process proceeds during fetal development and even during postnatal life. Each period of developmental establishment of DNA methylation patterns constitutes a "critical window" in which the environment, including nutrition, can affect the process.

Yet another feature of DNA methylation is that it requires dietary methyl donors and cofactors. And finally, and very importantly in Waterland's opinion, DNA methylation is mitotically heritable and researchers understand the mechanism underlying its mitotic heritability. He explained that a CG sequence on one strand is also a CG sequence in the opposite direction on the other strand, allowing for semiconservative replication of established DNA methylation patterns during mitosis.

Is Epigenetic Dysregulation Contributing to the Obesity Epidemic?

Waterland discussed evidence—mostly from animal models but also from humans—demonstrating how epigenetic mechanisms can affect obesity. For example, when mice and other mammals are cloned, they often are born with a slightly elevated weight and develop adult-onset obesity. Waterland showed an image from Tamashiro et al. (2002) of two genetically identical mice, one produced by cloning, with the cloned mouse also being obese, its obesity clearly an epigenetic, not a genetic, effect.

In humans, the neurodevelopmental syndrome known as Prader–Willi syndrome is a good example, in Waterland's opinion, of an epigenetic dysregulation that can cause obesity. Although the syndrome is most commonly caused by a genomic deletion of a large portion of chromosome 15, a subset of sporadic cases are caused by epigenetic silencing of the same genomic region.

Agouti mice are a third example of epigenetic dysregulation known to cause obesity. Again, Waterland showed an image of two genetically identical mice, this time two newborns who were indistinguishable at birth but who, because of an epigenetic difference at the agouti viable yellow (*Avy*) locus, grew up into very different phenotypes. One grew up to be yellow and obese, the other brown and lean (see Figure 2-1), with the obese mouse having a very low level of DNA methylation at the *Avy* locus and her lean sister being very highly methylated at the same locus.

Alleles that behave like the *Avy*, that is, with dramatic inter-individual variation in DNA methylation even among genetically identical individuals, are called metastable epialleles. Waterland and others have shown that nutrition and other environmental stimuli, both before and during preg-

nancy, can affect the establishment of DNA methylation at metastable epialleles with persistent and permanent phenotypic consequences.

A fascinating feature of metastable epialleles, in Waterland's opinion, is the systemic nature of the inter-individual variation, with essentially the same level of methylation present in all of the different cells of the body. Consequently, one could take a drop of blood from an agouti mouse, measure the methylation at *Avy*, and predict with absolute certainty whether the mouse would become obese in adulthood.

Obstacles to Understanding the Epigenetic Contribution to Human Obesity

While many clinicians and epidemiologists would like to have an epigenetic biomarker in humans like the differentially methylated *Avy* locus in agouti mice that could be used to predict who will become obese, Waterland cautioned that finding such a marker will not be a simple task. He identified several obstacles to understanding how epigenetic dysregulation contributes to human obesity, not the least of which is that genetic variation is an important determinant of epigenetic variation. If one was to conduct a case control study of obese versus lean individuals, one could certainly find epigenetic differences between the two groups, he said. However, it would be difficult to rule out that the observed differences in epigenetic regulation (and obesity) were caused by genetic differences between the two groups.

Another obstacle to understanding the epigenetic contribution to human obesity is the largely cell type–specific nature of epigenetic regulation. Although clinicians and epidemiologists would like to be able to study DNA from easily obtainable samples (e.g., peripheral blood or buccal swabs), Waterland observed that in most cases those samples will not provide much information about epigenetic regulation occurring in tissues of greater relevance to obesity, such as hypothalamus or adipose tissues.

Yet another obstacle is poor characterization of epigenetic regulatory regions, though the situation is improving, Waterland observed. He referred to just-published data from the National Institutes of Health (NIH) reference epigenome mapping project (Kundaje et al., 2015). One of the biggest insights provided by those data, in Waterland's opinion, is the importance of epigenetic regulation in enhancer regions. Most epigenetics researchers have been focused over the past couple of decades on promoter regions, that is, regions at the beginnings of genes. Enhancers are regulatory regions often located hundreds of thousands of base pairs away from genes. It appears now that epigenetic regulation at enhancers plays a critical role in tissue-specific and cell type–specific gene expression. In Waterland's opinion, inferring tissue-specific epigenetic dysregulation is going to be very difficult in human studies.

Also with respect to the poor characterization of epigenetic regulatory regions, while the general rule is that DNA methylation is a silencing mechanism, Yu et al. (2013a) reported a large class of genes in the human genome that are actually transcriptionally activated, not silenced, by methylation at the 3′ end of a gene. In sum, Waterland said, "We clearly have a lot to learn about how epigenetic regulation works."

Finally, the disease process itself can affect epigenetic mechanisms, raising questions about causality. The best example, in Waterland's opinion, is in cancer epigenetics. It has been known for decades that tumors are characterized by dramatic epigenetic dysregulation. However, it was unknown until recently whether epigenetic dysregulation actually caused the cancer. With respect to obesity, when epigenetic changes are observed in lean versus obese individuals, the direction of causality is still unclear.

The Way Forward

Waterland suggested some steps forward to help move the field past these many obstacles. First, controlled studies in appropriate animal models are urgently needed to advance researchers' understanding of epigenetic mechanisms underlying the developmental programming of obesity. For example, Waterland pointed to the significant advantages of using inbred mouse models: the removal of genetic variation as a factor, the ability to observe a single life span from embryonic development to adulthood in only 1 year, and the ability to obtain all relevant tissues.

In terms of human studies, Waterland reiterated the need to assess epigenetic variation in the context of genetic variation, to study appropriate tissues (or, if that is not possible, then at least confirmation of systemic variation), to focus on genomic regions with known functional inter-individual variation, and to conduct prospective studies that allow for causal inference (i.e., rather than just cross-sectional studies).

In closing, Waterland showed for a second time the image of the genetically identical but epigenetically different (at the *Avy* locus) agouti mice. He remarked that although it is customary to think that all phenotypic variation is genetically based, clearly epigenetics has a large role in determining phenotype.

CONCEPTUAL MODEL OF EPIGENETIC INFLUENCE ON OBESITY RISK[2]

Imagine a musical score with a very specific sequence of notes. Like DNA, the sequences of notes are translated into a phenotype, that is, music.

[2] This section summarizes information and opinions presented by Andrea Baccarelli, M.D., M.P.H., Ph.D., Harvard School of Public Health, Boston, Massachusetts.

Andrea Baccarelli showed an image of a music score and the great composer Herbert von Karajan at the podium, in front of an orchestra, translating the score into a phenotype (see Figure 2-2). If Baccarelli himself was at the podium instead of von Karajan, he said, the phenotype would change dramatically. That is an example, Baccarelli said, of how the exact same sequence of notes, or DNA, can yield completely different phenotypes. If Baccarelli had looked at the music score beforehand, he would have noticed that several parts of the score had marks written above the sequence of notes, marks indicating, for example, "louder" or "softer." Those marks, he said, are what epigenetics is all about: marks added to a sequence of notes, or a sequence of DNA, that do not change the sequence but do change the phenotype. The marks can be written in either pencil or pen, with notes written in pen representing, in the genome, permanent epigenetic markings established during fetal life. Notes written in pencil, in contrast, like the methylation markings on inflammatory genes, are reprogrammable and can change within a matter of minutes.

Baccarelli proposed a model to explain the epigenetic influence on obesity risk, with the fetus playing a central role and in utero exposures affecting the embryo and fetus in ways that potentially modify the epigenome

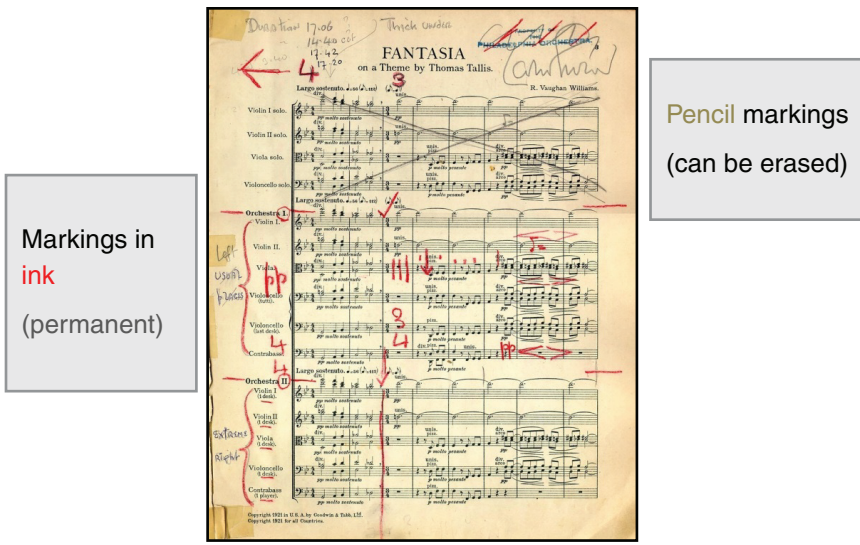

FIGURE 2-2 Comparison of the relationship between DNA, epigenetics, and a phenotype to the relationship between the sequence of notes on sheet music, marks scribbled on the score by the composer, and the music produced.
SOURCE: Presented by Andrea Baccarelli on February 26, 2015.

at birth and, in doing so, program risks associated with obesity (Fleisch et al., 2012). Baccarelli suggested that not only are in utero exposures important, but preconception exposures may be as well, particularly exposures of the gametes, given that gametes have their own epigenomes that can be influenced and modified by environmental exposures. In other words, what parents do before they have children, including what they do during their own childhood, potentially influences risks in their children. There is no clear evidence indicating that a parent's epigenome directly impacts the fetus, other than via gamete exposure; in fact, according to Baccarelli, some experts would argue whether such a direct impact (i.e., not via the gametes) is possible.

Based on this model, and borrowing concepts from Gillman and Ludwig (2013), Baccarelli proposed that fetal life exposure programs the epigenome at birth, but that the epigenome at birth is modifiable and can change in postnatal life. Fetal exposures can potentially be correlated to risk of obesity, and its sequelae, via the epigenome, include high maternal body mass index (BMI), high gestational weight gain, gestational diabetes, and other environmental factors.

Correlating DNA Methylation and Body Mass Index: The Challenge of Reverse Causation

In an epigenome-wide association study (EWAS), Dick et al. (2014) measured BMI and DNA methylation in blood samples from individuals participating in three different cohort studies: the Discovery in the Cardiogenics Consortium (n = 479), MARTHA (n = 339), and KORA (n = 1,789). Importantly, Baccarelli noted, both BMI and DNA methylation were measured at the same visit. The researchers identified three methylation sites in the hypoxia inducible factor 3a, or *HIF3A*, gene that were positively correlated with BMI across all three cohorts. Additionally, one of the three sites also correlated with BMI when DNA methylation was measured in adipose tissue (in addition to blood), but not in skin. However, based on a Mendelian randomization analysis, they concluded that perturbation of *HIF3A* methylation might play a role in the response to obesity, but is likely not a cause of obesity. (See the summary of Caroline Relton's presentation in the next chapter for a more detailed discussion of the use of Mendelian randomization to interpret the Dick et al. [2014] findings.)

Baccarelli explained that instead of the epigenome modifying the phenotype, in this case BMI, it is not surprising to find, that it is the phenotype modifying the epigenome (Relton and Davey Smith, 2012). He suggested that longitudinal studies might be a preferred study design for human epigenetics with respect to identifying the temporal, or causal, sequence of events.

Learning from Past Experience: Lessons from Genetics

One of the first genome-wide association studies (GWASs), published in 1990, reported an odds ratio of 8.7 for an association between alcohol dependency and a polymorphism in the dopamine receptor, with people who had the polymorphism 8.7 times more prone to be dependent on alcohol. Not only was the odds ratio high, Baccarelli explained, but so were the confidence intervals. Subsequent studies provided a different perspective (Smith et al., 2008). Over time, studies became larger, confidence intervals shrank, and the odds ratios being reported suggested that the increased risk associated with the polymorphism was actually only 40 percent higher. That odds ratio, Baccarelli said, is not nearly as high as 70 percent. There are other examples, he said, of odds ratios initially being reported as high as 9 but, over time, being reported as zero. He wondered whether the same phenomena, known as "the winner's curse," might be happening now with epigenetics. Are larger effects being reported that will become more moderate over time?

The winner's curse is similar to what happens on an Internet auction site, Baccarelli said, when an item is first auctioned and people overbid because they do not know the true value of the item. Initially, there is a high variance in the estimated dollar value of the item, and the "winner" is likely to be "cursed" because he or she has bid the most. In genetics, the first to publish positive GWAS findings often overbid, or overestimate, the effects. Again, he asked, is this happening now with EWAS findings? If so, how can it be avoided?

Additional Questions to Consider

In conclusion, Baccarelli observed that most NIH funding for epigenetic research is focused on DNA methylation, with less funding directed toward research on microRNA and histone modification (Burris and Baccarelli, 2014). He wondered whether opportunities exist to study other epigenetic mechanisms and what should be done to create those opportunities.

In terms of new research directions, he asked, which are the most promising? Which mechanisms are less explored? Is looking at the effect of the environment on the epigenome enough? Drawing another parallel between genetics and epigenetics, Baccarelli pointed out that most researchers are focusing on how the environment modifies the epigenome and how the epigenome, in turn, modifies disease risk. In genetics, researchers also examine how the environment modifies, in its case, the genome, for example, how carcinogens damage DNA. But geneticists have also been thinking a lot about how the genome makes individuals more or less susceptible to environmental influences via gene-by-environment interactions. Baccarelli

suggested that permanent epigenomic markers (i.e., markers "drawn in ink") might similarly be modifying the effect of environmental influences on phenotype (Bollati and Baccarelli, 2010).

Most importantly, in Baccarelli's opinion, is the need to consider the meaning of the research conducted. Why are researchers studying this? Are they looking for mechanisms or biomarkers? What are the benefits to patients and to society?

PANEL DISCUSSION WITH SPEAKERS

In the panel discussion following Baccarelli's presentation, workshop participants considered a range of topics: the tissue specificity of epigenetic markers and changes, nutritional exposure to dietary methyl donors, the impact of assisted reproductive technologies on epigenetic patterning, and the temporary nature of many epigenetic markers. This summary of the discussion is organized by topic.

Tissue Specificity

Kevin Grove of the Oregon National Primate Research Center asked how common it is for epigenetic phenomena to be tissue- or cell type–specific. He wondered whether heterogeneous epigenetic markers in different liver cells, for example, might contribute to the heterogeneity in observed responses. Robert Waterland responded that, yes, at least with DNA methylation, there are dramatic differences between different tissues and cell types. Regarding the liver, he said that he was unaware of the extent to which researchers have examined the heterogeneity of epigenetic regulation across different hepatocyte populations, but he suspected differences. While studying the hypothalamus in mice, he and his colleagues have observed dramatic differences between neurons and glia in epigenetic changes between birth and weaning (at day 21). Specifically, they have observed much greater increases in methylation in neurons compared to glia. He said that if they were to examine different classes of neurons in the hypothalamus, they would probably see dramatic differences there as well.

Baccarelli added that when measuring methylation in a tissue, researchers measured the proportion of cells that are methylated. When they measure it that way, there is a lot of variation, he said, between subjects.

Nutritional Exposure: Dietary Methyl Donors

An audience member asked Waterland which dietary methyl donors he and his colleagues have used in their research. Waterland replied that in the *Avy* agouti mice studies and in subsequent studies with related models, they

used what he referred to as a "methyl donor cocktail." They supplemented the mouse diet with extra folic acid, vitamin B_{12}, betaine, and choline. In his opinion, a combination of nutrients is required. They have unpublished data suggesting that folic acid supplementation alone does not produce the same pro-methylation effects in the offspring. He referred the questioner to a recent paper that he worked on in collaboration with Andrew Prentice showing that maternal nutrition status at around the time of conception affects DNA methylation at human metastable epialleles in offspring (Dominguez-Salas et al., 2014).

Assisted Reproductive Technologies and Epigenetics

When asked about assisted reproductive technologies and the risk of epigenetic dysregulation associated with it, Waterland replied, "The issue of assisted reproductive technologies is clearly very important. I find it just amazing that there seems to be more regulation about what goes into our breakfast cereal than what is the specific composition of the media that are used for these early embryos during the in vitro fertilization process." Early studies suggested that individuals conceived via assisted reproductive technologies run a higher risk of certain developmental diseases. However, according to Waterland, it is still unclear whether those diseases result from the process itself or from epigenetic aberrations that existed in either the sperm or the egg and that contributed to the infertility in the first place.

Reversible Epigenetic Markers (or Markers "Made in Pencil")

Grove asked if the reversibility of (reversible) epigenetic markers was due to cell turnover. He pointed out that there is a lot more cell turnover in the liver or skin, for example, than in the brain. Waterland responded, "Absolutely." He explained that there are two potential ways to reverse DNA methylation markers. One is active demethylation, which occurs via a ten-eleven translocation (TET) methylcytosine-mediated process, and the other is failure to maintain established methylation patterns during mitosis, which leads to erasure.

Waterland reiterated that metastable epialleles, which are established during early development, persist into later life. He and his team have data from mouse studies showing that measures of methylation and inter-individual variation in methylation obtained early in life are exactly the same as those obtained later in life. The same has been observed in humans, with methylation in one region measured at age 7 being predictive of what will be observed at age 17.

Related to the issue of reversibility, Caroline Relton of Newcastle University asked whether any epigenetic markers made early in life as a result

of early life exposure disappear later in life. She wondered whether if so, those transient markers may nonetheless set in motion a physiological chain of events that influence risk of obesity or other chronic disease. Moderator Matthew Gillman closed the session by suggesting that Relton's question be kept in mind for the remainder of the workshop. As he rephrased it, "Even if DNA methylation or other epigenetic processes are transient, could they set in motion a programmatic phenomenon?"

3

Etiology and Causal Inference

OVERVIEW

Following Robert Waterland's and Andrea Baccarelli's conceptual overview, workshop participants in Session 2, moderated by Karen Lillycrop of the University of Southampton, considered how the risk of childhood obesity can be affected by (1) maternal and paternal nutrition and other exposures before conception, (2) maternal and placental nutrition and health during pregnancy, and (3) postnatal maternal and infant nutrition and health. This chapter summarizes the Session 2 presentations and discussion.

"Obesity begets obesity," began Jacob Friedman of the University of Colorado, Denver. Friedman discussed animal and human data demonstrating that both prenatal and postnatal exposure to maternal obesity predispose infants to early-onset metabolic disease and childhood obesity. For example, studies conducted on obese pregnant mothers and their newborn infants indicate that pre-pregnancy body mass index (BMI) of the mothers, but not infant fatness, is predictive of higher liver fat at 2 weeks of age, which continues to increase during lactation. Friedman explained that excess maternal fuels in obese mothers crossing the placenta have nowhere to go but into the fetal liver, and suggested that this fatty liver transgenerational effect of maternal obesity may be accompanied by epigenetic changes in offspring liver and other tissues at 1 year of age. Postnatally, evidence from breastfeeding mothers indicates that maternal diet and obesity may also affect immune function and by different mechanisms have an impact on infant behavior, weight gain, and obesity risk.

Linda Adair of the University of North Carolina described inequities among different socioeconomic groups that cut across a range of obesity-related prenatal and postnatal exposures and outcomes. Examples of significant prenatal disparities among different socioeconomic groups include differences in parental overweight and gestational weight gain. Examples of significant postnatal disparities include differences in overweight and obesity in children under the age of 5. Adair noted that the fastest-growing rates of childhood obesity worldwide are in low-income groups. Adair also introduced discussion of the "mismatch hypothesis," that is, the notion that the risk of childhood obesity appears to be greatest among undernourished fetuses. The mismatch hypothesis was revisited several times later in the workshop.

While most of the workshop presentations and discussion focused on maternal exposure and its effect on the risk of childhood obesity, Stephen Krawetz of the Wayne State University School of Medicine shifted the focus to, in his words, what "Dad delivers." He provided an overview of the role of sperm in early development; described the many different types of RNA molecules that sperm deliver to the oocyte, including several that have been implicated as having an early developmental role in obesity; and discussed evidence of epigenetic-mediated transgenerational inheritance through the paternal line.

Caroline Relton of Newcastle University emphasized that both over-nutrition and under-nutrition during pregnancy can impact childhood adiposity. In her opinion, the evidence is compelling. The question for her is: What is the role of epigenetics? Relton stated that while identifying associations between methylation patterns and phenotypes has become straightforward, inferring causality remains a challenge. Revisiting and expanding on ideas introduced by Andrea Baccarelli in the previous section, Relton laid out the steps necessary to infer causality, gave numerous examples, and considered ways to improve those steps. Among other suggestions, she called for more refined measures of maternal obesity, an increased awareness of the pitfalls of association studies, and the use of triangulation among multiple studies to infer causality.

EPIGENETIC MECHANISMS FOR OBESITY RISK[1]

Maternal obesity in the United States has reached a point that compels Jacob Friedman to ask, "How could this not impact infant outcomes?" An estimated 60 percent of women between the ages of 20 and 39 years are overweight or obese (BMI > 25), with 33 percent being obese (BMI > 30)

[1] This section summarizes information and opinions presented by Jacob Friedman, Ph.D., University of Colorado, Denver.

and about 8.5 percent severely obese (BMI > 40) (Ogden et al., 2014). By race and ethnicity, almost 80 percent of Hispanic and black women in that age range are overweight or obese. There are known associations between maternal obesity and early-onset obesity, metabolic syndrome, and fatty liver and cardiovascular disease in children (Brumbaugh et al., 2013; Lawlor et al., 2011; Smith et al., 2009). Epidemiological data suggest that the maternal obesity effect on the offspring is not confined to neonatal life, affecting offspring across the life span, independent of lifestyle factors (Pirkola et al., 2010). However, surprisingly little is known about how maternal obesity may influence obesity risk in the human neonate.

Both animal and human studies have shown signs of aberrant methylation patterns—as well as mitochondrial dysfunction—in children born to mothers who are obese (Borengasser et al., 2013). Researchers have reported methylation changes in cord blood or in the placenta associated not just with intrauterine exposure to maternal obesity, but to maternal diabetes and famine as well (El Hajj et al., 2014). In Friedman's opinion, the epigenetic changes associated with intrauterine exposure to maternal obesity are probably tissue-specific, although it is not clear to what extent. The greater question for him is whether the epigenetic associations are causal. Regardless of causality, in a 2011 editorial based on a study showing that the methylation status of specific genes in human cord blood predicted subsequent development of childhood obesity, Friedman and his coauthor considered whether epigenetic analysis at birth may be useful for identifying future risk of obesity (Choudhury and Friedman, 2011).

It is not "just mom" that is affecting the infant epigenome, Friedman said. The placenta matters as well. The placental transcriptome can be highly different between lean and obese patients, with many inflammatory pathway genes being turned on early in the placenta in mothers who are obese (Basu et al., 2011). Fetuses derive their signals from the placenta, with their epigenome being informed by whatever crosses the placental barrier and with outcomes ultimately playing out in the infant metabolome, epigenome, and proteome.

Friedman's research group has focused many of its efforts on what obese women are eating when they become pregnant and how their diet affects fuel production, adipose tissue mass, and inflammation in the neonate. Using magnetic resonance imaging (MRI) data, he and his team are able to examine where the fat is accumulating in the infant. Additionally, by harvesting umbilical cord-derived mesenchymal stem cells from newborn infants exposed to maternal obesity, they are also able to detect epigenetic signatures associated with maternal obesity. Mesenchymal-derived stem cells (MSCs), Friedman explained, are a population of stem cells in the umbilical cord that can be programmed to develop into either myocytes or adipocytes, depending on what the stem cells are exposed to (Gang et al.,

2004; Janderová et al., 2003). MSC differentiation to either adipocyte or myocyte can be important for proper tissue development in utero, in particular because there is a large window where adipogenesis and myogenesis overlap during fetal development.

Kristen Boyle, a member of Friedman's research team, has shown that obese women have higher pre-pregnancy BMI, higher homeostasis model assessment-estimated insulin resistance (HOMA-IR) indexes, and higher lipid levels. Because all of those features affect the placenta, Friedman said, his research team has hypothesized that infants born to obese mothers are predisposed to early-onset metabolic disease due to "metabolic programming" events before birth resulting from fuel overload, impaired mitochondrial energy metabolism pathways (i.e., fatty acid oxidation and amino acid metabolism), and, ultimately, changes in the epigenome. Boyle and colleagues (data presented at the American Diabetes Association meeting, 2015) showed that epigenetic signatures associated with myocyte versus adipocyte differentiation are affected by maternal BMI, with suppressed expression of both epigenetic regulators, *DNMT1* and *KDM6A*, in the mesenchymal stem cells of infants born to obese women. Boyle and colleagues also showed that the stem cells from infants born to obese mothers had an increased adipogenic potential that correlated with percent fat of the infant. The pathways or mechanisms responsible for less lean mass and greater fat mass established during gestation are not well understood. Maternal diet and obesity impact fuels, hormones, and inflammation with powerful effects on fetal metabolic systems. Delving deeper into the mechanisms and molecules that differ in these cells on the basis of poor maternal health, including the potential epigenetic regulation of these differences, is an important area of future investigation and may hold the key to understanding how nutritional programming can lead to a susceptibility to obesity in adult life.

Metabolic Programming in the Fetus: Is It a Matter of Fat?

Friedman has been collaborating with Kevin Grove at the Oregon National Primate Research Center to develop a nonhuman primate model to study the effects of maternal diet, maternal obesity, and gestational diabetes mellitus on the development of metabolic systems in utero and on infant behavior and postnatal disease pathways.

A key finding from these studies is that the livers of fetuses from mothers fed a high-fat diet prior to conception are, Friedman said, "chock full of lipids" (McCurdy et al., 2009). The accumulation of lipids in the liver is not benign, Friedman explained. The livers are actually in a state of oxidative stress. The McCurdy et al. (2009) researchers fed a control chow diet to one pod of animals and a high-fat diet to the other pod, mated the animals, then performed a C-section and assayed the fetal livers. Because

they were also curious about how a change in diet would impact lipid accumulation in the liver, after the C-section they put the animals who had been on a high-fat diet on a healthy diet and conducted a second assay of fetal livers from subsequent pregnancies. In the second assay, they observed a reduction, but not a total reversal, of liver triglycerides. Friedman remarked that while the liver is one source of pathology related to a high-fat diet, in fact dietary exposure is affecting every single organ in the animal, including the brain.

To see if the same phenomenon occurs in humans, Friedman and his collaborators collected MRI data on infants born to mothers with gestational diabetes and estimated the amount of fat in the liver (i.e., using the ratio of water to fat in the liver). In a study of 13 infants born to mothers with normal weight and 12 infants born to mothers who were obese, Brumbaugh et al. (2013) found that hepatic lipids in infants born to obese mothers with gestational diabetes were 72 percent higher than in those born to normal-weight mothers with gestational diabetes.

The surprising finding in Brumbaugh et al. (2013), in Friedman's opinion, was that it was maternal pre-pregnancy BMI, not infant fat mass, that predicted liver fat mass. Unlike adults, who if developing a fatty liver have a lot of central obesity, infants are not born with central obesity and have very little visceral fat. Friedman interpreted this finding to mean that fuels in a mother who is obese have nowhere to go but into the fetal liver and that it is her obesity, not the infant's, that determines whether a child will be born with a fatty liver. Friedman observed that there is a high risk of fatty liver disease in children who are obese, with probably about 55 percent of children who are obese having fatty liver at the "first hit," that is, because of genetics, perhaps epigenetics, and in utero exposures (Anstee and Day, 2013). The "second hit," that is, when the hepatic lipid accumulation develops into a more severe form of nonalcoholic fatty liver disease (NAFLD), results from oxidative stress, hepatocyte injury, and inflammation (Vos et al., 2013). The second hit, Friedman explained, probably does not occur in utero.

The question for Friedman is, are the effects of maternal obesity on fat mass in offspring liver reversible? In a study with macaques, again using animals that were fed either a control diet or a high-fat diet, Friedman and his research team weaned offspring of mothers on a high-fat diet on to a healthy diet at 7 months. Then, at 1 year of age, they assayed the offspring livers. They found that juvenile livers from animals born to mothers on a high-fat diet but weaned on to a healthy diet had elevated de novo hepatic lipogenesis gene activation (Thorn et al., 2014). More importantly, when they looked more closely at who the mothers were, they found elevated activation of the liver lipid pathways only in offspring born to mothers on high-fat diets who were very insulin resistant.

On a molecular level, Friedman explained, the fuels from the high-fat diet are crossing the placenta and probably overwhelming the mitochondria, creating oxidative stress and a loss of control of the genes for de novo lipogenesis and triggering inflammation pathways that set the stage for high-risk NAFLD in obese teens and young adults.

Postnatal Influences of Maternal Diet and Breast Milk on the Infant Microbiome

It is widely known among scientists in the field that people who are obese have what is called a dysbiotic microbiome compared to people who are lean. Friedman asked, what does that mean? How does it occur? And when would it occur in an infant? The neonatal period immediately after birth is critical for programming the immune system. During breastfeeding, infants are exposed to novel nutrients, bioactive molecules, and bacteria; their brain neurocircuitry for gut-brain energy sensing systems is being established, and the gut and liver immune cells are receiving instruction from the diet in early-life aspects of immune protection (i.e., what things can get in and what things should be kept out). Part of this early programming has been shown to take place at the level of epigenetics, according to Friedman, and every aspect of it can be influenced by breast milk composition. If a mother is on a Western diet and has a dysbiotic microbiome, and if her infant is breastfeeding on that diet, many of the microbial products "setting up shop down in the infant gut" can readily cross into the liver because the gut is quite leaky in early development. As a result, what starts as simple steatosis can progress into the more severe fatty liver disease seen in obese adolescents, perhaps driven by early programming events in the infant immune system. While breastfeeding is generally associated with protection against rapid infant weight gain and later obesity, the mechanisms responsible are not known but likely involve the delivery of bioactive components that regulate infant appetite, metabolism, and weight gain and adiposity.

In what Friedman referred to as a "remarkable" paper, Koren et al. (2012) reported that when the normal microbiome of a woman in her first trimester of pregnancy is transplanted into a gnotobiotic mouse (i.e., a mouse with no microbiome), the mouse develops normally. But if the altered microbiota of a woman in her third trimester of pregnancy is transplanted into a gnotobiotic mouse, the mouse becomes fat and develops insulin desensitization. These findings raised a concern for Friedman, that is, what happens to their infants when women enter pregnancy with an already disordered microbiome?

As an example of what happens in primates, again with animals born to mothers fed a high-fat diet but then switched to a healthy diet at wean-

ing, Ma et al. (2014) reported that maternal diet during pregnancy altered the offspring microbiome such that, even when offspring were switched to a healthier diet, they retained the microbiome they received from their mothers one year later.

Friedman and his team are currently testing whether maternal obesity in humans directly affects the development of the infant microbiome and is associated with increased adiposity during the first 4 months of life. Specifically, they are evaluating four maternal phenotypes in the perinatal and gestational period: normal weight, overweight or obese, type 2 diabetes, and gestational diabetes. All of the infants are being delivered vaginally, with none of the mothers on antibiotics and all of them agreeing to breast-feed for at least the first 4 months. The researchers are assaying both the maternal and infant microbiomes and evaluating their associations with infant adiposity.

With respect to the relationship between breast milk from obese mothers and offspring inflammation, Friedman's colleague Bridget Young has shown that while the amount of triglycerides in breast milk is not significantly different at 2 weeks between obese and normal-weight mothers, there is a striking increase in levels of leptin, insulin, and the pro-inflammatory fatty acids (i.e., the n-6/n-3 fatty acid ratio). Moreover, leptin and insulin levels and n-6/n-3 fatty acid ratios, in turn, are associated with the composition of the infant microbiome. Friedman interpreted these findings to mean that exposure to maternal diet through the breast milk appears to be patterning the infant microbiome and, therefore, might be either protecting the infant gut or making infants more prone to inflammation and weight gain, but that is not yet known.

Final Thoughts

To conclude, Friedman shared some final thoughts:

- Humans share a core microbiome, yet they differ by genes, species, ecology, and gene count or richness.
- The gut microbiome is dynamic, yet its timescales are largely unknown.
- While changes in diet can lead to short-term changes in the microbiome, it is not clear which of those changes are reversible. In the nonhuman primate model studied by Friedman and his colleagues, some of the changes are reversible, but most are not.
- While microbiome gene richness is a key stratifier for response to dietary intervention, causing obesity in mice, the mice revert back, so the response is not permanent.

- Some microbiome-derived metabolites can have a positive effect on anti-inflammatory activity or energy harvest, while others are toxic to the host.
- A key question for Friedman is, can specific species or patterns of the gut microbiome be identified that might be relevant as targets for obesity?

THE ROLE OF DISPARITY IN THE ORIGINS OF OBESITY RISK[2]

Linda Adair defined disparity as a great difference or a lack of equality. Part of the challenge to understanding childhood obesity is the substantial variation in prevalence of obesity among children of different ages and races or ethnicities (Ogden et al., 2014) (see Figure 3-1). Using data from a very large database across the United States, Ogden et al. (2014) reported variation in obesity prevalence even in the 11-month age range. In children between 2 and 5 years of age, the highest obesity rates are in American Indians and Alaskan Natives, and the lowest rates are in non-Hispanic whites.

The wide variation observed in the United States is being observed globally as well, according to Adair, with the Department of Health and Human Services data from 26 different low- and middle-income countries showing a range of weight-for-length/height Z-scores from under 2.0 to over 14.0, with most countries having Z-scores greater than 2.0.

Disparities with Implications for Child Obesity

Adair identified several types of disparities with important implications for child obesity, not the least of which are disparities in resources, particularly health-promoting resources, with a wide range of socioeconomic factors (e.g., wealth, income, education, social status) creating disparities in both nutritional exposures (i.e., food availability, food security, and diet quality) and physical activity opportunities.

Also having important implications for child obesity are the many pronounced disparities in exposure, particularly pathogenic exposures related to poor water quality, sanitation and hygiene issues, and close living quarters. Often, Adair observed, individuals who are the most disadvantaged are also more likely to be exposed to pesticides and toxic metals. Additionally, Adair mentioned disparities in stress and social support arising from financial challenges, variation in physical environment, emotional factors, and different types of life events.

[2] This section summarizes information presented by Linda Adair, Ph.D., University of North Carolina at Chapel Hill.

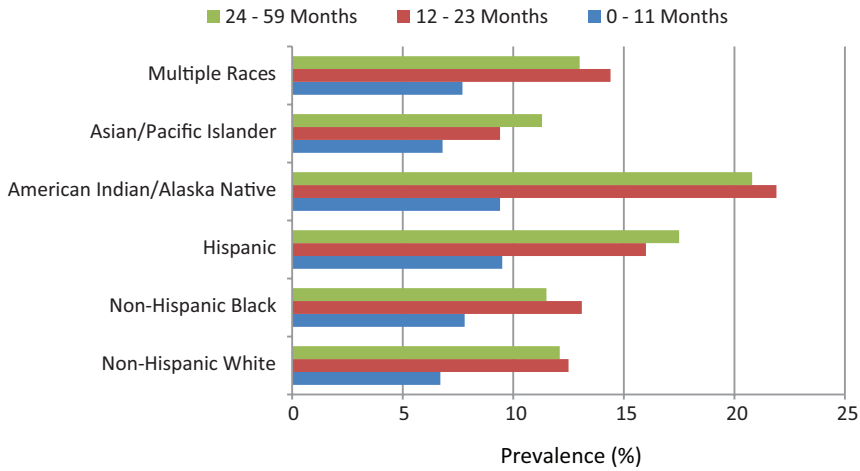

FIGURE 3-1 Prevalence of obesity, by age and race/ethnicity. Variations in the prevalence of childhood obesity in the United States among different races and ethnicities.
SOURCE: Presented by Linda Adair on February 26, 2015; modified from Ogden et al., 2012.

In terms of which of these aspects of disparity the fetus or young infant actually perceives, nutritional exposures are arguably one of the most important factors to consider. Disparities in nutritional exposure are reflected in maternal stores, that is, how fat he mother is, or, in the case of limited resources, how thin she is; maternal dietary intake of specific macro- and micronutrients associated with child growth (e.g., B vitamins, methyl donors); and several aspects of maternal metabolism, such as gestational diabetes. Adair stated that all of these various manifestations of disparities in nutritional exposure are highly critical in the first 1,000 days (beginning at conception).

In addition to variations in nutritional exposure, variation in maternal toxic exposures are also perceived in some manner by the young infant and developing fetus. For example, smoking (tobacco) is well known to be a very important factor that is differentially distributed across race and ethnic groups. Young infants and developing fetuses are also affected by maternal exposure to toxic metals, Adair observed. She did not elaborate, but she mentioned that she has been doing some work in South Africa with populations living close to mining communities and being exposed to high levels of arsenic and lead. She remarked that there has been a lot of recent concern about exposure to endocrine disruptors, which, like smoking and heavy metals, are differentially distributed according to socioeconomic status. According to Adair, researchers have also been reporting differential

exposures to growth and metabolic hormones, such as insulin and leptin, and to stress hormones, such as cortisol, again with the exposures ultimately translating into something that the fetus perceives.

Maternal Nutrition and Risk for Child Obesity

Adair discussed how both maternal under-nutrition and maternal excess nutrition have implications for child obesity and noted that both have been shown to be highly disparate among different socioeconomic groups or races and ethnicities.

First, with respect to maternal under-nutrition, underweight (BMI < 18.5 kg/m^2) and micronutrient deficiencies are more prevalent in low- and middle-income countries and in lower socioeconomic groups in high-income countries. Low pre-pregnancy BMI has, in turn, been associated with increased risk of low birth weight, small for gestational age, and preterm birth (Dean et al., 2014; Yu et al., 2013b). Micronutrient deficiencies—for example, iodine, zinc, and iron deficiencies—have been shown to increase risks of low birth weight, small for gestational age, and preterm birth (Ramakrishnan et al., 2012). More generally, Adair explained, when a mother is unable to supply nutrients to meet fetal demand, a cascade of metabolic events ensues involving the kidneys, liver, pancreas, bone and muscle tissue, the brain, and the hypothalamic-pituitary-adrenal (HPA) axis, with important implications for the development of obesity, particularly central obesity (Fall, 2011).

With respect to maternal excess nutrition, overweight (BMI > 25 kg/m^2), excess gestational weight gain, and dietary excesses have all been associated with increased risk of large-for-gestational-age deliveries and infant macrosomia (Siega-Riz et al. 2009).

In terms of what these maternal pre-pregnancy weight status disparities look like, Adair cited 2010 U.S. Pregnancy and Perinatal Surveillance System (PPNSS) data showing substantial race and ethnicity disparities in both underweight and overweight and obesity. As shown in Figure 3-2, the highest rates of underweight are in Asian and Pacific Islanders. While Asian and Pacific Islanders have the lowest rate of overweight and obesity, at about 30 percent, close to 60 percent of American Indians/Alaska Natives and non-Hispanic blacks show a maternal pre-pregnancy BMI greater than 25. Pregnancy weight gain data from the same database show similar race and ethnicity disparities (see Figure 3-3). Adair remarked that a large literature suggests that it is not so much race and ethnicity that vary, but rather the underlying social disparities (i.e., in wealth, education, etc.) that race and ethnicity represent.

In addition to disparities in maternal under- and over-nutrition, PPNSS data also show racial and ethnic disparities in maternal anemia, pregnancy-

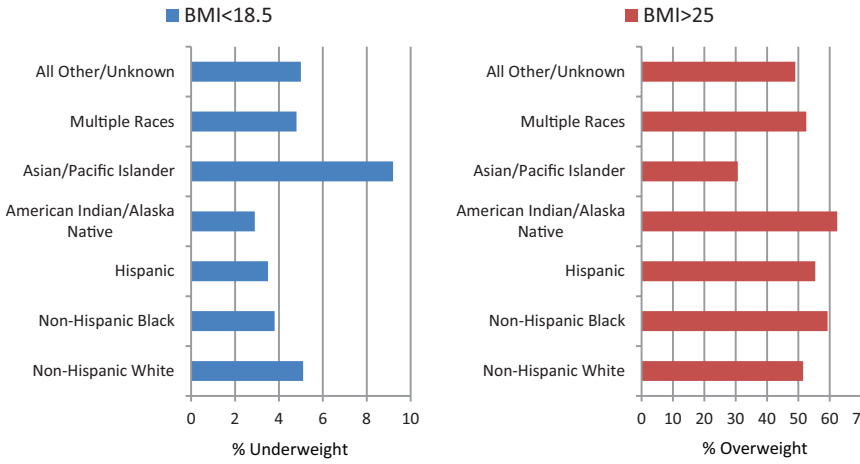

FIGURE 3-2 U.S. disparities in pre-pregnancy weight status among different races and ethnicities. Percentages of underweight (x-axis) by race/ethnicity (y-axis) are shown in the left panel, and percentages of overweight (x-axis) by race/ethnicity (y-axis) are shown in the right panel.
SOURCE: Presented by Linda Adair on February 26, 2015; modified from U.S. Pregnancy and Perinatal Surveillance Data (www.cdc.gov/pednss).

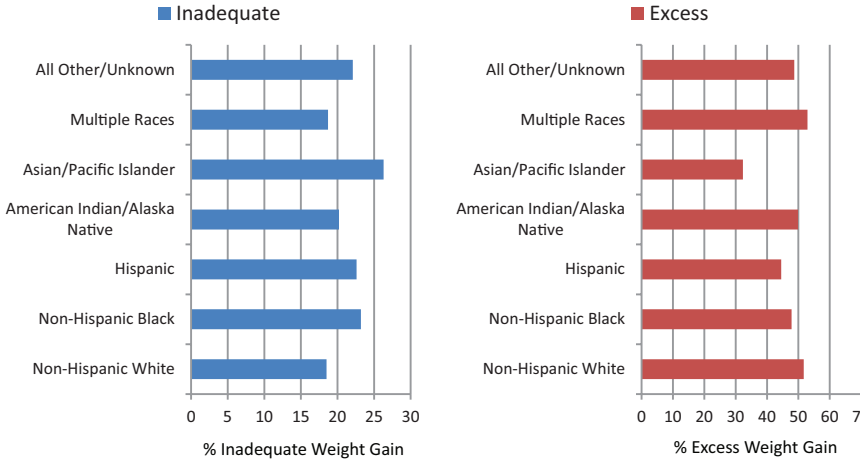

FIGURE 3-3 U.S. disparities in inadequate and excess weight gain during pregnancy among different races and ethnicities. Percentages of inadequate pregnancy weight gain (x-axis) by race/ethnicity (y-axis) are shown in the left panel, and percentages of excess pregnancy weight gain (x-axis) by race/ethnicity (y-axis) are shown in the right panel.
SOURCE: Presented by Linda Adair on February 26, 2015; modified from U.S. Pregnancy and Perinatal Surveillance Data (www.cdc.gov/pednss).

induced hypertension (PIH), and gestational diabetes, all with important implications for child obesity risk.

Globally, Black et al. (2013) reported that underweight has been declining, while overweight and obesity among women of childbearing age has increased fairly dramatically, with the Americas and the Caribbean, as well as Oceania, showing the largest increases worldwide.

Variation in Infant Outcomes

Data from PPNSS 2010 indicate that adverse birth outcomes in the United States, including preterm births, macrosomia, and low birth weight, are highly disparate across different races and ethnicities, with preterm births and low birth weight being highest in non-Hispanic black populations. Additionally, data from the U.S. Early Childhood Longitudinal Study Birth Cohort (2001–2007) showed dramatic differences in patterns of postnatal growth by race and ethnicity (Jones-Smith et al., 2014). Generally, according to Adair, the odds of being overweight or obese diverge among the different races or ethnicities at about 9 months of age. "So it's happening in infancy," she said, with breastfeeding and patterns of infant feeding having an effect and creating a divergence in obesity risk among race/ethnicity early in life.

With respect to breastfeeding, Adair referred to Jacob Friedman's remarks about the quality and quantity of breast milk being critical for infant development. That there are differences in the composition of breast milk based on maternal weight status is of concern in her opinion. Estimates of the prevalence of breastfeeding among different races and ethnicities vary depending on the source of the data. For example, PPNSS data from 2010 show less disparity in initiated breastfeeding than shown by the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) data from the Urban Institute. While the PPNSS data show that more than 60 percent of both non-Hispanic blacks and non-Hispanic whites initiate breastfeeding, the WIC data show that only a little over half of low-income non-Hispanic white women and only about 42 percent of low-income African American mothers initiate breastfeeding.

Researchers have reported substantial variations in infant and toddler feeding as well, including, in Adair's opinion, multiple aspects of infant and toddler feeding reflecting disparities in race, ethnicity, weight status, income, education, and age. Variation has been reported in initiation and duration of breastfeeding, timing of the introduction of complementary foods, and types of complementary foods fed to infants (e.g., fruits and vegetables, sweetened beverages, salty snacks) (Hendricks et al., 2006).

Global variation in infant outcomes is reflected in the very high rates of small for gestational age and low birth weight infants in South Asia,

whereas in sub-Saharan Africa the greater problem is large-for-gestational-age infants, or infant macrosomia (Black et al., 2013; Koyanagi et al., 2013). Macrosomia also occurs more frequently in China and Latin America. Additionally, Black et al. (2013) reported worldwide socioeconomic disparities in the prevalence of stunting, which, Adair noted, is still highly prevalent worldwide, and tracks with overweight and obesity in children under the age of 5 years. Specifically, they showed that rates of stunting are very different in low-income versus higher-income countries. While their reported disparities in obesity are not quite as great as they are for stunting, Adair believes, that trend is changing quite dramatically. In high-income countries, obesity prevalence is typically higher in disadvantaged groups. Historically, that has not been the case in low- and middle-income countries. It used to be that in low- and middle-income countries, obesity was found mainly in the upper socioeconomic groups. But today, even in low- and middle-income countries, the fastest rates of increase in both child and adult obesity are in lower-income groups (Jones-Smith et al., 2012). Adair emphasized the importance of this trend in the context of disparities because it is those low-income groups who are more exposed to all of the environmental risk factors she mentioned previously (e.g., low-quality diets, toxic substances).

Obesity Disparities and Epigenetic Mechanisms

According to Adair, there is evidence of an epigenetic association with almost every disparity with obesity implications. For example, disparities in maternal glycemia during pregnancy have been associated with altered methylation profiles of adiponectin genes in the placenta (Bouchard et al., 2012). Disparities in parental overweight, including both maternal and paternal overweight, have been associated with hypomethylation of *IGF2* (Soubry et al., 2013). Disparities in maternal diet, including carbohydrate intake, famine exposure, and vitamin and mineral uptake, have been associated with altered methylation patterns (Godfrey et al., 2011; McKay et al., 2012; Tobi et al., 2009). Although Adair did not go into detail, she said the same is true of stress (Brockie et al., 2013), environmental exposures such as arsenic (Marsit, 2015), maternal depression (Oberlander et al., 2008), and breastfeeding (Verduci et al., 2014), all of which differ substantially according to socioeconomic status, race and ethnicity, and geography. Evidence suggesting that all of these disparities are influenced by mechanisms associated with epigenetics provides a way, in Adair's opinion, to begin to understand the biology of how disparities translate into obesity risk for the child.

The Mismatch Hypothesis

Many of the developmental phenomena being discussed at the workshop can be viewed, in Adair's opinion, as adaptations that enhance survival in the environment to which an organism is exposed. It has been hypothesized that a mother provides information about her environment to the developing fetus and that the fetus, in response, develops a set of adaptations to cope with that environment (Bateson et al., 2014). The adaptations are also hypothesized to be anticipatory, that is, they are well suited to the environment that an individual will experience in the future as well. However, if the environment that is experienced postnatally is not what was predicted prenatally, then an organism will be maladapted and at an increased disease risk. For example, poor maternal diet, inadequate nutrient stores, or placental factors that limit transmission of nutrients to the developing fetus can all lead to fetal nutritional insufficiency and the metabolic differences that characterize under-nutrition (e.g., altered cell numbers, altered regulatory processes, altered epigenetics). Postnatally, if exposed to dietary excesses or sedentary behaviors, an individual that is programmed for under-nutrition may be maladapted, hyperresponsive, and at increased risk of obesity and chronic disease.

Adair pointed to the current situation in India to illustrate this notion of mismatch. India is considered a classic case of mismatch where young undernourished infants are growing up in an environment with nutritional excesses and with offspring having deficits in lean body mass but not body fat (Yajnik, 2004). Compared to a Caucasian baby that weighs an average of 3,500 grams, with 10 percent fat stores and 20 percent muscle mass, an Indian baby of average birth weight, which is 2,700 grams, has 20 percent fat stores and 10 percent muscle mass (Yajnik, 2004). According to Adair, Yajnik calls this the "thin fat Indian baby," that is, a baby who in utero developed a limited muscle mass and more adipose tissue and was well adapted to its maternal environment of constraint but who is at increased risk of obesity later in life.

According to data from the National Family Health Survey (NFHS), the World Health Organization (WHO), and UNICEF, conditions in India 30 years ago—that is, when today's mothers were being born—were such that low birth weight was common (30 percent), child stunting was highly prevalent (47 percent), and mortality under the age of 5 years was high (118/1,000). Contrast that to today's dramatic increase in maternal overweight and obesity among Indian women, with a 25 percent central obesity rate in women and 11 to 12 percent obesity in New Delhi among 14- to 17-year-olds (Garg et al., 2010). There have also been reports of increased rates of diabetes (Anjana et al., 2011).

Most studies of mismatch have focused on longer-term effects, Adair

said, with less focus on whether mismatch might help to explain short-term rapid infant weight gain. Adair mentioned having observed lower birth weights in infants born to first-time mothers, but with firstborns who are well fed experiencing rapid postnatal weight gain. That, in a way, Adair said, is mismatch. The infants experienced prenatal constraints and were prepared for under-nutrition, but experienced over-nutrition instead. She pointed to data from the Consortium of Health Orientated Research in Transitioning Societies (COHORTS), a collaborative among investigators conducting birth cohort studies in five low- and middle-income countries worldwide (Brazil, Guatemala, India, the Philippines, and South Africa). The data indicate that while low birth weight is associated with reduced risk of overweight at the age of 2 to 3 years, that association is modified in firstborn infants. Firstborn low birth weight infants gained more weight than higher-order low birth weight infants and were twice as likely as low birth weight infants of higher birth order to be overweight at 2 years of age. Again, that is mismatch, Adair said, with firstborns who were undernourished in utero experiencing very rapid postnatal growth.

Adair mentioned other studies of low birth weight or small-for-gestational-age infants who were deliberately being fed to catch up, with data indicating an increased risk of obesity associated with the catch-up growth. That situation may also represent mismatch.

Summary

In her summary, Adair made five key points: (1) wide disparities in socioeconomic status, physical environment, psychosocial factors, and stress contribute to substantial differences in fetal exposure to nutrients, toxins, hormones, and other regulatory substances; (2) these disparities may affect fetal and infant growth and susceptibility to later obesogenic factors through epigenetic and other pathways; (3) elevated risk of child obesity may result from prenatal under-nutrition as well as from nutritional excesses; (4) the risk may be greatest when the fetus is adapted to a maternal environment that differs from the environment faced as an infant and young child; and (5) understanding the exact nature of pathways of risk may lead to interventions to eliminate the adverse effects of health disparities.

THE FATHER'S EARLY CONTRIBUTION TO THE BIRTH OF THE CHILD: THE ROLE OF PATERNAL RNAs[3]

Stephen Krawetz of the Wayne State University School of Medicine expressed surprise that no one had considered Dad as an initial driver, given that the paternal genome and, in fact, everything carried in the sperm as well as a father's past can impact early development of a child.

Spermatogenesis

In order to understand the paternal contribution to childhood early development, Krawetz suggested starting with spermatogenesis (Wykes et al., 1995, 1997). Spermatogenesis, he said, is unlike any other system in the body. From puberty onward, it is continually replenished from the stem cells toward the lumen tubule through a series of developmental stages to eventually yield mature spermatozoa. As spermatozoa differentiate, each forms a little bag of cytoplasm, known as the residual body or cytoplasmic droplet, which Krawetz said is the sperm cell's way of getting rid of excess cytoplasm. Each mature spermatozoa is about 2 microns in diameter and, because of its large tail, up to 20 to 40 microns in length. The long tail is needed for motion and is what allows a sperm cell to reach the egg. It takes about 2 hours for the fastest sperm swimmer to make it to the egg. When the sperm reaches the egg, the entire sperm is taken in upon fertilization, including all components except the cytoplasmic droplet, followed by a rapid dissociation and integration.

The sperm genome is about 13 times more compact than the oocyte's, even though it contains the exact same amount of information. Its compactness is caused by a unique set of proteins called the protamines, which are autosomal but expressed only in men and without which a man is infertile. Using radiolabeled antisense imaging, Krawetz and colleagues have shown that not all spermatozoa are equal in terms of the amount of labeled protamines mRNAs present (Wykes et al., 1997). However, it is still unclear, Krawetz said, whether that is a reflection of relative content, namely, whether spermatozoa contain unequal amounts, or a reflection of penetration of the probe.

In addition to the paternal genome, the sperm delivers to the egg an organelle called the centrosome, which Krawetz said is critical for subsequent cellular divisions; a sperm oocyte-activating factor, over which Krawetz noted there is now some controversy; and an RNA component (Ostermeier et al., 2002, 2004). On a per cell basis, sperm deliver about

[3] This section summarizes information and opinions presented by Stephen Krawetz, Ph.D., Wayne State University, Detroit, Michigan.

50 to 100 femtograms of total RNA, including 0.3 femtograms of small noncoding RNA.

At the first cellular division, "Dad" plays a major role, in Krawetz's opinion, with paternal microRNA-181c being essential for dictating which of the first two dividing cells actually maintains a stem cell likeness, with the other cell being set on a course for developing the trophectoderm. Additionally, unlike in other mammals, zygotic genome activation in humans appears somewhat delayed, occurring by the four- or eight-cell stage, which means, Krawetz explained, that all of the information needed for the two prior cell divisions must be housed by either "Mom" or "Dad" because it cannot be made. This is in contrast to the mouse, in which zygotic genome activation occurs before the first cell division.

Evaluating Sperm RNAs

To study the biological relevance of sperm RNA, Krawetz and colleagues pooled RNA from the testes of 19 individuals, pooled RNA extracts from the ejaculates of 9 individuals, and collected RNA from the ejaculate of a single individual, and synthesized from all of these different samples a series of cDNAs (Ostermeier et al., 2002). Using microarray hybridization, they showed, first, that the sperm had a rich population of RNAs that were similar to those of the testes and, second, that all but 4 of the approximately 2,500 transcripts present in the single individual were present in the pooled ejaculate sample. Krawetz interpreted these findings to mean that, basically, all sperm carry a very similar load of RNAs. The question is, are all of those RNAs actually delivered? Using a hamster sperm penetration assay, Krawetz and collaborators showed that, in fact, the RNA was delivered (into hamster eggs) and retained for at least 3 hours, which is as long as they followed the delivery (Ostermeier et al., 2004). Krawetz interpreted this finding to mean that the RNA did have a chance to actually be utilized by the egg cell.

Much of the work being done to evaluate sperm RNAs is technology and now sequence-driven, Krawetz remarked. In the early 2000s, researchers thought they were doing a great job when they were able to collect 0.0012 gigabytes of information. Today, researchers can generate a terabyte of information over the course of just a couple of days.

Normally, when testes RNA is isolated, it yields two peaks, one at 28S and the other at 18S (representing two different ribosomal, or rRNA, species). Those are the two peaks all researchers look for when evaluating the quality of RNA numbers, Krawetz explained. But RNA isolated from sperm yields peaks that look nothing like that 28S-18S two-peak pattern. Initially, when he and his team did their early microarray work, the 18S RNAs appeared absent, suggesting that sperm were void of any ribosomal RNA.

But when they sequenced the rRNA, Krawetz and his colleagues were hit by what he said was "the shock of our life," which was that 89 percent of the RNA present was in fact rRNA, but fragmented rRNA (Sendler et al., 2013). Another 5 percent was mitochondrial RNA, another 5 percent other types of RNA, and about 1 percent was small noncoding RNA. Within the "other" category, approximately 50 percent of the transcripts had been described before as messenger RNA. One of the most abundant long noncoding RNAs present, *MALAT1*, is known to be involved in the regulation of chromatin structure. The functions of many of the "other" RNAs remain a mystery, Krawetz said, and they are "rife for discovery." A few clues indicate that they may have interesting functions.

In terms of the stability of the transcripts identified in sperm RNA, Sendler et al. (2013) divided the 1,000 most abundant sperm transcripts into quintiles based on how much of the RNA was present or absent among their samples. For example, all 13 exons of a transcript called *ACSBG2*, which is encoded by chromosome 19 and extends about 60 kilobases, were found to be represented in equal amounts in 10 different individuals, leading the researchers to classify that particular transcript as intact. Its function in early development is unknown, Krawetz said.

In terms of spermatozoal small noncoding RNAs (i.e., the 1 percent of transcripts identified in Krawetz et al. [2011]), the two major classes are the piRNAs and microRNAs. Four of the microRNAs identified (34c, 375, 184, 152) have been validated in other studies for their roles in controlling obesity or early development. There is also a series of tRNAs and tRNA fragments, the latter now known to be methylated and relatively stable.

Many functions have been proposed for spermatozoal RNAs, Krawetz continued, including confrontation and consolidation (i.e., in terms of how compatible the egg and the sperm genomes are), translation of intact paternal mRNAs, transcriptional regulation by paternal microRNAs, activation of paternal pre-microRNAs by maternal DICER (as shown from microRNA-181c), and transcriptional regulation by paternal microRNAs and RNA fragments (Jodar et al., 2013). Several studies have demonstrated that if microRNAs are delivered, they can have a fairly early and lasting effect. Rassoulzadegan et al. (2006) demonstrated that injecting either miR-221 or miR-222 microRNA into mice induced a mutated and heritable white-spotted phenotype. Subsequent work by Wagner et al. (2008) showed that injection of another microRNA, miR-1, which targets Cdk9, can induce heritable cardiac hypertrophy. Then, Grandjean et al. (2009) showed that injecting miR-124 microRNA, which targets Sox9, into mice yields a giant phenotype and twin pregnancies.

Transgenerational Epigenetic Inheritance

One role of epigenetics, in Krawetz's opinion, is to respond to a changing environment. Rather than making a change permanent, which would be difficult to undo when the environment changes yet again, epigenetics provides a way to transmit what Krawetz called a "responsive state" without altering the primary structure of the DNA.

In terms of implicating paternal microRNA in transgenerational epigenetic inheritance, the best evidence, in Krawetz's opinion, comes from studies on stress (Dias and Ressler, 2014; Gapp et al., 2014; Rodgers et al., 2013). Dias and Ressler (2014) showed that following intracytoplasmic sperm injection of microRNA-375 into the oocyte mice offspring exposed to the same odor that their parents had been exposed to before conception experienced the same averse response that their parents had. According to Krawetz, this has been demonstrated now with several different odorants.

In terms of transgenerational effects of paternal nutrition, in Krawetz's opinion the Overkalix study by Kaati et al. (2002) is among the top studies. The Overkalix study was conducted on a series of Swedish cohorts born in 1890, 1905, and 1920 and followed until 1995. The researchers extrapolated food access from historical data and asked whether an abundance of food during a child's slow growth period, that is, before the prepubertal peak, influenced descendants' risk of death from cardiovascular disease and diabetes. They found that limited access to food during the father's slow growth period limits a child's cardiovascular disease risk, but that a paternal grandfather surrounded by a bounty of food during his slow growth period increases the grandchild's risk of diabetes. The researchers concluded, "A nutrition-linked mechanism through the male line seems to have influenced the risk for cardiovascular and diabetes mellitus mortality" (Kaati et al., 2002, p. 682).

More recently, there has been a series of studies in mice on the intergenerational effects of paternal diet (Carone et al., 2010; Fullston et al., 2013; Lambrot et al., 2013). Carone et al. (2010) showed that offspring of male mice fed a low-protein, high-fat diet developed a fatty liver phenotype. In a study of diet-induced paternal obesity, Fullston et al. (2013) showed increases in adiposity and insulin resistance in both the F1 and F2 generations, with a heightened effect on female F1 offspring (67 percent increase in adiposity) and their F2 sons (24 percent increase in adiposity). The researchers isolated a series of microRNAs, including the paternally donated microRNA-205, which they suspect may play a mechanistic role.

Still to be resolved, Krawetz explained, is how paternal information gets relayed to the male gamete. He said, "It's really incredible to me that you could eat something, or you could smell something, or experience

something, and it would go from the brain to the testes." Also, is the change temporary or permanent? Does it cross the blood–testis barrier in order to be delivered to the sperm? Is it a modulated response, that is, does it occur by RNA production, stability, or acquisition? Using a mouse model xenografted with human cells expressing EGFP RNA, Cossetti et al. (2014) showed that even the murine sperm had traces of EGFRP RNA. Krawetz and collaborators have been looking at the structure of sperm and have observed that at high magnification the membrane is not a tight structure but looks almost like a series of vesicles. If in fact a series of vesicles, Krawetz believes, that structure may provide a mechanism for the exchange of genetic information. He and his team were very excited about that possibility until Chevillet et al. (2014) pointed out, if microRNAs are homogenously distributed across all exosomes, then each exosome would contain 0.01 copies of any particular microRNA, and microRNAs would have to be consolidated or concentrated in some way such that not all exosomes contain microRNAs. Krawetz and his team are currently exploring that possibility as a mechanism for the communication of genetic information.

In closing, Krawetz reminded the workshop audience that there are about 50–100 femtograms of RNA in a sperm cell, including 0.3 femtograms of small noncoding RNAs, compared to 40,000 femtograms of RNA in a somatic cell. "What this means," he said, is that "Dad has his challenges cut out for him, but he does deliver a few very important things."

MATERNAL INFLUENCES ON OFFSPRING'S EPIGENETICS AND LATER BODY COMPOSITION[4]

The evidence is compelling, Caroline Relton said, that both maternal over- and under-nutrition are associated with adverse consequences for offspring through their effects on adiposity. The question for her is, what potential mechanisms underlie those observational associations, and do epigenetic mechanisms play an important role in explaining those associations (Lawlor et al., 2012)?

When thinking about epigenetic processes as a potential mediating mechanism linking maternal over- or under-nutrition with offspring adiposity, Relton reminded the workshop audience that although her talk would be very much framed around epigenetic modifications—DNA methylation in particular as the mediator—one could very easily substitute "epigenetic modification" with microRNA expression, metabolomics profiles, or the microbiome. The same principles exist for consideration of a number of different mediating mechanisms.

[4] This section summarizes information and opinions presented by Caroline Relton, P.G.C.E., Ph.D., Newcastle University and the University of Bristol, United Kingdom.

While it has become increasingly straightforward to identify observational associations between a phenotype or an exposure and DNA methylation, Relton remarked, the challenge is to decipher whether that association is causal. There are several possible scenarios that could explain, for example, the observed association between maternal obesity (the exposure), offspring methylation (potential mediator), and offspring adiposity (the phenotype). Which is the right one? Is it indeed the case that maternal over-nutrition alters offspring methylation and subsequently has an impact on offspring phenotype? Or is it a case of reverse causation, where the exposure is altering the offspring phenotype, with the methylation change being a consequence of the altered phenotype? Or is it a situation of confounding, with exposure affecting both methylation and the phenotype?

Relton suggested a stepwise approach to addressing the challenge of determining whether epigenetic mechanisms are mediating maternal influences on childhood adiposity (see Box 3-1). First, establish an association between maternal factors (e.g., body weight and weight gain during pregnancy) and offspring adiposity. Relton reiterated that the observational evidence for such an association is very strong. Second, establish a relationship between the same exposure and a child's DNA methylation. Third, establish an association between the child's DNA methylation (i.e., the mediator) and the child's adiposity (i.e., the outcome). Fourth, implement a variety of different methods to strengthen the causal inference.

Later during her presentation, Relton described how she and her colleagues applied these steps to data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a Bristol, United Kingdom–based longi-

BOX 3-1
Epigenetics as a Potential Mechanism Mediating Maternal Influences on Childhood Body Composition

Caroline Relton identified four steps to determining whether epigenetics is a potential mechanism mediating influences on childhood body composition:

- Step 1: Establish an association between maternal factors (e.g., body weight, weight gain during pregnancy) and offspring adiposity.
- Step 2: Establish a relationship between the same exposure and a child's DNA methylation.
- Step 3: Establish an association between the child's DNA methylation and the child's adiposity.
- Step 4: Apply methods to strengthen causal inference.

tudinal cohort study of 14,000 pregnant women who were recruited in the early 1990s (Sharp et al., 2015). Both the women, who are now between the ages of 23 and 34 years, and their children have been followed extensively since their recruitment. Relton noted that the data generated throughout the life span of this cohort are publicly available for any bona fide researcher to access and use (www.bristol.ac.uk/alspac). The data cover a range of health and demographic factors, environmental exposures, behavioral factors, development and education, and parental psychological well-being.

In addition to the wealth of ALSPAC exposure and outcome data, Relton and colleagues were recently awarded funding to profile genome-wide methylation data using the Illumina Infinium® HumanMethylation450 BeadChip. So far, they have collected methylation data on 1,000 mother–child pairs, with data collected on the mothers when they were pregnant and again 17 years later and on the children when they were born, at age 7 years, and between 15 and 17 years. Relton and her team wanted to know whether the effects of gestational weight gain and/or maternal pre-pregnancy body mass index on birth weight, childhood adiposity, and adolescent adiposity were being mediated through altered methylation. Data generated from an epigenome-wide association study based on cord blood methylation in children at birth indicate a number of hits in relation to maternal underweight, very few hits in relation to maternal overweight, and some hits in relation to maternal obesity (Sharp et al., 2015). But again, the question is, are the associations causal?

Tools for Strengthening Causal Inference

Researchers have several tools at their disposal to strengthen causal inference: randomized controlled trials, cross-cohort comparisons, negative controls, parental comparisons, sibling comparisons, appraising temporal relationships through longitudinal analysis, and Mendelian randomization.

Randomized controlled trials are considered the gold standard, Relton remarked, but they are often either implausible or extremely expensive. Basically, they involve randomizing exposure and then comparing the risk of outcome. She mentioned that while some randomized controlled trials of nutrition interventions with epigenetic components are under way, they are not at the present time the most obvious choice in terms of trying to interrogate causal pathways in epigenetic pathways.

A complementary approach, the cohort comparison, involves analyzing two independent cohorts with different confounding structures. Researchers compare the magnitude of association in the two cohorts. While this approach has not yet been used in epigenetic studies, according to Relton, it has been used in observational epidemiology to determine whether breastfeeding has an impact on childhood adiposity. For example,

it was used to compare breastfeeding in relation to childhood obesity and intelligence quotient (IQ) as part of both the ALSPAC study and the Pelotas (Brazil) Birth Cohort Study (Brion et al., 2011), with evidence from both studies showing that childhood IQ but not childhood adiposity is higher in children who were breastfed. In Relton's opinion, the cohort comparison approach is potentially useful for epigenetic studies, but, again, it has yet to be applied.

The use of a negative control design, on the other hand, has been used for epigenetic studies, in particular in relation to maternal overweight and offspring methylation (Sharp et al., 2015). A negative control design involves using a second set of data with shared confounders and asking whether the same relationship is observed between exposure and offspring outcome by comparing the magnitudes of association. For example, Relton and colleagues postulated that maternal overweight would alter cord blood DNA methylation through an in utero biological event. If that truly was the case, then one would not expect to see the same association with paternal BMI and offspring phenotype. On the other hand, if the relationship was confounded by some other factor, then one would expect the two associations to be roughly the same magnitude. When Relton and colleagues compared their maternal overweight-offspring methylation association data with paternal overweight-offspring methylation association data, they observed robust associations between offspring methylation with maternal obesity (i.e., children born to mothers who were overweight were hyper- or hypo-methylated) but no significant associations with paternal obesity, suggesting that the effect of maternal obesity on offspring methylation is a biological in utero event.

Another helpful study design, one that Relton has not used but that has been reported in the literature, is sibling control. Sibling control involves exposure during the first pregnancy but no exposure during the second pregnancy, which overcomes the issue of familial confounding, and comparing the risk of exposure between siblings. An example is the comparison of adiposity or overweight in children born to mothers who were obese in their first pregnancy and then had bariatric surgery and were at a normalized weight in their second pregnancy (Kral et al., 2006).

Longitudinal modeling is yet another tool for strengthening causal inference with respect to DNA methylation. Of note, however, although a temporal association in a longitudinal study can help to overcome the issue of reverse causation, it may not overcome the issue of confounding. For example, Sharp et al. (2015) observed a difference in cord blood methylation between exposed and unexposed individuals (i.e., exposed to maternal factors) and were curious about how those differences tracked over time. They were able to track the differential methylation using multilevel modeling because they had DNA methylation data from the same

individuals at birth, at age 7, and between the ages of 15 and 17. They found one locus that was more highly methylated at birth among exposed individuals compared to unexposed individuals, but with the methylation attenuating over childhood and into adolescence in exposed individuals while remaining fairly static in unexposed individuals. In other situations—for example, when environmental influences come into play—unexposed individuals may also show changes over time. In Relton's opinion, when both exposed and unexposed individuals are tracked, longitudinal modeling is a good method for interpreting methylation patterns observed at birth and their changes over time. This approach has also been used to understand the changes in DNA methylation in offspring born to women who smoke during pregnancy (Richmond et al., 2015).

Mendelian Randomization

Relton spent the remainder of her presentation discussing Mendelian randomization, an approach introduced in the previous session by Andrea Baccarelli (see Chapter 2 for a summary of Baccarelli's presentation). The principle of Mendelian randomization is to use a proxy measure for an exposure that may otherwise be difficult to measure or that is subject to confounding or reverse causation. This measure is based on the application of instrumental variables analysis, used widely in the field of economics, and it allows for a better estimate of the effect of the exposure.

As an example of how Mendelian randomization could be applied to epigenetics, and how it was applied in the study that Baccarelli discussed (Dick et al., 2014), one could use as the proxy for maternal BMI an allele score generated from allelic variants known to influence maternal BMI and examine the relationship between this genetic proxy and a child's DNA methylation. Additionally, if one wanted to know whether the child's DNA methylation was causally related to the child's BMI, one could identify genetic variants strongly correlated with site-specific DNA methylation and use those genetic variants as a proxy measure for DNA methylation. Because a genotype is not subject to reverse causation or much confounding, it provides a much better and more secure causal anchor and allows one to make inferences about exposure with regard to outcome.

As Baccarelli explained, Dick et al. (2014) reported an observed association between DNA methylation and BMI. Although the authors included in their paper the principle of using a genetic proxy as a causal anchor and went so far as to identify a genetic proxy for their methylation site of interest, they did not actually conduct a formal Mendelian randomization analysis. Relton and her colleagues have done so. Using genome-wide methylation data generated from their 1,000 ALSPAC mother–offspring

pairs, they focused on methylation sites in the *HIF3A* gene, which was the differentially methylated locus reported in Dick et al. (2014). They wanted to know whether maternal BMI was causally linked to DNA methylation at different stages throughout childhood.

Following the steps outlined in Box 3-1, the first step in the analysis was to observationally link maternal body mass index with methylation of *HIF3A* in the child. Relton and her coworkers did this using their maternal BMI allele score (i.e., the genetic proxy for maternal BMI), which was generated from 97 small nucleotide polymorphisms (SNPs) known to be robustly associated with BMI and which was recently published through the Genetic Investigation of Anthropometric Traits (GIANT) consortium. They used the weighted scores generated from those 97 SNPs to test for differences between the observed and expected estimates of the association between the generated allele score and the child's BMI (i.e., expected if indeed the direction of effect was from BMI to DNA methylation change). Their results strengthened the causal inference that maternal BMI has a causal effect on childhood methylation.

The second step was to implement the same approach but using an allele score as a proxy for methylation and asking whether methylation was driving the child's BMI. Again, they tested for differences between observed and expected (i.e., expected if methylation is indeed a determinant) estimates of the association, in this case, between the allele score proxy and the child's BMI. Here, Relton and her colleagues concluded that there was no strong evidence that DNA methylation was having a causal effect on a child's BMI.

The third step, again using the same approach, was to test whether the child's own BMI had a causal effect on *HIF3A* methylation later in life. They found some evidence that, yes, a child's BMI has a causal effect on DNA methylation at *HIF3A*.

In sum, in terms of addressing the questions about whether maternal BMI changes offspring methylation at birth and whether offspring methylation at birth subsequently has an effect on childhood adiposity, it was postulated at the outset that, yes, maternal BMI alters cord blood or child methylation at the *HIF3A* gene and that methylation at that locus subsequently alters the child's BMI. However, having conducted the Mendelian randomization analysis, Relton and her team concluded that maternal BMI likely has a direct causal effect on the child's BMI and that the effect is not mediated by early life changes in methylation.

The Relton team's conclusion corroborates the conclusion made in the Dick et al. (2014) paper. That is, while there is an association between DNA methylation and BMI in adults, it is likely that the change in BMI is driving changes in methylation, not vice versa.

Strengthening Causal Inference: Improving the Evidence

To conclude, Relton considered ways to improve each of the four necessary steps toward strengthening causal inferences that epigenetics is mediating maternal influences on children's body composition (see Box 3-1). To improve the first step, she called for better observational evidence using more refined measures of maternal exposure. To improve the second step, she called for improved technology for the assessment of genome-wide DNA methylation. All of the data that she presented were heavily reliant on the Illumina array, which she said has obvious limitations. Improving the third step will require both better observational evidence and improved technology for the assessment of genome-wide DNA methylation. Finally, to improve the fourth step, Relton called for an increased awareness of the pitfalls of the association studies and approaches being used and for a more widespread implementation of what she referred to as "triangulation of evidence." She suggested not relying on one study design, but rather implementing a number of different tools to weigh the evidence.

PANEL DISCUSSION WITH SPEAKERS

Following Relton's presentation, audience members asked several questions about the differences between animal and human studies, the impact of genetic variation on epigenetic variation, sex differences in offspring measures, and obesity-related outcomes other than BMI.

Animal Versus Human Studies

There were two questions posed by workshop participants on the differences between animal and human studies. First, an audience member speculated that epigenetic processes are likely to be different for short-lived species (e.g., rodents) versus long-lived species (e.g., humans). The questioner wondered whether short-lived rodents, for example, need to be immediately adaptable to their environments, compared to long-lived humans, who need to be more immediately malleable and adaptable over generations. None of the panelists directly answered the question, although Jacob Friedman replied that many organ-specific developmental changes are species-specific.

Second, Robert Waterland pointed out that studies in mice have shown that leptin has an early postnatal effect on hypothalamic development. He asked Jacob Friedman if any evidence suggests that the same may be true in humans, that is, that leptin affects brain development. Friedman replied

that he is unaware of any such evidence. His work with leptin has focused on the infant gut.

The Effect of Genetic Variation on Epigenetic Variation

The effect of genetic variation on epigenetic variation came up several times during the course of the 2-day workshop. Here, in reference to Caroline Relton's demonstration of strong evidence indicating that maternal BMI is causally linked to child DNA methylation, Robert Waterland asked how Mendelian randomization rules out the alternative explanation that maternal genetics, which the child partially inherits, are causing the differences in the child's DNA methylation. Relton mentioned an analysis that she and her team recently undertook that involved quantifying the variance in methylation explained by common genetic variation (SNP heritability). Initial results indicated that only a small portion of the known genetic contribution to BMI is explained by SNPs that alter methylation. In contrast, a much greater proportion of methylated SNPs are implicated in, for example, type 1 diabetes and rheumatoid arthritis. These findings, she said, suggest to her that there is an interesting difference across traits regarding the proportion of genetic influence on epigenetic patterning. Waterland pointed out that the influence of genetics is also likely to be tissue-specific.

Sex Differences in Offspring Methylation Patterns

The panelists were asked to comment on differences between male and female offspring. Caroline Relton replied that she and her team have observed a huge number of genome-wide methylation differences between the sexes, but they have not looked at those differences in relation to adiposity. They have detected approximately 17,000 statistically significant "hits," all on autosomes, not on the sex chromosomes. They suspect that some of the hits are associated with sex hormones. In contrast, Stephen Krawetz added that he and his team have not found any statistical differences in the methylation patterns that they have directly targeted in sperm.

Beyond BMI: Other Outcome Measures of Obesity

An audience member expressed being in favor of seeking other measures of obesity besides BMI and asked the panelists if they had considered using metabolic disease correlates of BMI and not relying on size alone. Relton replied that she and her team have also used fat distribution data and other more subtle measures of adiposity at multiple time points. The beauty of BMI, she said, particularly in the context of Mendelian random-

ization, is that it is accompanied with a rich store of publicly available genotype data. One can gain a huge amount of leverage by querying data from large consortia such as GIANT with an SNP, or variant, that is known to be associated with methylation. The use of metabolic disease phenotypes as outcome measures instead of, or in addition to, BMI was an issue that was revisited several times throughout the workshop.

4

Opportunities for Intervention and Prevention

OVERVIEW

Moderated by planning committee member Leann Birch of the University of Georgia, Session 3 featured speakers who discussed potential opportunities for intervention and prevention based on rapidly advancing knowledge about the role of epigenetics and other factors in the early origins of obesity. This chapter summarizes the Session 3 presentations and discussion.

Reiterating what Linda Adair and Caroline Relton had voiced earlier about the importance not only of maternal over-nutrition, but also maternal under-nutrition in increasing the risk of childhood obesity (see Chapter 3 for summaries of their presentations), Karen Lillycrop of the University of Southampton summarized animal and human data suggesting that both maternal over- and under-nutrition can cause long-term metabolic changes in offspring and that many such changes are associated with altered DNA methylation patterns. She echoed Andrea Baccarelli's and others' hope that in the future DNA methylation differences at birth can be used as predictive biomarkers of later adiposity. Additionally, she speculated on the possibility of identifying stable epigenetic markers that could be used to monitor the success of obesity interventions over the life course. But several obstacles will need to be overcome and questions answered before the use of predictive or monitoring epigenetic biomarkers becomes a clinical reality, she said. For example, are the markers causal? What other epigenetic changes are occurring besides methylation? Which markers can be changed with nutritional or other intervention, and when and how?

Kevin Grove of the Oregon National Primate Research Center called attention to placental function and its association with a wide range of downstream metabolic complications. Based on research using a non-human primate model, evidence indicates that regardless of maternal body mass index (BMI), a high-fat maternal diet during pregnancy results in increased inflammatory cytokine production in the placenta and, because cytokines cross the placental barrier, in the fetus as well. Grove suggested that fetal systemic inflammation might be a potential target for therapeutic intervention. A high-fat maternal diet during pregnancy, when maintained postnatally through weaning, also has significant downstream effects on offspring neurochemistry and behavior. Grove emphasized the importance of testing any proposed intervention before implementing it, whether it involves diet and exercise, surgery, or pharmacotherapy. Results from pre-pregnancy bariatric surgery studies in rodents and humans have yielded conflicting risk–benefit results.

The perinatal period is sensitive not only to dietary fats, but also to leptin, a hormone produced by adipocytes. Marie-France Hivert of the Harvard Medical School discussed the physiology of leptin and summarized findings from animal and human observational studies suggesting that leptin exposure during the perinatal period is associated not only with early life weight gain, but also with long-lasting changes in the hypothalamus and other tissues. Additional human data from Hivert's laboratory group suggest that leptin levels in offspring are regulated, at least partially, by epigenetic changes in the placenta triggered by changes in maternal glucose levels.

According to Mark Vickers of the University of Auckland, leptin was one of the first obesity interventions tested in an animal model. When results suggested that administering leptin could stop overeating in animals on high-fat diets, there were calls to add leptin to infant formula. But the effects were also shown to depend on the nutritional status of the mother and to be sex-dependent. Vickers summarized results from mostly animal obesity intervention studies testing a range of strategies from leptin to exercise. In his opinion, it is reassuring that so many different animal models, from sheep to mice, demonstrated similar reversals in metabolic dysfunction resulting from early life interventions. But researchers need to gain a better understanding of short-term versus long-term trade-offs and how to apply results from animal studies to the clinic.

There was a great deal of discussion throughout the workshop about the human microbiome and how gut microbial metabolites can impact infant metabolism and risk of childhood obesity. William Nierman of the J. Craig Venter Institute provided an overview of the human microbiome and efforts over the past decade to increase awareness of its significance in human health. He emphasized that the microbiome's metabolic capabilities supplement the host's metabolic capabilities—for example, by digesting

complex carbohydrates—with consequences for cancer and other disease phenotypes.

Meredith Hullar of the Fred Hutchinson Cancer Research Center expanded the discussion of microbiome to epigenetics and observed that researchers have moved beyond simply reporting associations between the microbiome and host epigenetic patterning and are beginning to study how microbial metabolism actually mediates observed epigenetic changes. For example, many host genes associated with satiety are influenced by exposure to fatty acid metabolites of the gut microbiome. Although Hullar and others have found associations between the dominant microbial makeup of the gut microbiome and adult adiposity, it is difficult at this point to conjecture how the early life microbiome affects the risk of obesity. The field is limited, in her opinion, by small sample sizes and a lack of prospective studies.

While the focus of the workshop was obesity, several speakers advocated for thinking less about size, or BMI, and more about the metabolic dysregulation that accompanies or underlies obesity. It is especially helpful to think about metabolic dysregulation when thinking about the effect of obesity on the brain, said Antonio Convit of the Nathan Kline Institute and the New York University School of Medicine. Convit spoke about how metabolic dysregulation in adolescents leads to reduced cognition and reduced hippocampal volume, with insulin resistance being the primary driver. He suggested that retinal arterial width is a potentially useful biomarker for metabolic dysfunction in the brain and identified exercise and sleep, because of their known associations with insulin resistance and metabolic dysfunction, as two "easy public health handles" for obesity intervention in adolescents.

DEVELOPMENTAL PLASTICITY: SENSITIVE PERIODS AND RISK OF OBESITY[1]

Traditionally it has been widely accepted that genes determine phenotype and susceptibility to chronic diseases later in life. However, rates of obesity have risen dramatically over the past 20 years in both developed and developing countries, and they are projected to rise even higher by 2030. Such a rapid rise over such a short timeframe cannot be explained solely by genetic factors, said Karen Lillycrop of the University of Southampton. It suggests that the environment also plays a role. That role may be mediated, she suggested, through altered epigenetic regulation of genes. Lillycrop went

[1] This section summarizes information and opinions presented by Karen Lillycrop, Ph.D., University of Southampton, Southampton, United Kingdom.

on to discuss the effects of early life nutrition on the epigenome and how altered epigenomic processes might have an impact on risk of obesity.

Animal Model Evidence That Early Environment Can Induce Epigenetic and Phenotypic Changes

Increasing evidence suggests that a number of environmental factors, including nutrition, can affect the epigenome and that the epigenome seems most susceptible to those environmental factors during the prenatal, neonatal, and pubertal periods. Most of the evidence for this comes from work with animal models showing that maternal dietary and protein restriction, as well as maternal over-nutrition, can induce long-term metabolic changes within offspring. According to Lillycrop, both maternal under- and over-nutrition often induce similar metabolic changes, with both cases being associated with dyslipidemia, insulin resistance, and obesity in offspring and with the phenotypic changes in both cases being accompanied by changes in the methylation of key metabolic genes. For example, Lillycrop and her research team demonstrated that when rats were fed a protein-restricted diet during pregnancy, their offspring exhibited alterations in methylation of a range of genes, including a gene known as *PPAR-alpha* (peroxisome proliferated activated receptor alpha) that encodes for a nuclear receptor known to play a key role in lipid metabolism (Lillycrop et al., 2005, 2007). Specifically, the researchers observed decreased methylation in the promoter region of *PPAR-alpha* in the liver of protein-restricted offspring, compared to controls, with the decreased methylation being accompanied by an increased gene expression and increased levels of beta oxidation (i.e., the metabolic process controlled by the gene). The altered methylation appeared as early as embryonic day 18 and seemed to persist through adulthood, Lillycrop said.

It is not just under-nutrition that can induce that type of epigenetic and phenotypic change, Lillycrop said. Maternal over-nutrition can do the same. When she and her team fed pregnant rats a diet high in either saturated fat or fish oil, they observed an altered fatty acid composition in the livers of the offspring, specifically decreased levels of 22:6n-3 and 20:4n-6 (Hoile et al., 2013). Additionally, upon examination of methylation and expression of *Fads2*, a gene that encodes the rate-limiting enzyme in polyunsaturated fatty acid synthesis, they observed decreased expression in offspring from dams who had been fed a diet high in either saturated fats or fish oil, accompanied by an increased methylation within the promoter region of *Fads2*. Methylation at the –394 CpG site accounted for over 56 percent of the variation in *Fads2* mRNA expression among individual offspring.

Increasing evidence suggests that epigenetic plasticity extends into early postnatal life, Lillycrop stated. For example, Plagemann et al. (2009)

showed that over-nutrition in early life, which they induced by rearing the rat pups in small litters, triggered rapid fat gain and obesity in offspring and that the rapid weight gain and obesity were accompanied by alterations in the methylation of CpGs within the promoter region of the appetite control *POMC* gene. Specifically, they observed hyper-methylation of CpG sites in two binding sequences, Sp1 and NF-kappaB, known to be important for the induction of POMC by leptin and insulin.

Lillycrop remarked that many other studies have shown that variations in micronutrient intake, during either pregnancy or the peri-pubertal period, can similarly induce changes in gene methylation. Additionally, she said other research indicates that variations in paternal diet can induce altered methylation of a variety of genes. The affected genes often play key roles in metabolism and appetite control, suggesting to Lillycrop that the observed methylation changes underpin the long-term changes that have been observed in offspring metabolism and disease risk.

Human Evidence That Early Environment Can Induce Epigenetic and Phenotypic Changes

A number of research groups have begun to examine whether the same phenomena being observed in animals are occurring in humans. Much of the focus is on the effects of the Dutch Hunger Winter, a period of severe food shortage in the Netherlands in 1944–1945, when daily energy intakes dropped from around 1,800 kilocalories to between 400 and 800 kilocalories. Those studies, Lillycrop said, have shown that individuals born to mothers exposed to famine during pregnancy have an increased risk of heart disease, diabetes, and obesity later in life. Researchers have also reported alterations to the methylation of a number of genes in individuals exposed in utero. For example, Tobi et al. (2009) found an increase in methylation of the *IL10*, *GNASAS*, *IGF2*, *LEP*, *ABCA1*, and *MEG3* genes and a decrease in methylation of *INSIGF*. Interestingly, Lillycrop noted, those measurements were made 60 years after the famine, suggesting that variations in maternal diet induce a very persistent epigenetic change in offspring.

The Mismatch Hypothesis

Revisiting some of what Linda Adair had touched on during her presentation (see Chapter 3), Lillycrop elaborated on how the ability of early life nutrition to alter the epigenome has been suggested to be part of a normal adaptive process called developmental plasticity. That is, when an organism responds to its environment in early life, it adjusts its developmental path to produce a phenotype that confers a survival or fitness advantage

(Waddington, 1942). For instance, poor maternal nutrition might signal to the fetus that nutrients are scarce, in which case the fetus will adapt its metabolism by reducing energy demands, increasing its propensity to store fat, investing less in bone and muscle mass, and preparing for an uncertain life course. If that organism then finds itself in an environment where nutrients are indeed scarce, its metabolism will be matched, and it will be at low risk of metabolic disease. If, on the other hand, it finds itself in an environment where nutrients are abundant, its metabolism will be mismatched, it will have a propensity to store fat, and it will be at an increased risk for metabolic disease. This so-called mismatch between the pre- and postnatal environment, Lillycrop explained, has been suggested to account for some of the rapid rises in rates of obesity and diabetes in the developing world as populations move from rural to urban areas.

Biomarker Detection

Given increasing evidence from both animal and human studies suggesting that early life nutrition can alter the epigenome and that reset epigenetic markers persist and contribute to alterations in metabolism and disease risk, Lillycrop concluded that it should be possible to detect such changes early in life and use them to estimate metabolic capacity and disease risk. The challenge in humans, in her opinion, is limited tissue availability. Although there are several readily available tissues that could be accessed, including the umbilical cord, cord blood, placenta, buccal cells, and blood, she said that there is a real question as to whether methylation in those cell types adequately reflects methylation in more metabolically relevant cells. She speculated that if the environmental constraint (i.e., the constraint responsible for triggering the epigenetic change) occurred early enough in development, then perhaps yes, if all three germ layers were affected.

Lillycrop and her research team wanted to see if they could find methylation differences at birth in those peripheral tissues, specifically in cord tissue, that were associated with later adiposity (Godfrey et al., 2011). Using mother and offspring cohorts based in Southampton as their study population, they started their search by sequencing the promoter regions of genes known to play roles in adipogenesis in cord tissue. They found that methylation of CpG in the promoter region of the retinoid X receptor at birth was associated with percent fat mass at the age of 9 years in one cohort and at age 6 years in another cohort. Lillycrop interpreted the findings to mean that developmentally induced epigenetic markers might indeed make a significant contribution to later phenotype.

Ideally, in Lillycrop's opinion, methylation markers could be used not just to predict later phenotype, but also to follow the progression of an altered phenotype through the life course and to monitor the effectiveness

of interventions. Again, such a marker would have to be present in tissues that are readily accessible, such as blood or buccal cells, and it would have to be relatively stable over time. Lillycrop observed that although DNA methylation was originally thought to be a very stable marker maintained through life, a number of recent studies have suggested that epigenetic markers may be more dynamically regulated than previously thought. She mentioned that there is research indicating how just an intense bout of exercise, or even a weekend visit to the city, can change the level of methylation. If that is the case, she said, then there might be limits to the use of epigenetic markers for following the progression of a phenotype through the life course.

To address the question of stability, particularly during childhood, Lillycrop and her research team started looking at methylation of genes in blood samples collected annually from children ages 5 to 14 years. Initially they looked at *PGC-1a*, which plays a key role in energy sensing and in the coordination of the metabolic response. They found that methylation of the CpGs in the promoter region of *PGC-1a* was stable over the timeframe of the study, suggesting that, at least for these sites, methylation is set early in life—that is, before the age of 5, and stably maintained thereafter despite the onset of puberty and variations in exercise and pollution.

Having found some stable CpG methylation sites, the researchers wanted to see if the CpG sites were associated with a phenotype. They found associations between methylation of four of the CpG sites in the promoter region of *PGC-1a* at 5 to 7 years and percent fat mass from 9 to 14 years, again in both boys and girls. In the case of *PGC-1a*, the magnitude of the effect was such that, for a 10 percent difference in methylation at 5 to 7 years, percent body fat at 9 to 14 years differed from between 6.3 to 12.5 percent.

But the question remains, are the observed alterations in methylation simply markers of adiposity, or do they actually play a role in the development of obesity? To begin to address that question, Lillycrop's team has started looking at the effect of methylation of CpG sites on the function of *PGC-1a*. Thus far they have found that methylation of one of the CpG sites known to be associated with adiposity leads to a decrease in promoter activity of the gene. Additionally, they have found that methylation of the CpG site located at –783 altered the binding of the HOXB9/PBX1 heterodimer, a complex known to be important for adipogenesis and an important regulator of PGC-1a. These findings suggest to Lillycrop that methylation at these sites might have a functional consequence.

Summary

In closing, Lillycrop summarized by reiterating that growing evidence indicates that early life environment is an important determinant of later obesity risk, with two pathways: under-nutrition and over-nutrition. Evidence also suggests that early life environment can alter epigenetic markers and that the altered epigenetic markers appear to be an important contributor to later phenotype. Lillycrop suggested that it may be possible to detect such markers in early life and use them to identify individuals at increased risk of obesity and its sequelae.

But there is a lot to learn, she said. For instance, little is known about how either the under-nutrition or over-nutrition pathway leads to obesity or the epigenetic changes that occur and whether the epigenetic signatures are the same for both pathways. Lillycrop remarked that the epigenetic changes at play are likely not only methylation changes, but also non-coding RNA and histone changes. Moreover, it is not clear what factors drive the changes. Nutrition is obviously very important, Lillycrop said, but what other factors are operating? Finally, are there epigenetic markers that can be changed through intervention, and, if so, when and how?

Given that researchers are now in a position to begin to address these questions, either with current cohorts or new ones, Lillycrop expressed the hope that "we should then start to be able to stem the tide of this ever-increasing rise in rates of obesity."

MATERNAL HEALTH AND DIET'S EFFECT ON OFFSPRING'S METABOLIC FUNCTIONING[2]

The value added by his work, Kevin Grove of the Oregon National Primate Research Center began, is use of a nonhuman primate model to examine the impact of maternal obesity on offspring energy balance and risk of obesity. The monkey model he and his team use involves feeding adult Japanese macaques either a healthy diet or a high-fat, high-carbohydrate Western-style diet (McCurdy et al., 2009). After several years, the monkeys are bred, and sibling offspring are studied as they progress through different metabolic complications. While Grove and his group also conduct rodent studies, they are especially interested in the uncontrolled components of human studies that can be controlled using a nonhuman primate model. Initially, Grove said, he thought that the variability would make it impossible to detect anything. Then he realized, "that's the human nature of things." And it's actually what he likes about the model—that is, that he

[2] This section summarizes information and opinions presented by Kevin Grove, Ph.D., Oregon National Primate Research Center, Beaverton, Oregon, and Novo Nordisk, Seattle, Washington.

and his research team are able to look at not only phenotype by diet, but also phenotype by a distribution of metabolic outcomes (i.e., body weight, adiposity, insulin sensitivity, triglyceride levels, and so on).

Focus on the Placenta

In Grove's opinion, placental function is one of the most important components to understanding why many metabolic, inflammation, and brain development complications exist. There is a lot of clinical data demonstrating that clinical conditions that result in hyperinsulinemia or hyperglycemia, such as gestational diabetes, also cause complications in the placenta (e.g., Ragavendra and Tarantal, 2001). By taking a step back, Grove said, one can ask, what is it about the diet itself that might be contributing to these placental complications? Given his background in cardiovascular disease, and that he and his team study the effect of lipids on cardiovascular disease all the time, Grove views the placenta as "just a large vascular organ." It is no surprise to him that lipids known to cause inflammation and vascular dysfunction could have a direct impact on placental function, as elegantly demonstrated by Antonio Frias, a member of his research team at the Oregon Health and Science University (OHSU). Using traditional Doppler ultrasound techniques to measure placental blood flow during the early third trimester, Frias et al. (2012) demonstrated that regardless of whether a mother was obese or lean, a high-fat diet led to reductions in placental blood flow. The resulting placental insufficiency was great enough that experimentally inducing that level would cause negative outcomes. "So just being on the diet was a problem," Grove said.

When Frias et al. (2011) extended their study to look at some of the histological effects of a high-fat diet, they were able to characterize infarctions and calcifications in the placental tissue. A pathologist not named in the presentation was asked to analyze the blinded histological samples thought that the high-fat diet placental tissues were from preeclampsic patients, a "scary" response, Grove said, given that they were from simply either overweight or obese animals who had been fed a high-fat diet. That said, while the functional abnormalities observed by Frias et al. (2011) were caused by diet alone, the effects are exacerbated by obesity, insulin resistance, and high triglyceridemia.

Frias is currently trying to design more precise diagnostics using contrast-enhanced MRI, according to Grove. The Doppler ultrasound methodology provides a measure of average outcome across the placenta. But a dysfunctional placenta has areas that look rather normal. Rather than grinding up the entire tissue and examining the average, Grove and his team wanted to find a way to identify regional placental blood differences. So far, they have been able to identify differences between cotyledons

(subdivisions of the placenta) and have found quite a bit of variability. According to Grove, the technique can be used to image even individual spiral arteries. Preliminary results from Frias's contrast-enhanced magnetic resonance imaging (MRI) work indicate the presence of highly localized damage. So some areas are normal, others abnormal. While these differences are useful experimentally, Grove is unsure whether they will be useful clinically, except perhaps with very-high-risk patients. But, in his opinion, at least researchers can now start correlating outcomes with biomarkers.

Summarizing Frias's data, Grove stated that increasing dietary lipids in the maternal diet can cause placental problems, such as increased cytokine production, and that the problems can be exacerbated by adding other "hits" such as maternal obesity and hypertriglycemia. Cytokines produced in the placenta are shunted into the developing fetus, where, based on what has been learned from experimental models, they can cause all sorts of pregnancy and developmental programming complications. Other changes to nutrients in the diet—for example, a change in the n-6/n-3 fatty acid ratio—can also be transmitted through the placenta to the fetus, with important developmental consequences. N-3, for example, is important for brain development. Grove reiterated, "It may all start with the placenta." It is not just increased dietary lipids but all sorts of maternal health conditions that have been linked to placental dysfunction, including maternal obesity, inappropriate maternal pre-pregnancy weight and pregnancy weight gain, gestational diabetes, preterm delivery, and preeclampsia. In his opinion, focusing on the placenta may be a way to improve clinical outcomes.

Turning to the downstream metabolic effects on the offspring and touching on some of what Jacob Friedman had discussed during his presentation (see Chapter 3), Grove mentioned that signs of abnormal development of metabolic systems in very young animals include increased liver triglycerides, hepatic insulin resistance, muscle insulin resistance, decreased pancreatic alpha cell mass, and cardiac hypertrophy. In Grove's opinion, it is one thing to develop these complications at the age of 35 to 45 years, but it is another thing to "hit the ground" with that status and have to experience it throughout the entire life course. While nutritional manipulations can improve some of these metabolic outcomes, some of the underlying molecular changes still exist. Whether those changes are epigenetic in nature is unknown, Grove stated.

Turning his attention to more complex behaviors in juvenile offspring, Grove described how the offspring born either to mothers fed a healthy diet or to mothers fed a high-fat diet are kept with their mothers during the postnatal period but that upon weaning, the offspring can be maintained on either diet. So control offspring (i.e., those born to mothers who were fed a healthy diet) can be put on a high-fat diet post-weaning, and high-fat

offspring (i.e., those born to mothers fed a high-fat diet) can be put on a control diet post-weaning. Grove described some of the findings that he and his team have observed thus far as a result of switching post-weaning diets. Notably, the offspring of mothers fed a high-fat diet who were switched to a post-weaning control diet nonetheless experienced long-term persistent inflammation in the brain. That they experienced brain inflammation is not surprising, Grove said, given that cytokine production in the placenta can cause brain inflammation. But the fact that the inflammation appears to be sustained, as do some neurochemical imbalances known to be associated with hedonic feeding and appetite stress dysregulation (i.e., decreased serotonin levels, low dopamine levels, and abnormalities in the melanocortin system), suggests that the alterations persisted even after the offspring were put on healthy post-weaning diets.

Additional data from Elinor Sullivan, another Grove research team member at OHSU and the University of Portland, indicate that a high-fat diet in the same macaque model is also associated with long-term abnormalities in brain connectivity in cortical regions. Specifically, they have shown that functional connectivity in the frontal cortex decreases, while functional connectivity in the temporal cortex increases. The findings indicate long-term differences in brain function and raise questions about whether the affected regions are related to abnormal behavior.

They also raised the question for Grove, what is going on metabolically in juvenile monkeys born to mothers fed a high-fat diet? Interestingly, Grove said, animals put on a healthy diet after weaning actually normalize their body weight. So they look fairly normal, even though their liver molecular signals do not, as Jacob Friedman discussed during his presentation (see Chapter 3 for a summary of information presented by Friedman). But if the same animals are put back on a high-fat diet at the age of 1 year, they have accelerated weight gain due to increased overall food intake. Grove described the acute increase in food intake as one with almost binge-like eating episodes. If the animals are given something novel, they will lever-pull all day long, he said, and they will stuff their cheek pouches and armpits. That behavior suggests to Grove that the animals have a drive for palatable foods that they have not been getting. The control animals, on the other hand (i.e., animals born to mothers fed a healthy diet), showed no change in food preference when placed under the same conditions. Even though control infants eventually reach the same weight, Grove suspects that the acute increase in food intake in infants born to mothers fed a high-fat diet may have long-term consequences.

In terms of metabolic rate, the high-fat-diet offspring had an increased rate during their active period (i.e., during the day), and low metabolism during their inactive period (i.e., at night). While, on the one hand, the increased metabolic rate during their active period might explain why the

body weights of the high-fat-diet offspring end up being no different than those of the control offspring, Grove said that he suspects that the increased metabolic rate in the high-fat-diet offspring is a manifestation of anxiety and hyperactivity. Those offspring spend a lot of time avoiding social contacts. Not only does their metabolic rate decrease at night, but their metabolism uses fats at night, compared to carbohydrates during the day. It is not clear why they use a different fuel at night, Grove said.

Regarding the anxiety that Grove suspects may be driving the high metabolic rate during the day, Sullivan et al. (2010) showed a dramatic increase in anxiety behavior in female offspring of mothers fed high-fat diets, as measured by latency to interact with toys, and a dramatic increase in aggressive acts in males, as measured by a tendency to lash out. While the behavioral differences were sex-dependent, Grove said that he suspects that the underlying cause is probably the same, given that both sexes also had increased cortisol levels. Other social abnormalities detected in the monkeys born to mothers fed high-fat diets include more shrieks during novel peer interactions, which were not sex-dependent; less initiation of contact, but more successful contacts when they are not the initiator; more time spent alone, again regardless of sex; and less social play, in fact, almost nonexistent social play. According to Grove, evidence exists to suggest that multiple maternal health and dietary and other components are likely contributing to the abnormal behaviors.

Interventions

Grove concluded by voicing his opinion about potential interventions in humans. In his opinion, weight loss prior to pregnancy is optimal. Weight loss during pregnancy, on the other hand, typically should be avoided, although diet and exercise can be improved. The greater question for him is, can either pharmacotherapy or surgery play a role?

Regarding surgery prior to pregnancy, in a study on bariatric surgery in rats, Grayson et al. (2013) demonstrated improvements in outcomes after doing bariatric surgery prior to pregnancy, but there were some long-term complications. Several other research groups have studied bariatric surgery in women, with initial studies demonstrating dramatic improvements, including reductions in pregnancy complications and reduced macrosomia (Galazis et al., 2014; Willis et al., 2015). But more recent studies have shown a dramatic increase in preterm birth and small-for-gestational-age infants (Galazis et al., 2014; Willis et al., 2015). Grove stressed the importance of testing interventions before applying them—not just surgery, but pharmacotherapy too. He

mentioned but did not elaborate on work he and his team did with maternal resveratrol treatment during pregnancy (Roberts et al., 2014).[3]

The even greater question for Grove is what to do for children. He recommended against surgery and pharmacotherapy. Exercise, such as what has been tested in rodent models, may be helpful, but it needs to be tested in human, in his opinion. Some of the social issues associated with childhood obesity may be improved with enriched environments, but again, in Grove's opinion their actual effect on obesity needs to be tested. In conclusion, he stated, "I'm just going to end this by reminding people, this is a vicious cycle."

EARLY INFANT RAPID WEIGHT GAIN AND THE EPIGENETICS OF LEPTIN[4]

Marie-France Hivert of the Harvard Medical School began her presentation by asking, what is leptin? Leptin was discovered in 1994 in *ob/ob* mice (Zhang et al., 1994). *Ob/ob* mice are fully deficient in leptin, hyperphagic, and morbidly obese. Leptin was one of the first adipokine—a hormone produced by adipocytes—described in the scientific literature, Hivert said. Among the cases of leptin deficiency described in humans, Farooqi et al. (1999, 2002) demonstrated that, when provided with leptin, children fully deficient in leptin decreased their hyperphagic behavior, reduced their weight, and continued to grow in height to a point where they almost normalized their BMI.

From these early animal and human studies, researchers learned that leptin is an adipostat signal that sends messages to the hypothalamus indicating that if there is not enough adipose tissue and leptin levels fall, then one should increase his or her food intake to restore normal weight. Allard et al. (2013) demonstrated that, indeed, this negative feedback loop exists in lean individuals, and those young adults who are lean and who have relatively low leptin levels at baseline have a higher weight gain over the next 2 years. However, this does not seem to be the case in obese individuals, who seem to be resistant to leptin. Even when injected with exogenous leptin, no significant weight loss can be induced, according to Hivert (Heymsfield et al., 1999).

Leptin acts on many other systems, Hivert said, including the reproduc-

[3] During the panel discussion following Mark Vickers's presentation, Jacob Friedman noted that resveratrol is a natural supplement that he and his colleagues administered to offspring of mothers fed high-fat diets. They observed reduced triglyceride levels and improved placental function among the offspring administered the supplement, but with very odd alpha- to beta-cell ratios in the developing eyelets.

[4] This section summarizes information and opinions presented by Marie-France Hivert, M.D., M.M.Sc., Harvard Medical School, Boston, Massachusetts.

tive system, where it affects placental function and possibly fetal development. Leptin is actually produced by the placenta during pregnancy and not only by adipose tissue, greatly contributing to maternal circulating levels of leptin (Hauguel-de Mouzon et al., 2006). At the first trimester, long before there has been any significant pregnancy gain in adipose tissue, there is almost double the amount of maternal plasma leptin compared to the pregravid period. Within 48 hours after delivery, maternal plasma leptin levels drop significantly; the drop is due to the sudden drop in placental expulsion and the consequent lack of a placental source of leptin, Hivert said, not to a decrease in weight.

These findings raised the question for Hivert, why is the placenta producing so much leptin, given that leptin, in normal physiology, is part of a negative feedback loop on weight regulation? She and her research team have been investigating this question and trying to untangle the physiological roles of maternal versus fetal leptin. Based on data collected thus far, they think that during pregnancy, maternal circulating leptin is coming from both adipose tissue and the placenta in equal amounts, with the placenta also producing leptin that is being released on the fetal side, although Hivert sad most fetal leptin is probably being produced by fetal adipose tissue.

Using data collected from a longitudinal pregnancy cohort in Canada, Hivert and her research team measured maternal leptin levels at the first and second trimester and fetal levels at birth (circulating levels in cord blood). Hivert said that they were surprised to find that maternal leptin levels were not associated with lower subsequent gestational weight gain, but rather that higher leptin levels predicted a higher subsequent gestational weight gain, even after accounting for current maternal weight status (unpublished data). That is, the researchers observed what appeared to be a positive feedback loop, with the presence of elevated maternal leptin signaling to women that they should be eating more. The positive feedback signal was presented mainly in the second trimester and was of greater magnitude in women who were overweight. Additionally, Hivert and her team observed that maternal leptin levels were associated with both fetal adiposity, as measured by skin fold, and fetal leptin levels in cord blood at birth, once again independently from maternal weight status.

Hivert said that her interest in leptin levels in offspring in the perinatal period comes partly from animal studies published more than a decade ago that demonstrated that early leptin exposure is associated with hypothalamus development. Bouret et al. (2004) compared hypothalamic development in *ob/ob* mice versus normal mice and observed in the periventricular nucleus of the hypothalamus a definite lack of neuronal development in the *ob/ob* mice. When they injected leptin into the *ob/ob* mice during the perinatal period, they could rescue and reestablish density of

neuronal fiber and projection in the periventricular nucleus. When they tried to do the same in later life, again in *ob/ob* mice, they did not notice the same effect. Their findings suggested that leptin exposure in the perinatal period plays a critical role.

In a similar experiment, Bouyer and Simerly (2013) exposed *ob/ob* mice to a short course of leptin in the perinatal period and then followed the mice through life to observe their weight trajectory. Untreated *ob/ob* mice, who were fully leptin-deficient, became hyperphagic and morbidly obese, while *ob/ob* mice who were treated for only a few days in the perinatal period had a slightly lower weight trajectory. Hivert reiterated that the treated mice received only a few days of treatment and that the effects were lasting. When the researchers exposed the mice to leptin again later in life, those mice that had been exposed in the perinatal period were more sensitive to the later exposure compared to the ones who had never been exposed. The researchers also reported differences in adipocyte and adipose tissue development, with *ob/ob* mice exposed to leptin in the perinatal period having smaller, "healthier" adipocytes. According to Hivert, small adipocytes are more metabolically healthy and flexible than large cells. It seems to Hivert, based on these findings, that early leptin exposure is associated not only with hypothalamus development, but also adipose tissue.

In humans, as part of Project Viva, a research project led by Matthew Gillman that has been under way for more than 15 years, Parker et al. (2011) examined a series of factors in the prenatal period or at birth and their association with early infancy weight gain as measured by the change in weight-for-length from birth to 6 months. Among all of the factors that they examined, leptin emerged as a significant determinant of early-infancy weight gain, with higher leptin levels being associated with a slower weight gain between birth and 6 months of age. Mantzoros et al. (2009) reported a similar negative association at 3 years of age, with lower leptin levels in cord blood at birth being associated with a higher weight status at 3 years. However, Boeke et al. (2013) reported that higher leptin levels at 3 years of age predict a higher BMI at 7 years of age, indicating that the negative association observed earlier in life no longer exists.

So again, as with the animal data, human data suggest that the perinatal period is a critical period with respect to leptin exposure, with infants still being sensitive to leptin and with lower levels of leptin in infancy being associated with greater weight gain and adiposity at the age of 3. Hivert suggested that the association may be mediated through hypothalamic development, adipose tissue changes, or even the microbiome. Yet, the molecular mechanisms involved are still mostly unknown.

Epigenetics of Leptin in Early Life

Hivert remarked that her research group is especially interested in the impact of maternal glycemia on leptin. Bouchard et al. (2010) examined the association between maternal glucose and placental leptin gene DNA methylation in women classified as having gestational impaired glucose tolerance and found that the higher the maternal glycemia, the lower the DNA methylation in the promoter region of the fetal leptin gene but the higher the DNA methylation in the promoter region of the maternal leptin gene. Hivert suggested that if one believes that maternal leptin has a positive feedback on gestational weight gain, then the Bouchard et al. (2010) results suggest that higher maternal glycemia may lead to leptin gene DNA methylation adaptations that limit maternal leptin levels, with higher methylation in the leptin gene promoter region and therefore a lower expression of the leptin gene on the maternal side. But that possibility is only a hypothesis at this point, Hivert noted.

She and her research team used Mendelian randomization to better understand the observed association between maternal glucose and leptin epigenetic regulation. Using data from the Genetics of Glycemic Regulation in Gestation and Growth (Gen3G) cohort, the Mendelian randomization study they conducted showed that maternal glycemia is part of the causal pathway influencing offspring leptin epigenetic regulation (Allard et al., 2015) (see Chapter 3 for a summary of Caroline Relton's discussion of Mendelian randomization). Hivert expressed hope that Mendelian randomization might help researchers to identify additional factors that have an impact on risk of excess weight at birth and in childhood, including factors that might serve as potential targets for intervention.

Hivert's Take-Home Messages

Hivert highlighted two take-home messages. First, the perinatal period is critically sensitive to leptin, with leptin levels during that period likely affecting many tissues—not only the hypothalamus, but also adipose tissue. Based on results from both animal and human observational studies, perinatal leptin levels are associated with early life weight trajectory. Second, based on the current literature and Hivert's laboratory observations, it seems that maternal glucose modulates the epigenetic regulation of leptin in offspring.

THERAPIES TO REVERSE METABOLIC DISTURBANCES ARISING AS A CONSEQUENCE OF DEVELOPMENTAL PROGRAMMING[5]

Mark Vickers of the University of Auckland in New Zealand said that it is well established that alterations early in life, particularly nutritional exposures, increase risk for a range of metabolic disorders later in life, including obesity and heart disease. There is no single cause, he said; rather, it is a complex, multifactorial process. While scientists are beginning to understand some of the underlying mechanisms, there is still a great deal to learn about how interventions early in life can diminish the incidence and severity of later disease. More importantly, Vickers said, most of what is known is derived from experimental models with limited translation in the clinical setting.

A range of interventions have been tested, including dietary interventions (e.g., lipids, pre- and probiotics, taurine, vitamins, polyphenols, methyl donors), pharmacologic strategies (e.g., leptin, growth hormone, melatonin, GLP-1 analogs, nuclear receptor agonists), and behavioral and lifestyle interventions (e.g., exercise, dietary counseling). If efficacy of behavioral and lifestyle interventions was greater than it is, Vickers remarked, then that would be the ideal option given that it is the easiest strategy to implement and does not raise the same safety issues that the other two types of interventions do.

The question for Vickers is not only *how* to intervene, but *when* to intervene. The programming window of plasticity is difficult to time correctly, especially when translating across species (e.g., from rodents to humans). Godfrey et al. (2010) described how plasticity diminishes over time and argued that the earlier the intervention, the better the effect later in life. According to Vickers, some data suggest that the best window for intervention may even be during preconception. Unfortunately, Vickers said, in some areas of New Zealand 60 to 70 percent of pregnancies, particularly among high-risk groups, are unplanned.

Regarding nutritional exposure early in life, Vickers remarked that the risk of obesity among offspring is very much a U-shaped curve, with both under- and over-nutrition resulting in increased risks (Grattan, 2008). He reminded the workshop audience to keep in mind that many cases of overnutrition actually reflect micronutrient malnutrition and that many obese individuals are actually malnourished. He suggested that the similarity in phenotype may be due to malnutrition at both ends of the spectrum.

Before embarking on a review of intervention studies, Vickers commented on the similarity in phenotypic outcomes, in terms of efficacy in reversing metabolic disturbances, for a wide range of interventions tested

[5] This section summarizes information and opinions presented by Mark Vickers, M.Sc., Ph.D., University of Auckland, New Zealand.

across a range of animal models, including sheep, nonhuman primates, piglets, mice, and guinea pigs. As just one example, some of the leptin work that was originally undertaken in the rat has since been replicated in the piglet and several rodent strains.

Vickers also commented on the many different nutritional exposures, or manipulations (e.g., under-nutrition, high fat, high salt, low protein, and high sugar), that have been tested across a range of preclinical models and how they all induce a common metabolic syndrome phenotype (i.e., one featured by obesity, type 2 diabetes, heart disease, altered appetite, inflammation, and reproductive disorders). One nutritional exposure that is often overlooked, in Vickers's opinion, is the impact of high-fructose intake during pregnancy. There is very little literature on the effect of soda intake during pregnancy. He noted that he and his research team have demonstrated that as little as two cans of soda per day during pregnancy can rewire the arcuate nucleus (an aggregation of neurons in the hypothalamus) and program leptin resistance in offspring, independent of any change in maternal body weight or body composition.

Leptin

Leptin was one of the first interventions tested as a means to reverse metabolic disturbances arising as a consequence of developmental programming. Original work by Bouret et al. (2004), which Marie-France Hivert had mentioned in her presentation (see previous section), showed that replacing leptin in leptin-deficient *ob/ob* mice during the first few weeks of life could restore neural network pathways from the hypothalamus and result in essentially "normal" animals, while, in contrast, post-weaning treatment had no effect. Vickers and his research team conducted a similar experiment with outbred rats and found that maternally undernourished offspring, when fed a high-fat diet, became, Vickers said, "fatter and fatter and fatter over time" (Vickers et al., 2005). But when he administered leptin for 2 weeks during their neonatal development, the maternally undernourished offspring looked like control animals. Importantly, in Vickers's opinion, the effects were specific to animals born to undernourished mothers. Leptin had very little effect in control animals. These results have been replicated in a few other studies as well. Additionally, evidence exists to support Bouret et al.'s (2004) finding that leptin reprograms the arcuate nucleus.

Publication of the Vickers et al. (2005) report led to what Vickers described as a "big rush" of calls in the United Kingdom to add leptin to infant formula. However, subsequent studies suggested that the effects of leptin are dependent on the mother's prior nutritional status and sex (Gluckman et al., 2007; Vickers et al., 2008). Vickers stressed the importance of examining both male and female offspring and noted that many

researchers in this field do not consider the effect of the offspring's sex on outcome. Even Vickers et al. (2005) examined only female offspring. Leptin treatment in male neonates under the same Vickers et al. (2005) paradigm can elicit adverse metabolic phenotypes in later life, for example, with offspring becoming insulin resistant (Vickers et al., 2008).

With regards to leptin effects being dependent on the prior nutritional background of the mother, Gluckman et al. (2007) demonstrated that changes in *PPAR-alpha* and 11-beta-HSD2 methylation states as a result of leptin administration were directionally dependent on prior maternal nutritional status, with completely opposite effects observed depending on whether the mother was undernourished or not. Stocker et al. (2004) reported a reduction in placental 11-beta-HSD2 activity in low protein-fed mothers and a partial restoration of activity in mothers administered leptin.

GLP-1 Analogs

Vickers mentioned only one study relevant to the use of GLP-1 analogs, specifically extendin-4, as an intervention. In this study, Stoffers et al. (2003) showed that the neonate offspring of undernourished mothers had reduced beta-cell mass but that beta-cell mass and proliferation were normalized in neonates that were administered extendin-4. The normalization of beta-cell mass and proliferation was linked with a reversal of marked changes in epigenetic modifications to the pancreatic and duodenal homeobox 1 (PDX1).

Taurine

There has been quite a bit of work done on taurine, according to Vickers, including what he described as "hidden data" from two to three decades ago showing that in a rat model the administration of taurine supplement to mothers on a low-protein diet conferred completely normal pancreatic development. Taurine concentrations are known to be low in diabetic and prediabetic states, Vickers explained, with physiological plasma taurine levels playing an important role in maintaining adequate beta-cell function and insulin action. With certain liver disorders, such as maternal hepatic cholestasis, taurine is known to have a protective effect and appears to confer long-term benefits in offspring. As an example, Vickers said that members of his research group showed that 1.5 percent taurine-supplemented drinking water completely normalized the hyperinsulinemic effects of hepatic cholestasis in fructose-fed mothers (Li et al., 2015). Without taurine, the fructose diet induced a pro-inflammatory phenotype not just in the mothers but in the offspring as well, with both female and male offspring showing increased levels of inflammatory markers at birth. Importantly, in Vickers's opinion, taurine

supplementation in the mother's diet benefited not just the mother, but her offspring too, with normalized levels of inflammatory markers at birth.

Growth Hormone

Vickers's group has also done some work with growth hormone. Offspring born to undernourished mothers are typically hyperleptinemic as well as hyperinsulinemic, Vickers said. They also have very low insulin-like growth factor 1 (IGF1) levels. Gray et al. (2013) observed that rats born to undernourished mothers were fatter and their adipocytes were normally larger, but that offspring administered growth hormone during the first 2 weeks of life had normal-sized adipocytes. The offspring of undernourished mothers also had markedly increased systolic blood pressure, but the offspring that had been administered growth hormone as neonates were still benefiting with normalized blood pressure and fat mass as mature adults at 150 days of age (Reynolds et al., 2013).

In Vickers's opinion, while growth hormone administered during what, in rats, is a critical window of development is not likely to translate easily in a clinical setting, all of these findings in rats, not just with regard to growth hormone, but with leptin too, demonstrate proof of concept that a range of interventions can have lasting benefits over the life course of the offspring.

Maternal Lipid Supplementation

Vickers briefly mentioned work that his research team has done with conjugated linoleic acid, with offspring of obese mothers having significantly impaired insulin sensitivity, increased gut inflammatory markers, and altered gut taste receptors (i.e., they seem to have a preference for high-fat, high-sugar foods). But all of those effects are resolved in the offspring of mothers whose diets are supplemented with conjugated linoleic acid. The mothers benefited from the supplementation as well, with improved maternal insulin sensitivity.

Postnatal Dietary Omega-3 Supplementation

Vickers noted interesting work done by Wyroll et al. (2006) showing that offspring of pregnant rats treated with dexamethasone were hyperleptinemic by 6 months of age but that the hyperleptinemic phenotype could be completely prevented by feeding the offspring a postnatal diet rich in omega-3 (n-3) fatty acids.

Maternal Vitamin D Status

There is a high prevalence of vitamin D deficiency in New Zealand, Vickers observed, despite the amount of sun exposure, because people are always being told to cover up. The evidence for an impact of vitamin D supplementation on outcomes related to adiposity is conflicting, with the data being, in his words, "all over the place." What is known is that pre-pregnancy obesity predicts poor vitamin D status in both mothers and neonates. Recent studies have reported that vitamin D deficiency in pregnancy can result in insulin resistance, altered inflammatory profiles, and an increased risk of early postnatal obesity in offspring (Morales et al., 2015; Zhang et al., 2014).

Dietary Methyl Donors

A range of methyl donor supplements, including folic acid, glycine, choline, and mixed supplements, have been shown to have beneficial effects on long-term cardiovascular and other outcomes in offspring (Bai et al., 2012; Carlin et al., 2013; Jackson et al., 2002; Torrens et al., 2006). As just one example, Bai et al. (2012) showed that maternal choline supplementation in rats reduces low-protein-induced elevations in systolic blood pressure and fat mass in adult offspring.

Exercise/Lifestyle Interventions

Vickers identified two critical windows where physical activity has the potential to mitigate against increased obesity in offspring who were developmentally programmed to become obese: first, maternal exercise prior to and during pregnancy and, second, exercise during childhood for those at risk (Siebel et al., 2012). He mentioned that studies in rats have shown that early exercise in offspring can reduce the adiposity associated with both maternal under-nutrition (Miles et al., 2009) and maternal obesity (Santos et al., 2015). Other studies have reported that the effects of exercise are mediated in part by improved central leptin sensitivity (Sun et al., 2013) and that they are dependent on type and duration (Hopkins et al., 2010). With respect to dietary interventions, Zambrano et al. (2010) showed that dietary intervention in obese mothers prior to pregnancy reversed metabolic programming in offspring; the effects persisted into adult life, but they were sex-specific.

Catch-Up Growth Intervention

Preventing catch-up growth is, in Vickers's opinion, one of the easiest ways to prevent programmed postnatal obesity. Small offspring of undernourished mothers normally develop increased adiposity if they are allowed to catch up to controls. Howie et al. (2012) showed that keeping offspring born to undernourished mothers small during the pre-weaning period leads to them being completely indistinguishable from controls in adult life.

DHA Supplementation

With regard to docosahexaenoic acid (DHA) supplementation, Vickers said that studies suggest that the effects depend on dose, duration, and exposure (e.g., Carlson et al., 2013; Donahue et al., 2011), though he suggested that he and his colleague's findings published in Albert et al. (2015) might explain some of the variation in effects being reported. They tested 32 commercially available fish oil supplements in New Zealand and found that more than 80 percent were either heavily oxidized or contained far lower levels of n-3s than marketed.

Pre- and Probiotics

There has been a lot of work done on the use of pre- and probiotics as an intervention to reverse metabolic disturbances caused by developmental programming, according to Vickers, especially in terms of reprogramming the gut flora (Luoto et al., 2010; Thum et al., 2012). Because probiotic modification of the early gut microbiota may restrain excessive weight gain during the first years of life, it has been suggested that infant formula be supplemented with oligosaccharides to compensate for the lack of some of what is naturally present in human milk.

Vickers's Take-Home Message: One Size Does Not Fit All

The biggest take-home message from intervention studies, in Vickers's opinion, is that interventions in "intact" systems may lead to adverse outcomes, raising the question, how can those at risk of programmed disorders be identified? Additionally, some interventions yield quite profound sex-specific differences. For example, maternal methyl-deficient diets can result in metabolic disturbances in male, but not female, rat offspring.

Predictive biomarkers are being studied as a way to identify who and when to target for intervention. For example, Vickers cited Karen Lillycrop's work with cord blood methylation of RXR-alpha and its link to later childhood adiposity. The challenge, in his opinion, is what to do with

all the data, including how to distinguish between causative and associative links and how to weigh trade-offs. According to Vickers, epimutations with short-term trade-offs are likely associated with later negative health outcomes. For example, while maternal methyl donor supplementation can lead to a reduction in fatty liver disease, it can also lead to increased adipose tissue storage in offspring later exposed to a high-fat diet.

Vickers concluded with some comments about the generational nature of environmental exposure. A single maternal insult can affect not only the development of the fetus (the F_1 generation), but also the germ cells that form the next (F_2) generation. Many early studies went only to the F_2 generation, which Vickers said is not truly transgenerational because F_2 effects reflect the original insult. Most studies that have examined the F_3 and later generations have show that in many cases the phenotype is eventually resolved, although more work in that area needs to be done in Vickers's opinion.

In addition to what is known about maternal exposure, a large amount of data show that paternal exposure and transmission affect disease risk. For example, Ng et al. (2010) showed that a chronic high-fat diet in the father programs beta-cell dysfunction in female rat offspring. The effect can be partially reversed by exercise (McPherson et al., 2014).

In summary, Vickers said, the early life period of developmental plasticity offers the most effective avenue for intervention. Although reversal has been shown in a number of experimental models, both maternal and neonatal, direct translation to the clinic may prove difficult. Nonetheless, he said, the results will inform possible intervention strategies.

THE MICROBIOME AND OUR GENOME[6]

Recognition of the presence and significance of the human microbiome started to emerge around 2000, according to William Nierman of the J. Craig Venter Institute. Nierman provided a general overview of the human microbiome, beginning with what he said were "highly speculative" numbers, that is, that it contains an estimated 10 trillion bacterial cells, which amounts to about 10 times more cells than human cells and 150 times more bacterial genes than human genes. Bacteria are not the only microbial component of the human microbiome; fungi and viruses occupy our bodies as well. Altogether, the human microbiome weighs a total of approximately 3 pounds, he said.

Two striking features of the human microbiome, in Nierman's opinion, are, first, that probably half of the microbial genes in the human body are functionally unknown and, second, that the vast majority of organisms in

[6] This section summarizes information and opinions presented by William Nierman, Ph.D., J. Craig Venter Institute, Rockville, Maryland.

the human microbiome have never been cultured and therefore have never been studied in the way that scientists traditionally study bacteria.

It has been clearly established by now, Nierman continued, that a healthy gut microbiome provides many metabolic capabilities that supplement its human host's metabolic capabilities, such as digestion of complex carbohydrates, with diet playing an important role in the process. Healthy microbiomes also protect against disease. For example, a healthy stomach microbiome protects against *Helicobacter pylori*–mediated stomach cancer, and a healthy digestive tract microbiome protects against *Clostridium difficile* infection. Many disease states, particularly infectious disease states, are characterized by collapses in microbiome diversity, according to Nierman, which predisposes the human host to a number of problems, including enhanced inflammation, loss of barrier function, and increased immune dysfunction.

Of particular interest to Nierman because of his interest in the respiratory microbiome is the fact that when someone has a severe flu, the flu virus modulates the host immune system, which results in a high incidence of streptococcal pneumonia following the flu. He explained that the streptococcal bacteria are already present in the respiratory tract microbiome. In other words, people do not "catch" bacterial infections in their lungs. They might "catch" the flu virus, but streptococcal bacteria are commensal organisms that naturally live in the respiratory tract. Rather, something happens to "bring out these things."

Because of its role in maintaining health, the microbiome can be modulated in dietary and other ways to have a therapeutic effect. For example, fecal transplants have been considered as one way to treat recurrent *C. difficile* infections (van Nood et al., 2013). Nierman pointed out that the idea of fecal bacteriotherapy is not new and referred workshop participants to a 1989 paper (Tvede and Rask-Madsen, 1989). Sarah Highlander, a colleage of Nierman's at the J. Craig Venter Institute, has been exploring the use of pure cultures of lactobacilli to suppress *C. difficile* overgrowth and to cause quick remission of symptoms.

Humans acquire their microbiomes beginning at birth. Dominguez-Bello et al. (2010) demonstrated that the mode by which a baby is delivered (i.e., vaginal or Cesarean) affects the population structure of the infant microbiome. Koenig et al. (2011) followed infants for 2 years after birth, sampling changes in their gut microbiomes along the way, and Palmer et al. (2007) demonstrated that the gut microbiome approaches adult status by 1 to 2 years of age. The respiratory microbiome, on the other hand, shows what Nierman described as "wild variation" month to month even at 2 years of age.

Nierman emphasized the site-specific nature of the human microbiome—for example, with the respiratory, gut, and skin microbiomes each being

very different from each other. Body sites are like ecosystems, he said. You would not expect to find the same species in the tropical rainforest that you find in the desert. The same is true of the human body. Indeed, the goal of the Human Microbiome Project (HMP) was to characterize the microbiomes of healthy individuals at selected body sites in the oral cavity, skin, nasal cavity, gut, and vagina. The only observed overlap, according to Nierman, was between the external nasal site and the skin, which he said was not surprising, given that the external nares are so close to the skin. A striking finding in the oral cavity, in his opinion, was the dramatic differences between nearby sites, such as dental plaque and gums. While HMP data showed no apparent relationship between the microbiome at any site and gender, age, weight, ethnicity, or race, it did reveal tremendous variability at each site among individuals.

Regarding the usefulness of 16s rRNA profiling, one of the HMP techniques used to characterize the bacterial complexity of the microbiome, Nierman observed that such profiling provides a good view of relative abundance at the genus level but not at the species level (Keseler et al., 2013). A listing of what is present at the genus level is not very informative, in Nierman's opinion, because of the very important functional differences that exist not just between species within a genus, but also between strains within a species. For example, the *E. coli* K12 strain, a lab strain, is benign and is usually found in the lower intestine, whereas the *E. coli* O157:H7 strain is a highly virulent pathogen that can cause severe diarrhea and kidney failure.

Obesity and the Microbiome

Although there have been several studies of an association between the microbiome and obesity, many of them in animal models, Nierman stated that no single taxonomic signature of obesity in the microbiota of the human gut has been found (Finucane et al., 2014). Generally, however, obese individuals harbor less diverse bacteria communities, and Nierman suggested that even though the mechanisms through which gut microbes influence obesity or BMI are unknown, novel therapies aimed at restoring the gut microbiota balance could potentially help to treat obesity.

In conclusion, Nierman proposed what he called the obesity–host–pathogen–microbiome–innate immune system paradigm, modeled after the host–pathogen–microbiome disease paradigm. In his "fantasy" obesity paradigm, host genetics, microbiome metabolic function, and the innate immune system would be assembled into an obesity-causing interaction triad. He suggested, based on information being presented at this workshop, that the category of host genetics should include an epigenetic component as well.

THE EPIGENETICS OF THE MICROBIOME[7]

"We are not alone," Meredith Hullar of the Fred Hutchinson Cancer Research Center began. A large proportion of genomic DNA associated with humans is bacterial DNA located either within or on the human host. Hullar provided a brief overview of the epigenetic mechanisms of bacteria in the human gut, with a focus on DNA methylation; discussed how microbial metabolites of the host diet may influence host health via epigenetic mechanisms involving DNA methylation, noncoding RNAs, microRNAs (miRNA, in particular), or histone deacetylase inhibition; and concluded with a discussion of her research in obesity and the microbiome.

Epigenetics Within the Microbiome

Unlike methylated human DNA, which is mostly N^5 methyl cytosine,[8] bacteria have three types of methylated DNA, with the predominant one being N^6 methyl adenine and the others N^4 and N^5 methyl cytosine. The nucleotide bases are methylated by DNA methyltransferases, with the methyl group transferred from S-adenosyl-L-methionine.

In bacteria, the predominant role of DNA methylation is to protect the host bacterium against phage attack. It does so, Hullar explained, through restriction modification systems composed of methyltransferases and restriction endonucleases. The restriction endonucleases cut DNA at the internal phosphodiester bonds and recognize unmethylated four- to six-base-pair palindrome sequences. When a phage injects its DNA into a host bacterial cell, the phage DNA, because it is not methylated, is cleaved by the restriction endonucleases so that it cannot be incorporated into the host genome. Sometimes the phages escape detection and when they do, their DNA is incorporated into the microbial genome. The phage DNA ends up with the same methylation pattern as the microbial DNA, thus, the phage escapes detection by host restriction endonucleases and the phage DNA is replicated as part of the bacterial cell cycle.

Restriction modification systems within bacteria have diversified and are broadly distributed across multiple phyla, with more than 2,000 different systems involving approximately 43,000 restriction modification enzymes (Korlach and Turner, 2012). Modification of the endonucleases creates new functions for the restriction modification systems, Hullar explained, such as the DNA adenine methylase (Dam) post-replication mismatch repair system in *E. coli*. The Dam system is dictated by a methylated base in the CTAG

[7] This section summarizes information and opinions presented by Meredith Hullar, Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington.

[8] An N^5 methyl cytosine has a methyl group attached to the fifth atom in the six-atom cytosine ring.

region and affects microbial gene expression. It has been hypothesized that the changes in gene expression are associated with genes involved in phage infectivity, DNA replication, stress response, culturability, and host association (Chen et al., 2014). "These are very dynamic systems that may influence microbial function in the human gut," Hullar said.

Methylation patterns between two bacterial strains can vary substantially and may alter bacterial fitness. In a methylome comparison of *H. Pylori* 26695 and J99-R3, Krebes et al. (2014) found many different methylation markers in the *H. pylori* genomes. They hypothesized that although methylation is a key driving force in genetic diversification, the methylation in *H. pylori* may reduce natural transformation. Disparate methylation patterns were also found in two strains of *Bacteroides dorei*, the predominant genus found in the microbial gut microbiome. One was highly methylated (20,551 methylated sites), and the other lacked a DNA adenine methyltransferase gene and lacked methylation (Leonard et al., 2014). How CTAG methylation affects these two strains is unknown. Regardless, Hullar suggested that methylation patterns may influence microbial gene expression and add another layer of complexity in understanding how microbial metabolism in the human gut affects host health.

Epigenetics Between the Host and Microbiome

Both pathogenic and commensal bacteria exploit a diverse set of epigenetic mechanisms such as DNA methylation, histone modifications, and noncoding RNAs to alter host gene expression. These epigenetic mechanisms can occur directly through infection or indirectly through microbial metabolites that interact with host genes. Altered gene expression has far-reaching implications from maintenance of gut homeostasis to changes in gene expression of human host pathways associated with inflammation and adiposity.

Commensal bacteria influence gut homeostasis through differential methylation of host genes. Although exposed to a large number of commensal gut bacteria, intestinal epithelial cells are relatively unresponsive to bacterial cell wall antigens that usually promote inflammation by interacting with host innate immunity via toll-like receptors (TLRs). Takahashi et al. (2009) found that the presence of intestinal commensal bacteria increased the methylation of host TLR genes, specifically TLR4, which in turn blunted the host inflammation response. The patterns vary between the small and large intestine in mouse models. Regulation of the host gene expression via epigenetic modifications may maintain symbiosis in the large intestine because methylation was dependent on colonization with commensal bacteria. In the small intestine, methylation was independent of bacterial colonization, and the degree of methylation varied. TLR4 genes

were more methylated in the differentiated epithelial cells at the top of the crypts than the bottom of the crypt (Takahashi et al., 2011). According to Hullar, others have found this to be the case with other TLRs (Kellermayer et al., 2011).

Some pathogenic bacteria also escape host surveillance by altering host miRNA associated with inflammation and immune response. Upon infection, miRNAs associated with some genes, Hullar explained, are up-regulated, while other miRNAs linked to inflammation response are down-regulated. This approach is employed by the well-known pathogens *Listeria*, *Salmonella*, *Helicobacter*, and *Mycobacterium* species (Maudet et al., 2014). Hullar emphasized, however, that how a host responds to a pathogen is influenced by the commensal microbiome. In a comparison of germ-free versus conventionalized mice, Archambaud et al. (2013) showed that all of the miRNAs in response to an oral *Listeria* infection were down-regulated in the presence of commensal bacteria, suggesting that healthy commensal microbiota can provide a barrier against some pathogens.

Host exposure to microbial fermentation products varies, in part, based on the distribution of the different microbial metabolic pathways present in the gut and may influence disease risk associated with obesity. This moves beyond the idea of caloric excess causing obesity and incorporates the idea that microbial fermentation of dietary compounds may alter host gene expression (Hullar and Fu, 2014) in host pathways associated with obesity. Compounds that escape metabolism in the small intestine enter the colon where they undergo microbial metabolism. The metabolites produced by microbial fermentation influence host pathways, and increased fermentation products from fiber have been inversely associated with obesity. Fiber is fermented into short-chain fatty acids, acetate, butyrate, and propionate. However, the fermentation process is complex, Hullar said. For example, the short-chain fatty acid butyrate can be produced via four different metabolic pathways by different groups of bacteria, although not all human guts have a microbial community with all four pathways (Vital et al., 2013). Host exposure to microbial fermentation products varies, based in part on the distribution of the different microbial metabolic pathways present in the gut, and may influence disease risk associated with obesity.

Hullar described several biological consequences of microbial fermentation in the colon and epigenetic mechanisms that influence gene regulation in host pathways in obesity. Short-chain fatty acids produced in the gut can move into the blood, enter systemic circulation, and increase lipogenesis, fatty acid oxidation, and adipogenesis. They have also been associated with increased production of the satiety peptide YY (PYY) and alter gut epithelium integrity, according to Hullar. Short-chain fatty acids modulate adiposity via a direct effect on several genes, including fasting-induced adipose factor (Fiaf) involved in fatty acid metabolism,

G-protein coupled receptor 43 (Gpr43) involved in obesity, and peroxisome proliferator-activated receptor gamma (Pparγ), which regulates fatty acid storage, and glucose metabolism histone deacetylase (Hdac) involved in epigenetic modification of the lysine residue in histones. Recently, Lukovac et al. (2014) reported that many genes associated with host fatty acid metabolism in gut tissue are influenced by exposure to short-chain fatty acids. More specifically, butyrate and propionate significantly enhanced the expression of genes associated with histone deacetylation (Hdac3, Hdac5) and fatty acid metabolism (Fiaf) and decreased expression of genes associated with fatty acid storage (Pparγ) and satiety (Gpr43).This suggests that microbiota trigger specific transcriptome responses in host epithelium, most likely depending on the metabolic products produced by different bacteria and fermentation pathways.

The studies Hullar presented pointed, she said, to a potential role of the microbiome in the epigenetics of obesity. She presented microbiome-mediated epigenetic mechanisms that alter host gene expression through three mechanisms: methylation, miRNA, and histone deacetylase (HDAC) inhibitors via microbial metabolites derived from diet. Westernized diets, which are high in fat and sugar, select for a gut microbiome composition that is associated with disease risks common to industrialized societies, such as obesity, cardiovascular disease risk, and certain cancers. Exposure to microbial metabolites from a Westernized diet may influence the epigenetic regulation of gene expression in host pathways associated with higher disease risk (Lukovac et al., 2014). In addition, several studies have shown that the consumption of a Westernized diet in primates or mice alters the microbiome composition in the offspring (Ma et al., 2014; Myles et al., 2013). Because the microbiome is transferred to the offspring from the mother, there may be a generational influence on gene expression through epigenetic modification by the microbiome, although this has not been shown in humans. Hullar said that the role of epigenetic mechanisms that influence the control of gene expression both within the microbiome and between the microbiome and host is a "burgeoning area." She encouraged greater consideration of the interaction between microbial metabolism of the human diet and epigenetics. In her opinion, efforts are hindered by small sample sizes and the lack of prospective studies. Without prospective studies, it is difficult to know how the early life microbiome may influence obesity later in life or transgenerational effects of the microbiome on gene expression modulated by microbially mediated epigenetic mechanisms.

Diet, Obesity, and the Microbiome

Diet affects adiposity through changes in fuel availability that alters energy balance and increase lipids, adipocytes, macrophages, and inflamma-

tion. The microbiome also plays a role, and early work by Turnbaugh et al. (2009) showed an increase in microbial genes associated with carbohydrate metabolism and fatty biosynthesis in individuals who were obese. This suggested that people who are obese have different energy-gathering or energy-yielding capacity than lean people based, in part, on the composition and function of the gut microbiome. These findings led Hullar and her research team to ask whether the microbiome is associated with obesity.

To study the association between the microbiome and adiposity, Hullar and Johanna Lampe (unpublished data) collected fecal samples, measured adiposity by dual-energy X-ray absorptiometry, and obtained a 3-day food record from a cross-sectional sample of 107 women, aged 40 to 45 years. Based on an examination of 16S rRNA genes from the fecal samples, the researchers were able to group the women by the dominant microbe found in their gut. Most participants' microbiomes were dominated by *Prevotella*, *Bacteroides*, or *Pyramidobacter*, and the groups were associated with dietary intake (Hullar et al., 2015). The researchers also found that the women in the *Bacteroides* group had increased animal protein intake and decreased fiber and vegetable protein intake and that the women whose guts were dominated by *Firmicutes* had increased fiber and vegetable protein intake, similar to other reports (Wu et al., 2011). Even after correcting for fiber intake (because of an observed inverse relationship between fiber intake and adiposity), Hullar and Lampe still observed a significant association between the microbiome and adiposity.

Hullar concluded by mentioning another study that she is currently collaborating on—a multiethnic cohort study on disease risk, obesity, and fat distribution. Health risk due to obesity varies among ethnic and racial groups in association with the distribution of adipose tissue (Cleary and Grossmann, 2009; Conroy et al., 2011; Cummings and Schwartz, 2003; Lim et al., 2012). The researchers will be analyzing four different potential predictors of adiposity: the exposome, genetic variation, metabolomics, and the microbiome.

TOXIC STRESS AND CHILDHOOD OBESITY[9]

Antonio Convit of the New York University School of Medicine remarked that he would be talking about the toxic effects of over-nutrition on the brain rather than toxic stress per se. He agreed with earlier remarks that the greater focus of discussion around childhood obesity should be on metabolic dysregulation, which he said is "certainly what has the biggest impact on the brain." For example, data on lean versus obese children with

[9] This section summarizes information and opinions presented by Antonio Convit, M.D., Nathan Kline Institute and New York University School of Medicine, New York.

or without insulin resistance or type 2 diabetes reveal a step-down function of cognitive performance. Specifically, lean children performed the best in all of four school-relevant cognitive domains tested (spelling, arithmetic, general memory index, and psychomotor efficiency), obese children without marked insulin resistance performed second best, and obese children with insulin resistance had the worst marks among obese children with type 2 diabetes.

Compared to adults, Convit said, children with metabolic dysregulation are much more cognitively impaired. In a study of a group of a little more than 100 children, with the control group consisting of individuals who may meet one or two of the criteria for metabolic syndrome but not three or more criteria, Convit and his research team found that several cognitive domains were affected by metabolic syndrome: academic achievement (i.e., based on Wide Range Achievement Test), executive function, attention, and psychomotor efficiency. As the number of criteria for metabolic syndrome increased, cognitive performance decreased. With adolescents, researchers use slightly modified criteria to define metabolic syndrome, Convit explained. Instead of impaired fasting glucose, they use insulin resistance, which is a more sensitive measure obtained from the Quantitative Insulin Sensitivity Check Index (QUICKI). The other criteria are blood pressure, triglyceride levels, abdominal obesity, and high-density lipoprotein (HDL).

These findings raised the question for Convit, what is happening in the brain in children with metabolic dysregulation? He and his research team decided to address the question by using a noninvasive MRI technique called diffusor tensor imaging to examine microstructural integrity of the brain's white matter. White matter is what connects different parts of the brain. Convit explained that a molecule in a huge vat of water can move in all directions equally. That movement is known as isotropic diffusion. But if the movement of a water drop is constrained, as it is at the top of a pipe for example, the water will move preferentially along the long axis of the pipe. Axons in the brain are basically pipes that happen to be covered with myelin. Movement of water in a pipe, or axon, is known as anisotropic diffusion, with diffusion along the x-axis (the long axis of the pipe, or axon) greater than diffusion along the y-axis (the narrow width of the pipe, or axon). Anisotropic diffusion can be measured in a magnet (i.e., a magnet in the MRI system) and a map of anisotropy produced (i.e., in the MRI image). For example, Figure 4-1 shows the difference in fractional anisotropic (FA) maps between a 16-year-old male with metabolic syndrome and a 16-year-old control male, with the red indicating where the white matter fibers are most compact (in the corpus callosum, internal capsule, etc.), that is, where water is most constrained. The yellow indicates less tightly packed white matter. The green areas are where water is least constrained, that is, in the

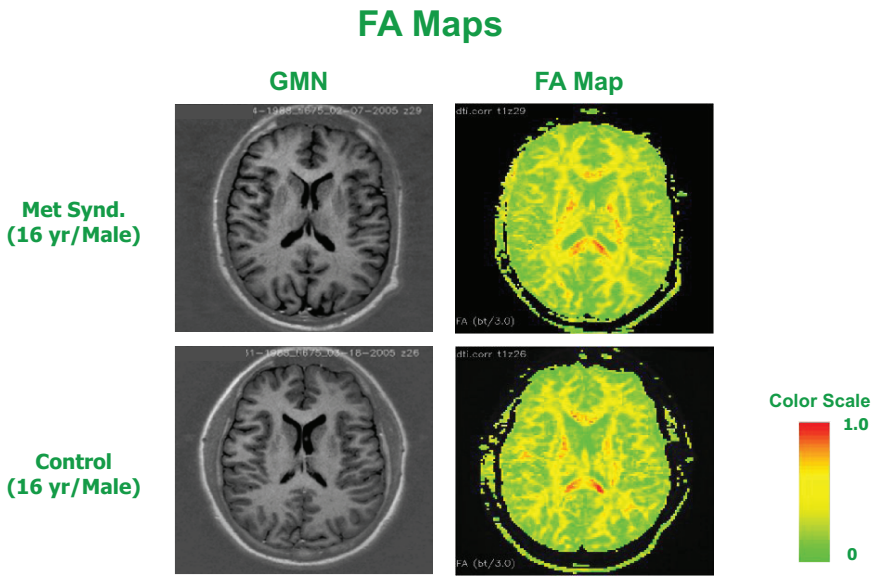

FIGURE 4-1 Gradient moment nulling (GMN) images and fractional anisotropic (FA) maps of the brains of a 16-year-old male with metabolic syndrome and a 16-year-old male without metabolic syndrome. The reduced red and yellow areas in the FA map of the child with metabolic syndrome reflect reduced white matter microstructural integrity.
SOURCE: Presented by Antonio Convit on February 26, 2015.

gray matter where the cerebrospinal fluid and neurons are located and where only membranes can prevent water from moving freely.

Using FA mapping, Convit and his team compared, voxel by voxel, the brains of children with metabolic syndrome to those without and found a significant reduction in white matter microstructural integrity in children with metabolic syndrome (Yau et al., 2012). They also measured hippocampal volume and found it was reduced in children with metabolic syndrome compared to control children. Although the same finding has been reported for both children and adults with type 2 diabetes, according to Convit, the Yau et al. (2012) results suggest that even children who are just prediabetic already have reduced hippocampal volume and more brain atrophy than children who are not.

Not all factors that contribute to metabolic syndrome are equal, in Convit's opinion. Based on a multivariate analysis of all factors, insulin resistance appears to be the driver, not cholesterol, blood pressure, or any other measured factor. The higher the QUICKI score—so the more insulin

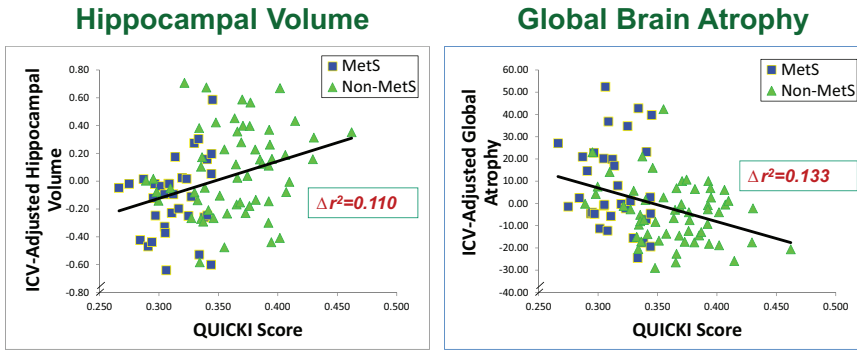

FIGURE 4-2 Associations between insulin sensitivity and hippocampal volume (left) and global brain atrophy (right).
NOTE: ICV = intracranial vault; Mets = metabolic syndrome.
SOURCE: Presented by Antonio Convit on February 26, 2015, from Yau et al., 2012.

sensitive a child is—the greater the hippocampal volume and the less global brain atrophy, with both outcomes adjusted for intracranial vault size (see Figure 4-2) (Yau et al., 2012).

In terms of a mechanism that might explain metabolic dysregulation–associated brain problems in adolescents, Convit suggested that microvascular dysfunction likely plays a role. He described what happens to your brain when you are trying to leave the house but cannot find your keys: the parts of your brain that are trying to help you find the keys get activated and start using glucose extremely avidly. However, it is known from microdialysis experiments in animals that the glucose transporter at the blood–brain barrier has a low Km value,[10] meaning that it is fully saturated at normal glucose levels and, Convit said, "can't move any faster to get you more juice to those parts of the brain that need it." The only way to shuttle more glucose to those parts of the brain that need it is to expose more glucose transporters by relaxing the capillary bed. But with insulin resistance, endothelial dysfunction compromises the ability to relax the capillary bed. The same may be true with inflammation, he said.

One way to noninvasively examine microvascular dysfunction in children is by examining their retinas and measuring their retinal vessels. Convit explained that, developmentally, retinal vessels come from the same place that cerebral microvasculature does, and they are controlled by similar physiology. By having a child sit in a dimly lit room and dilating his or her pupils, researchers can take a picture of the eye and measure both the veins and

[10] The Km value is an indicator of the affinity of the transporter for glucose molecules, with a low Km value suggesting a high affinity.

arteries across the retina; the larger the artery, the healthier the individual. High blood pressure and dyslipidemia are just two of many factors that reduce the size of the retinal arteries. In a study of adolescent boys and girls, Convit and colleagues found that, when controlling for high blood pressure and other confounding factors (which together explained about 9 percent of the variance) as well as for vessel size (which explained about 33 percent of the variance), BMI and insulin resistance explained about 20 percent of the variance in arterial size (Tirsi et al., 2013).

In summary, Convit reiterated that retinal arterial size is potentially a very noninvasive way to obtain a cheap biomarker for microvascular dysfunction and, by extension, metabolic dysfunction. Using MRI technology and other methods, he and his team have found huge vascular responses in the brain under certain conditions, but with children who are insulin resistant showing blunted vascular responses.

Potential Public Health "Handles" for Intervention

Convit identified two easy public health "handles" to reduce or prevent metabolic dysfunction in adolescents: fitness and sleep. Convit and colleagues measured fitness in children in a laboratory setting and related their fitness measures to weight–height ratios and found that weight–height ratios accounted for 85 percent of the variance in fitness. The findings suggested to Convit a "very tight relationship between the weight–height ratio in the child and how fit the child is." More importantly, in his opinion, when fitness was plotted against insulin sensitivity (QUICKI score), there was a direct correlation, with fitness increasing as insulin sensitivity increased. According to Convit, more sophisticated measures of insulin sensitivity have yielded the same results. He said, "No matter what measure of insulin sensitivity we use, we get the exact same measurements."

Convit observed that when school budgets are cut, it is usually gym and art that are cut first, with more investment in "academic" time. The problem with that strategy, Convit said, is that according to a New York City (NYC) Department of Health and Mental Hygiene study of 550,000 students in NYC public schools, children with higher BMIs are less fit than children with lower BMIs (Egger et al., 2009). In addition to finding a correlation between BMI and fitness, when the researchers compared children with the top third fitness scores to those with the bottom third, they found statistically different grades and, more importantly in Convit's opinion, statistically different standardized state exam results. When they compared children with the top 5 percent fitness scores to those with the bottom 5 percent, they found that the more fit children scored 36 percentile points higher on standardized tests than the less fit children. "That's the difference between an A and a D," Convit said. He noted that those children are at

the extremes of the distribution and that some of the unfit children may have had other illnesses, such as asthma, that could have contributed to their being unfit. Nonetheless, in his opinion, the results demonstrate that fitness is a key predictor of academic performance.

Regarding sleep, Convit said that the sleep of a healthy athletic individual can be manipulated such that within 1 week the individual will become insulin resistant. Researchers can do that in a sleep lab by disrupting sleep every time an individual enters slow wave sleep. Slow wave sleep is recuperative sleep that occurs during the first 3 to 4 hours of sleep. Based on a recent animal study, slow wave sleep appears to play an important role in cleaning toxins out of the brain. According to Convit, slow wave sleep is important not just for recuperation and cleaning toxins out of the brain, but has also been shown to be associated with insulin resistance and inflammation. The more slow wave sleep that a child has, the more insulin sensitive he or she is and the less inflammation he or she has.

Conclusion

In conclusion, Convit summarized that there are clear brain abnormalities associated with obesity and metabolic dysregulation and that the abnormalities appear to be driven by insulin resistance, with the impact of insulin resistance on cerebral vascular reactivity a plausible mechanism. In his opinion, researchers may be able to use something as simple as retinal arteriolar width as a biomarker in field trials and in other situations requiring noninvasiveness. Meanwhile, he views fitness and sleep as two interventions that could be implemented to incentivize children to stay healthier.

PANEL DISCUSSIONS WITH SPEAKERS

The Session 3 presentations were accompanied by two separate panel discussions, one following Mark Vickers's presentation and the other following Antonio Convit's presentation. Both panel discussions are summarized together here.

Phenotypic Outcomes Associated with Maternal Under-Nutrition Compared to Over-Nutrition

Matthew Gillman asked Karen Lillycrop and Mark Vickers why they thought similar phenotypes emerge at both "ends" of prenatal nutrition, that is, why the risk of obesity and other metabolic dysfunctions is similar for both under- and over-nutrition during pregnancy. Vickers replied that although many people associate obesity with macrosomia and fetal overgrowth, his models have associated maternal obesity with fetal growth

restriction and placental insufficiency in both structure and function. Moreover, most rat models of maternal obesity do not lead to any overgrowth or macrosomia at all. He suspected that the pathways may be similar for both under- and over-nutrition, resulting in similar phenotypic outcomes. With respect to what those outcomes are, he described offspring at both ends of the spectrum tending to be hyperleptinemic and hyperinsulinemic, with reduced birth weight and length.

Lillycrop added that it would be very interesting to compare the under-nutrition and over-nutrition pathways in humans and examine epigenetic changes, or signatures, associated with each pathway. If over-nutrition is in fact a consequence of malnutrition in terms of micronutrients, she agreed that the pathways might not be so disparate.

The Mismatch Hypothesis

Gillman asked whether a high-fat diet in utero followed by a high-fat postnatal diet would be "good" based on the mismatch hypothesis, saying, "That's a perfect match." Kevin Grove replied that it depends on the severity. With significant placental insufficiency and a small-for-gestational-age outcome, he suggested that, yes, long-term fat may be "the answer." The metabolic outcomes being observed suggest that high-fat-diet offspring may deal better with fat than control-diet offspring. However, the other complications that eventually emerge in association with a high-fat diet, such as inflammation and brain complications, probably end up in the long-term causing more damage.

"We don't know," was Vickers's answer. He observed that many people argue that the mismatch hypothesis works for only one side of the equation, that is, under-nutrition. But, in his opinion, no one has done enough work to see if a high-fat in utero diet matched with a high-fat postnatal diet could result in healthy infants.

Lillycrop added that her research group has bred mice over four generations on a high-fat diet and has observed some adjustment in terms of metabolism. But when the mice start to age, she said, the dysfunction begins to appear.

The Placenta

The speakers were asked whether placental shape or length and width, which could be measured by ultrasound, might be useful as an indicator of risk of obesity. Grove replied that he had spoken many times with David Barker about that possibility. The challenge, Grove said, is that measurements of the size and shape—or as Barker described it, the "ovality"—of the placenta and their association with dysfunction are typically determined

postpartum. It is not clear what a dysfunctional placenta looks like in pregnancy. Moreover, placental shape and size are highly variable among women, and they depend on how many pregnancies a woman has had. He said that such an indicator would be possible, yes, but he encouraged finding a better biomarker.

Testing Dietary Interventions in Animals Versus Humans

In Jacob Friedman's opinion, the next necessary step to studying all of the various nutritional interventions being studied in animal models is to test them in "higher" organisms and to be aware of what might be different in humans. In an effort to translate some of what has been learned in animal models to humans, his research team conducted what he said was the first randomized control diet–treated study (Hernandez et al., 2014). They randomized pregnant women diagnosed with gestational diabetes into either a "conventional" diet, that is, one that was low carbohydrate and high fat, or a high-complex-carbohydrate, low-fat diet. They found that, over time, patients on the high-complex-carbohydrate, low-fat diet had better insulin resistance scores, suppressed fat cell inflammation (based on biopsy), and fewer fat infants. Freidman emphasized that as part of the study, patients were given every single calorie of their respective diets. They did not make their own choices and then tell the researchers what those choices were. He implied that dietary interventions work, or can work, when people know what to eat. "If you're trying to get into the real world," he said, "this is where you have to start."

Grove added that, in his opinion, more than being told what they need to eat, mothers who are obese need hope. He described obese mothers as feeling helpless, anxious, and depressed that their children are going to experience some of the same problems they have experienced. Part of giving them hope is telling them that something can be done and that diet is something that can be changed quickly. Then they become motivated. "I think that has to be the place we start," he said.

In response to Friedman's comments, Vickers remarked that the order of experiments is an interesting issue, that is, whether animal model or human studies are conducted first. He noted that a small clinical trial is under way in New Zealand testing Viagra in women at risk of very low birth weight infants, despite all animal data pointing to long-term negative consequences.

The challenge with dietary interventions in humans is not just *what*, but *when*, Marie-France Hivert reminded the workshop audience. She mentioned that screenings for gestational diabetes or gestational hyperglycemia are typically conducted in the middle of pregnancy or later. But given that the early phases of fetal development are especially sensitive to epigeneti-

cally mediated programming, perhaps these conditions should be screened and treated earlier.

Hivert's comment prompted Lillycrop to mention a couple of diet- and exercise-based intervention trials currently under way in the United Kingdom that target obese mothers about midway through pregnancy. She noted some success with those interventions at that point in pregnancy. Given that the most plastic period of development may be earlier, she wondered if the interventions should be targeting mothers earlier during pregnancy—for example, at 6 or 7 weeks. But the challenge with that, she said, is that most high-risk women are not aware at that point that they are pregnant.

Hivert added that when conducting dietary intervention studies in humans, researchers need to start thinking about longer-term outcomes. Most outcomes being measured for dietary interventions are short-term maternal or birth outcomes. Hivert's comment prompted Friedman to ask about the metformin trial, which he said is following children whose mothers were administered metformin and measuring outcomes. The children, he said, should be about 7 or 8 years old now. Vickers replied that while he did not know much about that particular trial, he was under the impression that the long-term outcomes were not optimal. He agreed with Hivert that, too often, people do not see past the immediate effect (of an intervention). But there are long-term trade-offs, he said. Again, he mentioned the Viagra trial under way in New Zealand and remarked that while administration of the drug allows for a fetus to be born and viable, by maturity the child will be hypertensive, obese, and insulin resistant. The long-term trade-offs are, he said, "pretty bad." He said that the metformin trial sounds similar. He also mentioned some vitamin supplement studies in the United Kingdom, where researchers are observing long-term outcomes opposite to what was expected.

Strain Specificity in the Microbiome

Given the hundreds of thousands of microbes living in the human body, Stephen Krawetz was curious about ways to identify other functional genes besides those identified via 16S rRNA sequencing. William Nierman replied that one can analyze the microbiome at different levels depending on resources. 16S rRNA sequencing is the quickest and less expensive way to obtain information. At the other end of the scale, he said, is whole metagenomic sequencing, which provides very good coverage of most of the organisms present. Alternatively, polymerase chain reaction (PCR) assays can be used to isolate and sequence individual genes. Meredith Hullar added that in one of the studies she had described—the Vital et al. (2013) study on the production of the short-chain fatty acid butyrate by four different metabolic pathways—the researchers created sequencing primers

specific to those pathways. That seems to be the trend right now, she said. She also replied that in order to identify the functional genes identified by metagenomic sequencing, it is helpful to relate the sequences to a database of known bacterial representatives. Historically, genome sequencing was dominated by human pathogens, while the genomes of bacteria in healthy individuals were underrepresented. Despite the great progress of the HMP, Hullar replied that there is more work to be done to expand the microbial genome reference database.

Microbiome Transplants

Jacob Friedman expressed fascination with an observation made in the Netherlands that transplanting a bacterial microbiome from a healthy teenager into an individual with metabolic syndrome can lower the recipient's insulin resistance and improve his or her health. He observed that the same thing can be done with a gnotobiotic mouse. However, eventually, with both humans and gnotobiotic mice, their health reverts to what it was before the transplant, raising the question for Friedman, how far down, taxonomically, does one need to go to understand which microbe to transplant and is it even possible to go down to the sub-species level?

Nierman replied that the work he had mentioned by Sarah Highlander involved single species management of, in many instances, near fatal *C. difficile* infections in antibiotic-treated humans. As far as he was aware, she identified three or four individual strains of *Lactobacillus* that brought about rapid remission of symptoms. He emphasized, however, that even without an intervention, after a disturbance, whether that disturbance is a severe episode of antibiotic treatment or a dramatic alteration in diet, the microbiome eventually returns on its own to something resembling its former state. "But it takes a bit of time to achieve that," he said. He explained that the transient nature of the change observed after a microbiome transplant is a consequence of the transient proliferation of whatever it is that has been put there to compete with everything else that is there. Eventually, over time, the microbiome returns to its former state.

Effects of Metabolic Dysfunction on the Brain

In Kevin Grove's opinion, metabolic phenotypes are "the least of our worries." He thinks that some of the behavioral responses being observed in his animal studies are of greater concern. He said, "When you start messing with social cues and cognition, you've got lifelong problems in your whole community."

Antonio Convit was asked if he had redone any of his retinal arterial or sleep analyses on adolescents after significant weight loss to see if the

weight loss affected any of the measured parameters. Convit said that he has not. He is currently in the process of trying to obtain funding for a study to longitudinally track people before and after bariatric surgery and to assess the effect of surgery on the brain. He said that existing evidence suggests that bariatric surgery and massive weight loss improve cognition, but the mechanism is unknown.

Robert Waterland asked Convit if there are any national data suggesting that the increase in adiposity over the past few decades, including among children, has affected academic performance. Convit replied that he was unaware of any nationwide longitudinal data. He noted that the National Health and Nutrition Examination Survey (NHANES) has been tracking children and contains some educational information, but he did not know what those data have shown. Also, he noted that the difference in cognition that he and his research team reported among NYC public school children was only about 10 percent. So children who have metabolic dysregulation are not necessarily performing abnormally, he said. But they are not performing as well as those who do not have metabolic dysregulation. He reiterated the importance of fitness in adolescents. Even if parents have to pay their children to play sports, he said, "that's going to cost you a lot less than dealing with all the complications of not playing the sports."

5

Real-World Application

OVERVIEW

Given the dynamism, complexity, and context-dependency of childhood obesity etiology, it is difficult to translate research results, especially findings from animal experiments, into real-world application. In Session 4, moderated by Debra Haire-Joshu of Washington University in St. Louis, the speakers explored in detail some of the challenges—as well as the opportunities—for real-world application of research into childhood obesity. This chapter summarizes the Session 4 presentations and discussion.

According to Aryeh Stein of Emory University, the Dutch Hunger Winter of 1944–1945, which Karen Lillycrop had briefly mentioned in her earlier presentation, has long been recognized by researchers as a useful period for studying the effects of short-term hunger on subsequent generations. Stein summarized evidence from those and other studies on "real-world" human prenatal exposure to famine and its effects on obesity-related outcomes. In addition to the Dutch Hunger Winter and other periods of famine during World War II, researchers have examined next-generation obesity-related outcomes of the China famine of 1959–1961 and the Biafra, Nigeria, famine of 1967–1970. Stein emphasized that all of the studies he surveyed are flawed by fundamental confounding factors, the main one being that women become amenorrheic when food is restricted, so fertility drops, making it impossible to know how children born to fertile women are different from those who would have been born if fertility had not dropped. Additionally, none of the studies can separate the effects of hunger from the effects of other stressors, such as the extremely cold temperatures of the

Dutch Hunger Winter. That said, Stein observed two general conclusions: (1) female offspring appear to be more vulnerable to prenatal exposure than male offspring, and (2) gestational exposure appears to have a greater effect than later exposure.

Following Stein's presentation, the focus of the workshop shifted to the social, health policy, and clinical implications of the wide range of animal and human evidence being discussed. Sarah Richardson of Harvard University explored the implications for women, particularly poor women of color, of the focus on female reproductive bodies as the central site of obesity-related epigenetic programming. She expressed concern that unless some critical issues are attended to, the public discourse around epigenetics and obesity is likely to become deterministic and stigmatizing in a way that threatens women's reproductive autonomy. When communicating about epigenetic science in the public sphere, she urged greater emphasis on the complexity of the science, differences between animal and human studies, the role of paternal as well as maternal effects, and the need to seek societal changes rather than individual solutions.

In his discussion of the role of scientific evidence in Developmental Origins of Health and Disease (DOHaD) health policy development and implementation, Matthew Gillman of the Harvard School of Public Health agreed with Stein that obesity prevention calls for a greater focus on the environment and policy and less emphasis on the individual. He emphasized the importance of evidence in policy decision making and identified ways to improve DOHaD etiological evidence from both animal and human studies. He urged researchers who rely on animal models to harmonize experimental designs and measures and to report more null results. He suggested that more innovative experimental designs and analyses and more comparing and contrasting across studies could help overcome the confounding limitations of human observational research. He also discussed how prediction models, risk–benefit and intervention studies, long-term simulation modeling, and natural evaluation experiments can help inform DOHaD policy.

Finally, Shari Barkin of the Monroe Carell Jr. Children's Hospital at Vanderbilt University brought the discussion full circle by reiterating what Sandra Hassink had emphasized in her opening remarks, that is, the clinic setting is a ripe environment for implementing childhood obesity interventions. The challenge is, how? Based on recent research, Barkin identified several potential clinical interventions for improving offspring obesity and metabolic dysfunction outcomes. For example, evidence suggests that maternal poor nutrition during pregnancy combined with rapid infant catch-up growth leads to increased metabolic dysfunction in offspring. A potential clinical application of this knowledge is the promotion of appetite regulation during infancy. But again, how? One of the objectives of the Greenlight Intervention Study, Barkin said, is to develop methods for

training providers to teach parents how to recognize hunger and satiety cues in their infants and how to soothe infants in ways that do not involve only feeding. Based on results from that and many other studies, Barkin suggested several interventions that could be implemented in the pediatric clinic, such as providing group visits or health coaching calls to pregnant women and setting nutrition and activity goals for parents as well as for toddlers.

PRENATAL EXPOSURE TO UNDER-NUTRITION AND OBESITY RISK IN ADULTHOOD[1]

Aryeh Stein of Emory University reviewed the current scientific literature on prenatal exposures to famine and food shortages and their effects on adult body weight and obesity risk. He used as his starting point a review study coauthored by him and L. H. Lumey of Columbia University (Lumey et al., 2011). For this workshop presentation, his research assistant, Bernice Thomas, supplemented the Lumey et al. (2011) findings with a PubMed search. The search yielded 11 relevant studies covering 3 famines or periods of famine: (1) World War II, (2) China, and (3) Biafra. Stein did not conduct a formal meta-analysis, but rather he reviewed some of the central themes across the 11 studies.

Famines of World War II

The most famous famine episode of World War II was the Dutch Hunger Winter, a 5-month period of food shortages during the last winter of the war (1944–1945). According to Stein, the Dutch Hunger Winter has long been recognized as a great period for studying the short-term effects of famine and food deprivation on the next generation. First among those studies was Ravelli et al.'s (1976) analysis of the medical examination data of 19-year-old males born during the famine who were categorized as being either exposed or unexposed to the famine based on where in Holland they were born. Individuals born in the urban areas of western Holland were considered exposed. Those born outside those areas were considered unexposed. Individuals were also grouped according to the timing of exposure during gestation. The authors reported that individuals exposed during early and middle gestation had an increased prevalence of obesity at age 19 compared to individuals born in unaffected areas and that individuals exposed during very late gestation had a lower prevalence of obesity compared to individuals born in unaffected areas (i.e., obesity as defined

[1] This section summarizes information and opinions presented by Aryeh Stein, M.P.H., Ph.D., Emory University, Atlanta, Georgia.

by 120 percent of the standard set at the time by the Metropolitan Life Insurance Company). Stein pointed out that the increased prevalence of obesity among 19-year-old males exposed to famine during early or middle gestation was a doubling (from 1 percent to 1.9 percent). However, according to Stein, Lumey has access to the original data, and he replicated the analysis using more current standards of obesity—that is, BMI—and found no differences in BMI among the groups.

The Ravelli et al. (1976) study was a retrospective cross-sectional sample of 19-year-olds. Stein's literature search yielded two relevant Dutch Hunger Winter studies, both of which used prospective birth cohort approaches. The first, Ravelli et al. (1999), followed a group of men and women born in the main Amsterdam maternity hospital up through the age of 50. The study included a total of 741 men and women, 298 of whom were exposed to the famine at some period of gestation. The data were stratified by early, middle, and late exposure in non-overlapping cohorts and were further stratified by gender. The researchers reported that women exposed to famine during early gestation were 7.9 kilograms heavier than unexposed women, representing a 7.4 percent increase in BMI. They reported no detectable differences in men.

Stein's research group independently replicated the Ravelli et al. (1999) study a few years later among what were then 59-year-olds (Stein et al., 2007). They included in their analysis three additional birth institutions, including two maternity hospitals and one midwife training school. The study included a total of 956 individuals, 350 of whom were exposed to the famine at some period of gestation. They stratified the data into four partially overlapping gestational exposure periods. They were unable to replicate the large increase in body weight in the early gestational group. Instead, they found a consistent 4 percent kilogram increase among women exposed to famine at any time during gestation relative to the unexposed controls. They found no evidence of any effect in men except in men exposed very early in pregnancy. Stein reminded his audience that sexual differentiation in humans occurs in the first few weeks of gestation.

In addition to the Dutch Hunger Winter, the Leningrad siege of 1941–1944 has also been studied for its association with adult body weight. Stanner et al. (1997) collected data among survivors of the siege who were recruited in and around Leningrad in the 1990s, with an average age of 52 years. The authors grouped the recruits into one of three groups: (1) those born in the siege area (i.e., German-occupied areas of Leningrad) before the siege, (2) those born in the siege area during the siege, and (3) those born outside the siege area. They were unable to identify any differences in BMI among the three groups. They reported a somewhat lower skin fold ratio in women in the unexposed group compared to the other two groups.

Finally, Stein identified a fourth relevant World War II study pub-

lished in the *Israel Medical Association Journal* (Bercovich et al., 2014) on individuals born in Nazi-occupied parts of Europe during the war and therefore whose mothers could be assumed to have been exposed to food restrictions as well as other stresses associated with being Jewish in German Europe. Stein referred to the sampling of exposed individuals as "snowball sampling" among individuals living in Israel and known to be Holocaust survivors by Holocaust survivors' organizations. The control group included individuals from the Israel National Health Interview Study. It was a small study, Stein said, with 70 individuals in the exposed group and 230 individuals in the control group. The mean age at assessment was 69 years. The researchers reported a higher prevalence of dyslipidemia, diabetes, and hypertension in the exposed group, compared to the unexposed group, and about a two-unit increase in BMI among males in the exposed group compared to males in the unexposed group (i.e., 29.1 in the exposed group compared to 27.0 in the unexposed). They reported no difference in BMI for females.

China Famine, 1959–1961

There have been several studies of the long-term consequences of being born during the 2-year China famine of 1959–1961. In Stein's opinion, probably one of the best is Huang et al. (2010). The authors used a variety of approaches to try to model the deficit in population count attributable to infertility during the famine by examining how urban versus rural areas fared. Both areas suffered famines, but because mass shipments of food were sent from rural to urban areas, rural areas suffered a more severe famine. The researchers reported that individuals born before the famine had higher BMIs than individuals born after the famine, but that individuals born during the famine had lower BMIs than individuals born after the famine. Stein pointed out that the famine caused perhaps 50 million deaths, most of which were children, and that one of the limitations of the study is that it did not account for differences between the children who died during the famine versus those who survived and became part of the study sample.

In a second study of the China famine, Yang et al. (2008) reported a significantly higher BMI among women in three different famine groups (born in 1959, 1960, or 1961) compared to control women born in 1964. A limitation of that study, Stein pointed out, was uncontrollable confounding by age, that is, as people age they tend to put on weight.

In another study, Wang et al. (2010) examined individuals at approximately 50 years of age who were born before the famine (the toddler group), during the famine (gestational), or after the famine (controls). They reported that among women, but not men, those born before the famine had a more pronounced odds ratio for overweight (1.48) than those born

during the famine (1.26) and a significantly greater odds ratio for obesity (1.46) than those born after the famine. The authors reported no significant differences in men.

In a fourth study of the China famine, Wang et al. (2012) reported lower odds of obesity in men exposed during fetal development compared to a unexposed control group.

Biafra, Nigeria, 1967–1970

Stein found one study of the long-term effects of exposure to the Biafra famine, a consequence of the Nigerian Civil War in the 1970s. Hult et al. (2010) conducted a house-to-house recruitment of individuals who were roughly 40 years old and then categorized the individuals by their exact age and whether they were born in the region during the famine. They reported that the unexposed, being younger (born after 1971), were thinner than the exposed. Those conceived and born during the famine had a somewhat higher BMI than the unexposed.

Addressing Questions of Selectivity

To address questions of selectivity, Stein said, van Ewijk et al. (2013) examined data from the Indonesian Family Life Survey and tried to identify individuals who may have self-selected to change their diets during Ramadan. The researchers analyzed the data based on presumed exposure to Ramadan fasting while in utero and found that Muslim individuals who were exposed to Ramadan fasting early in gestation were thinner than Muslim individuals who were not in utero during Ramadan. They found no association between non-Muslim individuals born in utero during Ramadan and either BMI or weight.

Summary of the Evidence

In summary, Stein found three relevant studies of the Dutch Hunger Winter. All three studies suggested that at least one group of individuals exposed to the famine in utero showed an increased prevalence of at least one measure of overweight later in life, with early gestation tending to have stronger associations than later gestation and with stronger associations observed in women than in men (when the researchers were able to analyze both sexes). Among the four Chinese famine studies, the results are highly divergent. It is possible to make any determination one wants, Stein said, depending on the study picked. The Leningrad study authors reported no major associations between in utero exposure to famine and later obesity risk. The Nigeria study demonstrated an increased waist circumference

and increased risk of overweight in those exposed to the famine. Finally, the Ramadan study demonstrated that those exposed to Ramadan in utero were thinner.

Conclusion

"I am an epidemiologist," Stein said. "The first thing epidemiologists always say is, 'The study is fundamentally flawed.'" He remarked that even his study is fundamentally flawed (Stein et al., 2007). The main reason for this, he said, is that when food is restricted, the first thing that happens to women is that they become amenorrheic. So fertility drops. In Holland, during the Dutch Hunger Winter, fertility dropped 50 percent. In China, fertility dropped 60 to 70 percent in some places. In Leningrad, fertility dropped 80 percent. If fertility drops, but some women are still having children, it is impossible to know what makes children born to those women different from the children who were not born to women who were not fertile (but who otherwise might have been born if the women were fertile). Any study that compares the general population before and after a famine to the group of individuals who conceived during the famine is fundamentally confounded by that problem, which with most experimental designs Stein described as "not resolvable." In his opinion, his study (Stein et al., 2007) came closest to resolving that problem because he and his colleagues recruited siblings of the affected birth cohort. But none of the other studies have been able to do that, he said.

Nor have any of the famine studies been able to separate the effects of food shortages from the effects of all the other wartime stressors, such as the particularly cold temperatures of the Dutch Hunger Winter. It is impossible to know how many of the observed effects were due to the famine versus the cold temperature.

Where there are male versus female data, Stein observed that female offspring tend to be more vulnerable than male offspring. He said that the data do not lend themselves to an explanation for why that is the case.

The data also suggest that the risk of obesity is increased following early gestation exposure. But the only famine for which researchers have really been able to examine early gestation effects is the Dutch Hunger Winter. The other famines were too long to differentiate early exposure from either other periods of exposure or whole gestation exposure.

MESSAGES TO WOMEN ABOUT EPIGENETICS AND CHILDHOOD OBESITY[2]

Sarah Richardson of Harvard University spoke about how fetal origins research focuses on female reproductive bodies as a central site for epigenetic programing and public health policy intervention and the implications for women of this rising discourse, with a particular emphasis on the implications for poor women of color and their children. "If we do not attend to these issues," she said, "epigenetics can become another stigmatizing, determinist discourse that threatens women's reproductive autonomy and contributes to fat stigma and moral panic about the mothering practices of poor women of color."

She emphasized, first, that epigenetics has a life well beyond the laboratory and the halls of the Academies. "It has riveted the public," she said. In her opinion, it has been received in a largely celebratory manner, categorized variously by mysticism, poetic wonder, hype, and humor, with popular writing on epigenetics often projecting very nascent scientific findings into practical advice for everyday living. Epigenetics is poorly understood by the public, Richardson observed, and has generally been received in a way that mirrors the genetic determinism of previous eras by offering switches that can turn genes on and off through simple lifestyle modifications. She remarked that she has been following the trope of headlines that say, "You can change your DNA. You can change your genes." Those headlines are a reminder, in her opinion, that there is a high degree of scientific illiteracy around both genetics and epigenetics.

Richardson observed that while the presentations at this workshop had been well balanced, much of the other discourse around epigenetics tends to convey a larger effect size and much greater confidence in the solidity of the science in humans than is reflected in the scientific literature. She remarked that part of this hype can be attributed, of course, to the ways in which maternal fetal epigenetic science speaks to urgent public health priorities in the areas of infant mortality, early childhood development, and the prevention of complex and resource-intensive public health problems, such as obesity. There is a great amount of hope that epigenetics can help to address what have been intransigent problems, presenting a rich translational context for attracting investment and interest in epigenetics research and allowing epigenetics research to circulate to much broader publics than other areas of science.

Epigenetic programming also resonates publicly, Richardson continued, because it raises philosophically riveting questions of the possibility that

[2] This section summarizes information and opinions presented by Sarah Richardson, M.A., Ph.D., Harvard University, Boston, Massachusetts.

one's own experiences and behaviors can amplify into future generations, connecting with traditional notions of inheritance as patrilineal and matrilineal. This intergenerational kind of claim has focused largely on females. One reason for the focus on females, Richardson explained, is that the maternal body in fetal programming types of explanations is conceptualized as an adaptive environment for the growing infant who is receiving crucial developmental cues. Because this programming can imprint on the female fetus's own gametes, it has been suggested that the effects of the maternal environment may be intergenerationally passed through the maternal line to grand-offspring.

Historically, Richardson observed, this kind of formulation of public health risk is, on the one hand, rooted in long-standing concerns to improve society by reducing the number of problem people through the management and control of human reproduction and, in particular, by calling on mothers to raise sound and productive citizens. That movement reached its heights in the eugenic era when, using what Richardson referred to as the very new genetic science, public health officials urged that certain maternal bodies were so harmful to society that they should be called upon not to reproduce. Women in the tens of thousands were subjected to forced sterilizations, Richardson said. The effects of this were particularly harsh on women of color and had a legacy well into the 20th century—and, some would say, Richardson observed, even to this day. From that legacy came a new profound commitment to reproductive autonomy and a new humility about directing human choices through very new science.

The science of maternal fetal epigenetic programming converges with several major trends in 20th- and 21st-century science, gender, and culture, Richardson continued, from an understanding of motherhood as instinctual, selfless, and intrinsically moral to a notion of motherhood as an agential project of the self, in which the mother's interests are often perceived to be in tension with the child's; from a psychosocial model of human development to a genetic and neurological model of child development; and from a concept of birth as that moment of personhood and medical concern to a concept of conception and preconception as the focal points of political interests and biomedical interventions in reproduction.

Epigenetic studies of maternal effects, given this backdrop of history, raise vital social, ethical, and philosophical questions for Richardson and others. Is there a potential for this new research to heighten public health surveillance of and restrictions on pregnant women and mothers through a molecular policing of their behavior? How might this new research participate in the often troubled history of notions of the supreme role of the mother in normal and pathological development? What are the empirical and methodological implications of a research focus on maternal effects largely to the exclusion of the larger social environment and, of course, paternal effects?

In the case of claims of obesity, added to these concerns is a worry about replicating harmful stereotypes and misconceptions that contribute to stigma about fat children, which in itself can harm their mental and physical health and imperil their safety. Richardson pointed to childhood bullying issues as an example. The notion that a fat child is something to mourn and to avoid at all costs is reflected in images known as headless fatties, which, Richardson noted, appeared on a few slides at this Institute of Medicine (IOM) meeting. Such images suggest that fat is such a horrific thing that a mother and child would experience shame if their faces were shown. Critics have pointed out the profoundly dehumanizing effects of such images.

Countering this stigma is a growing and important critique of the use of the language of health and science and the concept of an obesity epidemic to tag certain bodies as a threat to the nation in ways that are often laced with the language of moral panic. Who Richardson referred to as "fat activists" and their allies are not always looking to science and medicine for help and hope and are increasingly wary of their use of health to control and limit their autonomy and contribute to fat stigma. Their view is that a world in which all bodies are accepted is the ideal one for overweight and obese people, and they wish to have maximal autonomy to live their lives as they wish.

Regarding problematic considerations of race and class, Richardson said that they do not occur only in the popular communications of the science. They also exist in the scientific literature. She highlighted an example from Jonathon Wells, who she described as an influential theorist of early developmental programming in the prenatal maternal environment who has been very interested in the prenatal development of metabolic disorders and obesity as somatic manifestations of the intergenerational transmission of health inequalities. Wells (2010) modeled how features of the mother's social and environmental context during her own development, including her social class, may be transmitted to the growing fetus, conditioning the fetus for a life of inequality even before birth. According to Wells, Richardson explained, the maternal body serves as a transducing medium for health inequalities from one generation to another. In Wells's model, what he calls "maternal capital," including public health policies, education, and health care, is corporealized in the maternal–fetal relation, Richardson explained. She remarked that Wells has quite provocatively used the term "metabolic ghetto" to refer to spaces of disadvantage within the mother's body and has argued that public health policies must target multiple interventions through the maternal medium.

While there is a huge heterogeneity in discourse in the obesity literature, some of that literature has replicated the focus on mothers as both the agential causes of undesirable outcomes and the point of intervention. In fact,

the focus extends not just to mothers, but to all women, even beginning at birth or, according to Wells's words, the total developmental period of mothers (Wells, 2007). For example, Kuzawa (2005) suggested that "public health interventions may be most effective if focused not on the individual, but on the matriline" (p. 5). These discourses often include a representation of great responsibility combined with little agency, which Richardson referred to as the "urgent, nonspecific expansive intervention model." She pointed out some hyped language in the literature based on this model: the "feed-forward cycle of maternal to offspring obesity transfer," the "importance of addressing the risk of obesity before females enter the childbearing years," and "intervention during pregnancy merits consideration" (Olson, 2007, p. 435).

The Maternal Body as an Epigenetic Vector

Richardson has argued in some of her writing on maternal fetal epigenetics that in scientific research the maternal body emerges as an epigenetic vector—an intensified space for the introduction of epigenetic perturbations in development. She discussed some of the ways that this notion is being mobilized in public discourse around the research findings.

First, she referred to what she called a "deficit model," with the research principally advancing, or being seen as advancing, a model of epigenetic modifications as a source of error, adverse effects, or disease risk. So while scientists acknowledge that epigenetics may provide a route to human enhancement or therapy, the main body of work at this time—and the central object of concern—is how to prevent the adverse effects of impaired or maladaptive maternal environments that are seen as causing epigenetic lesions in human lineages.

Second, maternal bodies are regarded as the central targets of epigenetics-based health intervention. Graphically, time and time again, a mother's body is shown at the center of a causal scheme or scheme of intervention, with public health interventions being seen as most effective if they target women. Again, Richardson noted, what counts as a maternal body in this literature is potentially quite large and includes all premenopausal women, including young girls. She remarked that the point had been made repeatedly at this workshop that paternal effects have been neglected. Richardson observed that males of many species provide paternal care. Male gametes, as Stephen Krawetz discussed in his presentation (see Chapter 3), are also subject to environmental exposures and may carry critical developmental cues to future generations. While paternal effects are increasingly being recognized by scientists, with several studies substantiating the existence of intergenerational effects in mammalian paternal lines, Richardson said that it is fair to say that there is an overwhelming focus on

maternal effects in the research literature. She noted that in the highly cited Zhang and Meaney (2010) review article, the term "maternal" or "mother" was used 137 times, the term "parental" or "parent" 11 times, and the term "paternal" or "father" only three times.

While one can defend this imbalance by arguing that maternal phenotype has a greater capacity to shape offspring phenotype, in Richardson's opinion, that argument is largely a working assumption rather that something that has been fully validated. As a cultural study scholar and historian, she also pointed to the fact that despite evidence that paternal behaviors and life experience, such as alcohol use, smoking, and pesticide exposure, can impact the health of offspring from conception and over time, public health interventions to reduce fetal harm have historically consistently focused on the mother and minimized paternal effects. This asymmetry, she said, originates in long-standing Western cultural and ideological convictions that include, on the one hand, a belief in the greater vulnerability of female rather than male bodies, and, on the other hand, a belief in the primary responsibility of the mother for child development and resistance to notions of male reproductive vulnerability and paternal responsibility for the development of embryos and infants.

Third, while the target of intervention is the maternal body, the desired outcome of epigenetics-driven health interventions is usually improved fetal health. Researchers, of course, do hope that a collateral effect of their policies will be to enhance resources for pregnant women. However, most proposed interventions are directed toward the most efficient methods to ensure developmentally optimum outcomes for the fetus. Richardson commented that symbols favored by some of the major texts and societies represent this, with fetuses encapsulated in headless, legless maternal abdomens. She pointed to insignia of the International Society for Developmental Origins of Health and Disease and the cover of one of the field's leading textbooks (Gluckman and Hanson, 2004) as examples. The maternal body in this discourse is a transducing and amplifying medium necessary to get to the fetus—an obligatory passage point, not a primary end point or subject of the research.

Finally, while maternal bodies are conceptualized as having great power to influence future generations and are positioned at the center of the intervention model advanced by many in the field, in fact the model accords individual women very little power to influence their own outcomes. Richardson considered this an interesting feature of these kinds of feed-forward or mismatched models. While women are instructed to do all they can to prevent harm to their fetus, at the same time, with the epigenetic feed-forward cycle hypothesized by maternal fetal effects research, adverse effects originate in a misalignment between a fetus's initial epigenetic programming and eventual environment. So the system has this kind of inertial

quality, Richardson said, where short-term investments may not lead to long-term benefits that are beyond conscious or individual control. The fact that change manifests at the level of intergenerational lineage, rather than the individual female, advances a shifting and mixed message regarding maternal agency and responsibility. In Richardson's opinion, the system exhorts mothers to make changes that are unlikely to bring them or their offspring benefit. At the same time, it produces a model of the maternal body that suggests that maternal experiences, exposures, and behaviors may have very significant amplified consequences for the woman's offspring, her descendants, and society at large.

Conclusion

In wrapping up, Richardson reiterated that epigenetic research on maternal effects advances a model of human inheritance and development in which the wider social and physical environment can be seen as heritable and as a determinant of future biomedical outcomes via discreet biochemical modifications introduced by the amplifying vector of the maternal body. She stated that while the hope is that this literature would move the field away from forms of deterministic explanations, in fact the language strongly resembles that of genetic determinism. The multivalenced concept of a vector points to a causal mechanistic explanatory landscape in which genes remain very much at the center, with environments, nutritional factors, toxins, social policy, and stress all collapsed into molecular mechanisms acting at the level of the DNA. Rather than challenging genetic determinism and biological reductionism, Richardson suggested that present-day research programs in human epigenetics could strategically appropriate and modify the discourse to produce a new form of determinism, a somatic determinism or an epigenetic determinism, one that places the maternal–infant relation at the center.

Richardson drew attention to a research comment published in *Nature* in 2011 that she, Matthew Gillman, and several others in the field coauthored, where some of the issues that she discussed at the workshop are highlighted and where concrete suggestions are offered for the translation of this research into the public sphere in ways that she and her coauthors considered to be non-stigmatizing and that appropriately clarify the state of the science (Richardson et al., 2014). They made four suggestions: (1) avoid extrapolating from animal studies to humans without qualification, (2) emphasize the role of both paternal and maternal effects, (3) convey complexity, and (4) recognize the need for societal changes rather than individual solutions. Richardson expressed concern that too often there is an impulse to simplify in order to quickly move scientific findings into the public sphere. When that happens, many of the efforts outlined in her suggestions are neglected: differences between animal and human

data are not clarified, the role of both paternal and maternal effects are not emphasized, and the complexity of the findings are not conveyed (e.g., that an effect size is small or that there are many still poorly understood factors at play). She emphasized that focusing on societal-level changes, instead of instructing individuals to change their behavior under conditions of structural inequality, would be best for everyone involved and also for addressing issues of stigma.

THEORY TO POLICY[3]

Matthew Gillman of the Harvard School of Public Health began his presentation by expressing gratitude to, among others, David Barker, "without whom," he said, "none of us would be here" (Gillman and Jaddoe, 2013). He also showed what he said would be his "only slide on theory," that is, that earlier intervention, during developmental periods of plasticity, sets individuals on better lifetime health trajectories than later intervention does (Godfrey et al., 2010). For the remainder of his talk, he discussed ways to improve the evidence base for Developmental Origins of Health and Disease (DOHaD) health policy.

Impact of Developmental Origins of Health and Disease and Health Policy

Gillman described health policy as strategies to improve health via government actions at the national, state, or local level through legislative or regulatory means. Health care policy is a subset of health policy that is aimed at improving individuals' health via the organization, financing, and delivery of medical care services. Because pregnant women and infants see medical providers for routine care more often than they do in any other period in the life course, health care policy is a key piece of DOHaD health policy. Examples of DOHaD health policies are Massachusetts's regulation of child care centers requiring each child to participate in at least 60 minutes of physical activity daily and baby-friendly hospital initiatives to promote breastfeeding initiation, duration, and exclusivity, or to screen and provide treatment recommendations for gestational diabetes. Gillman explained that DOHaD health policies also include government policies, such as cigarette tax policies that reduce smoking both outside of and during pregnancy, and mixtures of government and health care policies such as the IOM guidelines for gestational weight gain, which could be transmitted either through health care or outside of health care.

[3] This section summarizes information and opinions presented by Matthew Gillman, M.D., S.M., Harvard Medical School, Boston, Massachusetts.

The role of evidence in health policy is to both develop and evaluate policies, Gillman remarked. While evidence is only one of several factors that drive policy decisions, he said, "If we have the wrong evidence or the wrong type of evidence, we might be outside the loop." Ideally, Gillman said, evidence is at the center of decision making, as it for the Robert Wood Johnson Foundation (RWJF, 2009). The question for him is, "How can we have the best types of evidence to drive policy?"

Although improving DOHaD evidence for use in policy development begins with etiology, which Gillman noted was the subject of most of this workshop discussion, it can also involve prediction, risk–benefit analysis, interventions, long-term effects, and policy evaluation. Gillman discussed each of these types of policy-relevant evidence in turn.

Etiology

While animal experiments have provided very helpful information on exposures, timing, mechanisms, and effects on outcomes, and while they have effectively, in Gillman's opinion, "proved the programming principle," they could be more helpful. What is often missing, he said, are certain features of randomized controlled trials in humans, including specification of the source population, a sampling frame and eligibility criteria, recruitment and retention rates, blinding, intention to treat analyses, attention to missing data, and cluster methods for litter sizes greater than one (Muhlhausler et al., 2013).

Ainge et al. (2011) attempted a systematic review of rodent models of maternal high-fat feeding and offspring glycemic control but, Gillman said, they were unable to summarize or meta-analyze the data because of the low quality and variability among the studies. Starting with 1,483 studies, they identified only 11 that met their criteria. Among those 11, quality scores were low, and there was a large amount of variability in maternal diet, with some being hypocaloric, others hypercaloric, others not stated, and none isocaloric, and a wide range of fat and carbohydrate content. Additionally, there was large variability in postnatal feeding regimens and age at outcome assessment.

Another way that animal studies could be more helpful, Gillman said, is by following, not just leading, epidemiology. In particular, he suggested that animal studies follow epidemiology's lead in addressing pre-pregnancy obesity, gestational weight gain, low-carbohydrate and high-protein diets, glycemic index or load, vitamin D, and smoking. In his opinion, it would be great to have all of these translated into animal models.

In sum, animal experiments would, in Gillman's view, be greatly improved by more harmonizing of interventions and measures across studies and by "translating up." He also encouraged the publishing of null results.

Gillman reminded the workshop audience that DOHaD is an interdisciplinary field with multidirectional communication among several different fields, with evidence deriving from population-based studies, animal models, in vitro studies, and clinical studies. Just as there is room for improvement in animal models, the same is true of population-based studies. Both observational studies and randomized controlled trials have a number of disadvantages. In Gillman's opinion, confounding is the main disadvantage of observational studies. In fact, the main reason randomized controlled trials are conducted is to minimize confounding. There are a number of what Gillman called "epidemiological tricks of the trade" to manage confounding. He noted that his list of innovative study designs or analyses was very similar to Caroline Relton's list (see Chapter 3 for a summary of Relton's presentation): judicious multivariable analysis, sibling-pair design, cohorts with different confounding structures, comparing maternal versus paternal effects, long-term follow-up of randomized controlled trials, Mendelian randomization, the use of biomarkers, and quasi-experimental studies (which Gillman noted are not really cohort studies, but usually repeated cross-sectional studies). Each of these has its own strengths and weaknesses. Gillman stressed that it is the totality of evidence that should be judged when using evidence to develop policy.

As an example of judging the totality of evidence, in 2011 Gillman was asked to write an editorial on breastfeeding and child obesity, in which he summarized the evidence for and against the hypothesis that having been breastfed reduces the risk of obesity (Gillman, 2011). In the editorial he categorized the evidence based on type of study design: cluster randomized controlled trial of breastfeeding promotion, cohort studies of mostly white European descent individuals, cohort studies in developing countries and in racial/ethnic minorities, sibling-pair analyses, comparisons of cohorts with different confounding structures, reverse causality studies, and studies of biological mechanisms. He identified which studies supported the hypothesis, which suggested that it may be true, and which indicated that breastfeeding does not reduce the risk of obesity. He described his conclusion as "fence-sitting."

Gillman (2011) was published before the 11-year results of the Promotion of Breastfeeding Intervention Trial, or PROBIT, were made available. PROBIT was a cluster randomized trial in the Republic of Belarus that examined baby-friendly hospital initiatives versus usual care in 31 maternity hospitals and affiliated pediatric practices. The results of that trial indicated that breastfeeding promotion did not reduce adiposity at age 11.5 years. If anything, Gillman noted, the relative risks for the intervention sites, compared to the control sites, were above 1, not below 1 (Martin et al., 2013). The PROBIT study, Gillman said, was wonderful for a couple

of reasons, one being that it was a long-term randomized controlled trial and therefore a good test of causality.

In Gillman's opinion, policy about breastfeeding and obesity has been based on the wrong evidence. While earlier studies suggested that breastfeeding afforded considerable protection against the risk of obesity, more recent studies, like the PROBIT study, cast doubt.

Impact of Epigenetics and Health Policy

Gillman asked how epigenetics might be translated into health policy. He explained that the conceptual model upon which the Project Viva cohort study is based is that prenatal exposures result in health outcomes mediated through epigenetics. That is, epigenetics plays an intermediate role between pre- and perinatal exposures and obesity-related outcomes. Those intermediate epigenetic biomarkers could be used as surrogate outcomes, Gillman suggested, as long as the markers are indeed causally related to the outcomes, making some studies more feasible because pregnant women and their children would not need to be followed in the long term. Additionally, if epigenetic biomarkers are causally related to outcomes, for Gillman, they serve as a rationale for what he called primordial prevention. That is, if certain pre- and perinatal factors are related to epigenetic markers that themselves are related to certain outcomes, the question for Gillman then becomes, "How can we prevent those pre- and perinatal risk factors in the beginning?" In a recently published editorial on primordial prevention of cardiovascular disease, Gillman wrote about optimizing the socio-behavioral milieu starting with conception, or even preconception, as a way to avoid exposures known to be related to offspring obesity, such as maternal obesity, excess gestational weight gain, gestational diabetes, and smoking (Gillman, 2015).

For Gillman, epigenetics is a reminder that, quoting Hertzman and Boyce (2010), "experiences get under the skin early in life in ways that affect the course of human development. . . . [E]pigenetic regulation is the best example of operating principles relevant to biological embedding [of societal influences]." Also for Gillman, epigenetics is a reminder that many solutions will not be at the individual level. Individual behavior is hard to change in restrictive environments. Instead, efforts should be aimed at changing people's physiology or even their behavior through environmental and policy solutions. Finally, Gillman viewed epigenetics as an "easy" science to communicate to policy makers. For him, it is at what he described as the "right archaeological level" to explain how pre- and perinatal factors cause obesity. He remarked that even he understood the epigenetic differences between fat, yellow, short-lived agouti mice and lean, brown, long-

lived brown mice (see the summary of Robert Waterland's presentation in Chapter 2 for a description).

Regarding the discussion concerning the use of epigenetic biomarkers as predictors, Gillman cautioned that prediction means separating the risk of one individual from another. It has a high bar of proof and requires a high sensitivity and specificity. A predictive signature cannot indicate a mildly elevated relative risk. Additionally, he warned that much of the talk about prediction in general revolves around the development of drugs based on what is learned about who is susceptible. Developing drugs for pregnant women and infants is, he said, "really the last thing that we want to do."

Prediction

As an example of DOHaD prediction evidence for policy translation, Gillman described a Project Viva study on the risk of obesity at age 7 to 10 years that he and a coworker performed (Gillman and Ludwig, 2013). This study found that the optimal levels of four potentially causal and modifiable pre- and perinatal risk factors (smoking, gestational weight gain, breastfeeding, and sleep duration in infancy) were associated with a 4 percent predictive probability of obesity. At adverse levels of all four risks, the predictive probability was 28 percent. The population attributable risk (PAR) percentage (the proportion of disease incidence in a population that would be eliminated if exposure was eliminated) was 20 to 50 percent. In other words, eliminating the risks would eliminate 20 to 50 percent of the observed obesity. These results suggested to Gillman that multiple risk factor interventions in early developmental periods hold promise for preventing obesity and its consequences. Prediction studies can also be used to quantify the overall potential benefit of intervening early and to distinguish the most important determinants, which may vary by population or subgroup.

Risk–Benefit Analysis

Risk–benefit analysis provides a way to take into account different exposures and outcomes among populations. For example, it has been used to determine optimal gestational weight gain by taking into account both short- and long-term outcomes for both the mother and child. Associations between gestational weight gain and different outcomes vary. For example, the association with preterm delivery is U-shaped, whereas the association with small for gestational age is indirect and the associations with large for gestational age, postpartum weight retention, and child obesity are all direct. Oken et al. (2009) examined the average probability of all five outcomes and, based on all five outcomes, determined risk curves for

obese, overweight, and normal-weight women. They concluded that optimal weight gain lies at the nadir of each risk curve (i.e., with gestational weight gain on the x-axis and average predicted probability of adverse outcome on the y-axis).

Risk–benefit analysis has also been used to tease apart multiple outcomes among both mother and child associated with rapid weight gain during infancy. While rapid weight gain in infancy is known to be related to later obesity, it may also be beneficial for neurodevelopment. Belfort and Gillman (2013) summarized the evidence for differing effects of rapid infant weight gain on obesity and neurodevelopment, depending on gestational age, and they reported some positive associations, some evidence of no association, and insufficient evidence for some associations.

Interventions

Many DOHaD intervention studies focus on efficacy. But for policy translation, Gillman encouraged researchers to move beyond efficacy and focus more on implementation. He listed some prevention interventions that the Harvard Pilgrim Health Care Institute's Obesity Prevention Program has been involved with, many of which are cluster randomized controlled trials. Cluster randomized controlled trials can be very useful, in his opinion, because they focus on groups, not individuals. The features of the interventions vary. For example, one relies on health information technology, another is based on a chronic care model, yet another invokes interventions within an integrated clinical and child care system. Despite the variation in features, most are based on only one or a few kinds of determinants or one or a few kinds of settings.

In Gillman's opinion, the "most bang for the buck" obesity prevention intervention will likely come from a whole-of-community approach, one that focuses not only on families and individuals, but also on the environment and policy (IOM, 2005). He said that he is excited about a whole-of-community project he is coleading with Ross Hammond at the Brookings Institution. The project, known as COMPACT, is aimed at obesity prevention from birth to 5 years of age: what works, for whom, and under what circumstances? At the time of this workshop, the research team had completed two intervention studies, one in the United States and the other in Australia, and had a cluster randomized controlled trial under way in Australia. The team will be using the results to design a new obesity prevention intervention for children in the United States under the age of 5 years.

Long-Term Effects

Long-term effects of interest to policy makers include effectiveness, safety, costs, and cost-effectiveness. The only way to integrate such data from multiple sources, Gillman said, is via simulation modeling. But there has not been much DOHaD simulation modeling. Instead, as an example, Gillman pointed to a study on the effectiveness and cost-effectiveness of blood pressure screening in adolescents (Wang et al., 2011). The researchers used a two-stage model structure. One stage was based on available data from blood pressure tracking, screening, and treatment among 15- to 35-year-olds. The other involved use of an existing coronary heart disease policy model for individuals aged 35 to death to estimate costs and effects of blood pressure screening. The researchers applied their two-stage model to the U.S. population of 15-year-old adolescents in 2000 and compared several blood pressure screen-and-treat programs versus population-wide strategies and concluded that population-wide policy approaches, including salt reduction and an increase in either physical education classes or individual exercise programs, were more effective and less costly than any of the screen-and-treat strategies. Gillman interpreted these results to mean that shifting a distribution of risk factors by a small amount over an entire population can do just as much for prevention as screening and intervening on a high-risk population.

Policy Evaluation

Gillman provided two examples of natural policy evaluation experiments. The first was a Project Viva time-series analysis of fish intake among pregnant women following warnings to eat less fish that was known to have high levels of mercury (Oken et al., 2003). The researchers demonstrated that fish intake had gradually increased before the warning, then dropped noticeably immediately after the warning, and declined steadily in the subsequent year. The analysis showed that even a weak intervention, in this case a policy promulgated through obstetricians' offices and without a great implementation strategy, can have an effect on pregnant women's eating habits. In Gillman's opinion, the findings also suggested that pregnant women are concerned about their growing fetuses and may be willing to change behaviors that they might not be willing to change at other times in their lives.

A second example was Hawkins et al.'s (2014) use of a quasi-experimental approach to analyze tobacco control policies and birth outcomes. Analyzing repeated cross-sections of U.S. natality files from 28 states, they found that increases in cigarette tax were associated with improved health outcomes related to smoking among the highest-risk moth-

ers and infants. Gillman said, "Considering that states increase cigarette taxes for other reasons, not to improve birth outcomes, this is a very good side effect of this policy."

Conclusion

In summary, to strengthen etiological evidence for policy translation, Gillman encouraged more consistent methods and harmonization of designs and measures in animal studies and more innovative designs and analyses in human observational and intervention studies. He also encouraged greater comparing and combining across human studies. In his opinion, epigenetics should help with science-to-policy communication of the results of human studies. In addition to etiological studies, several other types of studies can produce policy-relevant evidence. Prediction models can help to identify potential intervention targets; risk–benefit analyses can help inform guidelines; intervention studies that look beyond efficacy to implementation can be helpful; long-term simulation models that include cost-effectiveness can play a large part; and evaluations can be used to measure the impact of current policies.

In closing, Gillman emphasized that racial and ethnic disparities in childhood adiposity and obesity are determined by factors operating in infancy and early childhood and that changing risk factors early in life has great potential to reduce obesity disparities (Taveras et al., 2013).

THEORY TO CLINICAL PRACTICE[4]

If scientists know the effects of what happens in utero and early in life, such as that excess gestational weight gain and rapid infant weight gain in the first 6 months of life place a child at greater risk for insulin resistance, hypertension, and obesity, then for Shari Barkin of the Vanderbilt University Medical Center, the key question is, how can clinicians apply that knowledge in their practices? Barkin elaborated on ways to apply science discussed at the workshop to clinical practice, given caveats about the context and population specificity of much of the evidence. She considered potential applications of the science based on developmental period (i.e., what happens during pregnancy versus infancy versus toddlerhood). In addition to highlighting evidence where it exists, she presented additional ideas that have yet to be tested but that she thinks should be considered.

[4] This section summarizes information and opinions presented by Shari Barkin, M.D., M.S.H.S., Vanderbilt University Medical Center, Nashville, Tennessee.

From Science to Clinical Application: Pregnancy

Scientists know that offspring exposed to both under- and overnourished mothers during gestation have similar metabolic dysfunction, including not just obesity but also insulin and leptin resistance. Given this knowledge, it would make sense, in Barkin's opinion, to set clear and balanced nutritional patterns early in life, maintain those patterns during pregnancy, and reinforce them consistently in clinical settings both prior to and during pregnancy. Considering what is known about epigenetic paternal effects (see the summary of Stephen Krawetz's presentation in Chapter 3), pediatricians should be talking to boys about nutrition. Talking with pregnant women and other family members about nutrition can occur in multiple settings, not just in the obstetrician's office.

Scientific evidence also indicates that regardless of whether BMI changes as a result, exercise is beneficial to health and exercise during pregnancy can mitigate the development of metabolic dysfunction. A potential clinical application of this knowledge is to encourage mild to moderate exercise daily, for example, by recommending exercising or, in the case of children, playing for 30 minutes per day. Barkin commented that, given her experience as chief of the Division of General Pediatrics at Vanderbilt University Medical Center, where she and her staff see some 42,000 outpatient visits and supervise about 5,000 births yearly, while nothing is as straightforward as telling someone during a visit to exercise 30 minutes per day and that doing so will offer a greater health benefit than anything else would, simply conveying knowledge is not likely to change behavior. For most of their patients, Barkin said, "It is not about knowledge. Knowledge is necessary, but not sufficient. It is about skills and context."

Barkin stated that there have been many pregnancy interventions aimed at educating women during pregnancy about physical activity and nutrition. They vary in approach and in how information is delivered. For example, some are group sessions, others individualized. Some health calls or coaching sessions are with a trusted adviser, others with someone unknown who is a part of an insurance plan. The interventions also vary in dose (how many) and timing (when).

With all of this variability as a caveat, Barkin highlighted two pregnancy interventions focused on physical activity and nutrition education during pregnancy. First was the Behaviors Affecting Baby and You (B.A.B.Y.) study. The participants, 110 prenatal care women, 60 percent Hispanic, were randomized during the second trimester into either a 12-week exercise intervention arm or a health and wellness arm (Chasan-Taber et al., 2011). For the exercise intervention group, researchers matched participants' physical activity goals with their willingness to exercise, with the overall goal being to increase time spent in moderate activity by 10 percent each week until,

ultimately, participants were exercising 30 minutes on 5 or more days. The intervention involved one face-to-face visit, weekly mailed surveys and individually tailored reports, and 12 weekly telephone calls providing motivational-based individualized feedback. Barkin explained that with motivational interviewing, the interviewer does not always know the interviewee's context, and therefore even though the interviewer is conveying expertise and knowledge, if that knowledge does not meet with any agency, the interview is unlikely to change behavior. This study took that into account as part of the study design, said Barkin. The researchers measured outcomes using a pregnancy physical activity questionnaire. They found that the exercise arm experienced a smaller decrease in physical activity in the second trimester compared to the control arm. Barkin said, "Sometimes we are talking about how to get more. Perhaps just getting to 'better' can affect outcomes, especially if you see patterns of decreasing physical activity during pregnancy."

Barkin highlighted centering pregnancy as a second example of a pregnancy intervention focused on physical activity and nutrition education. She noted that centering pregnancy has been around for quite a long time and serves as a national model of group prenatal care. Applying adult learning theories that highlight the importance of group work and participatory process, it involves bringing together groups of 8 to 12 pregnant women at similar gestational ages to meet 10 times over 6 months, with the meetings facilitated by a nurse practitioner or midwife, and usually the same facilitator over time to ensure continuity. The meetings are 2-hour sessions covering nutrition and activity, stress reduction, relationships, and parenting. Picklesimer et al. (2012) showed that participation, which the researchers defined as attending even just one group session, led to a consistent reduction in preterm birth. In another recent study, Benediktsson et al. (2013) compared women who recalled receiving information during a centering pregnancy meeting to women who recalled receiving information during a typical prenatal visit. They found that recall for information about nutrition, cigarette smoking and secondhand smoke, and alcohol consumption during pregnancy was statistically different between the two types of women. Multiple other evaluations of centering pregnancy are under way, according to Barkin. "Stay tuned for more details," she said. "We are in the evolving stages of examining evidence related to this type of clinical application."

In addition to the potential clinical applications of scientific evidence related to physical activity and nutrition during pregnancy, Barkin discussed potential applications of what is known about maternal excess gestational weight and its interaction with pre-pregnancy weight to alter early infant growth trajectories. The science suggests that clear goals for weight gain during pregnancy should be set, perhaps by utilizing group visits or health

coaches during and after pregnancy. Barkin also encouraged more focus on appropriate weight loss after pregnancy by linking to effective community weight programs.

Barkin pointed to the IOM's release in 2009 of a report on pregnancy weight gain guidelines (IOM, 2009) and remarked that it is known that women who experience excess gestational weight gain have children with an increased risk of high BMI and high systolic blood pressure at 3 years of age.

In a study aimed at understanding how excess gestational weight gain interacts with pregnancy weight to alter early infant growth trajectories, Barkin and colleagues used linked electronic medical record data for about 500 mother–child pairs and objective measurements of infant weight (as opposed to self-reported weights) (Heerman et al., 2014). Their main finding was that the infant growth trajectory was different for women who were obese prior to pregnancy and who also had excess gestational weight gain compared to mothers who were either obese prior to pregnancy but who did not have excess gestational weight gain or had excess gestational weight gain but were not overweight prior to pregnancy. Specifically, there was a 13 percent difference in the infant weight/length ratio at 3 months of age that persisted at 1 year of age. Also of interest to Barkin and her coauthors, and something that Barkin said they are trying to replicate, was the rapid increase in the first few months of life for infant weight gain, followed by a dip, then another increase, such that by the end of the first year of life, infants born to mothers who were obese and who also had excess gestational weight gain had greater weight/length ratios than infants born to mothers who were overweight prior to pregnancy.

Barkin considered post-pregnancy weight loss a "lever point." Ideally, if mothers could reach a healthier pre-pregnancy weight for subsequent pregnancies, the interaction that she had just described, that is, between pre-pregnancy obesity and excess gestational weight gain, would not exist. Reaching a healthier pre-pregnancy weight would also prevent the development of the pro-inflammatory state described by Jacob Friedman and other speakers (see Chapter 3 for a summary of Friedman's presentation). Barkin suggested that the Practice-based Opportunities for Weight Reduction (POWER) trial, although not intended to test post-pregnancy weight loss, nonetheless shows what an effective weight loss program looks like and perhaps ought to be utilized for post-pregnancy weight loss (Appel et al., 2011). The trial involved six primary care practices in Baltimore and 415 obese patients, 41 percent of whom were African American and the majority women. The patients were randomized into three conditions: (1) a self-directed weight loss control group, (2) a remote support-only group (i.e., patients received remote support from health coaches), and (3) an in-person support group (i.e., patients received face-to-face group and individual support as well as phone call health coaching). What was most surprising to

the investigators, Barkin said, was that both average weight loss and the percentage of patients who lost at least 5 percent of their body weight at 24 months were similar for the two treatment groups (10.1 pounds and 38 percent, respectively, for the remote group, and 11.2 pounds and 41 percent for the in-person group, compared to 1.8 pounds and 19 percent for the controls). In other words, the treatments were clearly and similarly effective despite their different durations and doses. In Barkin's opinion, it is important to keep in mind that different durations and doses of an intervention can have similarly effective outcomes, particularly when thinking about clinical applications. In the case of the POWER study, participants received a higher dose earlier in the trial than in the later phases, and this still resulted in clinically significant outcomes. It does not appear that the same degree of participation throughout a study is necessary, Barkin said.

In a subsequent focus group study, Bennett et al. (2014) asked providers how they would use the POWER trial results. Providers replied that their role was to refer patients to effective programs and provide endorsement, to provide patient accountability, and to cheerlead when things are going well. They viewed their role in actual weight management as limited and, instead, preferred to maintain long-term trusted relationships with their patients so that they could connect them to the appropriate programs.

From Science to Clinical Application: Infancy

Barkin identified two potential clinical applications of scientific evidence indicating that poor nutrition during pregnancy, with rapid infant catch-up growth, leads to later increased offspring adiposity, hyperphagia, and hyperinsulinemia. First is the promotion of infant appetite regulation, perhaps by training providers to discuss with parents how to recognize satiety cues. Second is a change in the pediatric paradigm for catch-up growth. She suggested that perhaps weight gain velocity in early infancy should be slowed down and the expectations of early growth for providers and parents reassessed, although more science is needed to know what a safe velocity would be with respect to unintended consequences (e.g., effect on neurodevelopment) (see previous section for a summary of Matthew Gillman's discussion on the use cost–benefit analysis to analyze trade-offs associated with rapid infant weight gain).

As an example of the promotion of infant appetite regulation, Barkin highlighted the Greenlight Study, a health-literature, health-numerate intervention being conducted by herself and Russell Rothman and colleagues. The researchers taught residents how to talk with families about how to recognize satiety in their infants and provide soothing strategies that do not involve only feeding. The conversations started early, when an infant was 2 months old. The materials provided to families were easy to view, with

pictures illustrating ways to tell when a baby is hungry versus full. The trial had been completed at the time of this workshop and the data were being analyzed. As with the results of ongoing centering pregnancy trials, Barkin said, "Stay tuned."

As another example, Paul et al. (2011) tested the effects of two nurse home visits, one occurring 2 to 3 weeks after birth and the other after the introduction of solids to the infant diet, on infant weight velocity. During the first home visit, or intervention, parents were instructed on how to identify hunger versus satiety cues and how to soothe their infants. During the second visit, they were taught more about hunger and satiety cues and how to handle infant rejection of healthy foods through repeated exposure. The investigators randomized 160 mother–newborn dyads into four treatment groups, with each group receiving both interventions, one or the other intervention, or neither. The researchers found that those receiving both interventions had infants with a lower weight-for-length percentile compared to those receiving just one of the interventions or neither intervention.

Other recent work conducted by Rothman and his group has shown that larger bottles tend to result in increased consumption. "This is not normal," Barkin said. She stated that, based on her clinical setting experience, infants and toddlers used to be the prime examples of individuals who regulate themselves. They knew when they were full and would stop eating and throw or push their plate away. Today, she and other clinicians are not seeing that behavior as much. Infants and toddlers are behaving like adults, she said, eating extra servings even when full, with their hedonic drive appearing to supersede their homeostatic regulation.

From Science to Clinical Application: Toddler

The Paul et al. (2011) study raised the question for Barkin, how can the findings be applied to toddlers? Like infants, toddlers used to have very good self-regulation thermostats. But again, clinicians are not seeing that anymore. Toddlers also tend to imitate the world around them, including how others are eating and playing. Barkin suggested that two potential clinical applications of these observations are, first, to support and reinforce toddler self-regulation and, second, to set normative habits in nutrition and physical activity. Barkin emphasized the importance of focusing on the community and the role of the clinical setting in making connections to community-based programs. She also encouraged clinicians to examine emerging science on how families, not just the child, are affected by interventions. When the goal is to improve the health of the parents as well as the child, clinicians create a much different dialogue. Additionally, Barkin encouraged considering how to utilize social networks to reinforce healthy habits.

As an example of research in this area, the Salud con la Familia (Health with the Family) study tested a family-based, community-centered intervention to prevent and treat obesity among Latino parent–preschool child pairs. The study was a skills-building dyadic intervention where the focus was on improving the health for both the parents and child and helping them to use their built environment to reinforce healthy habits. The researchers found that 41 percent of Latino preschoolers were already overweight at the time of entry into the study, a prevalence that is much higher than any Centers for Disease Control and Prevention statistic, Barkin said, and that those who participated in the intervention group were twice as likely to change their weight category (Barkin et al., 2012). For example, children in the intervention group who were obese at the start were twice as likely to be overweight by the end of the 3-month study, whereas the BMIs of those in the control group continued to increase even within the 3 months of the study (see Figure 5-1).

The Barkin et al. (2012) study raised the question for Barkin, why did the intervention work? To help answer that question, Gesell et al. (2012) measured both the intervention and control group social networks—not social media networks, Barkin explained, but rather bidirectional relationships through which new knowledge and new behaviors could be introduced. The researchers found that both the control and intervention groups started with some initial ties and that both groups developed additional ties over the course of the 3-month study, but the intervention treatment had been designed so that by the end of the study, the intervention group had a much more significant and dense social network.

- 41 percent of Latino preschoolers already overweight
- Those that participated in the intervention group were 2× as likely to change their weight category to normal
- Those in the control group increased their BMI

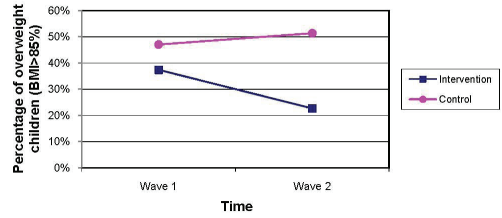

FIGURE 5-1 Change in percentage of overweight children following a skills-building intervention where the focus was on improving health for both the parents and child and helping them to use their built environment to reinforce healthy habits.
SOURCE: Presented by Shari Barkin on February 27, 2015; modified from Barkin et al., 2012.

Summary

In summary, Barkin reiterated that the clinical setting is one of many environments that should be used to prevent childhood obesity. Ways to use it include providing more group visits and/or health coaching calls; addressing nutrition and physical activity with both pregnant women and fathers (in all clinic settings); linking to effective programs for postpartum weight loss to better prepare women for a next pregnancy; teaching parents about satiety versus hunger cues and about soothing approaches that can be used during early infancy; reassessing recommendations for appropriate weight gain during infancy and rethinking catch-up growth parameters for infants who are premature or small for gestational age; including families in setting nutrition and physical activity goals during toddlerhood for themselves and their child; and linking to trusted, effective community programs.

PANEL DISCUSSION WITH SPEAKERS

Following Shari Barkin's presentation, a panel discussion with the speakers led to further discussion of breastfeeding and its association (or lack thereof) with the risk of offspring obesity, the famine studies, differences between animal and human studies, and calls to focus more on fetal and placental health.

Breastfeeding and Risk of Offspring Obesity

An audience member asked about breastfeeding data and what researchers know about the effects of breastfeeding on potential prevention of rapid infant weight gain and offspring metabolic dysfunction. The questioner was especially curious about the concept of dose, which Barkin had emphasized during her presentation. Specifically, what is known about exclusive versus partial breastfeeding in terms of preventing obesity and obesity-related metabolic disorders? Barkin commented on the complexities of that question, noting that bringing a baby directly to the breast, compared to bottle-feeding a baby expressed milk, not only affects the microbiome of the child in a different way but also encourages recognition of infant satiety. In her opinion good data indicate that exclusive breastfeeding for 6 months confers many benefits, such as immune globulin transference and its associations with reduced risks for cancer and type 1 diabetes. However, based on available data, in Barkin's opinion exclusive breastfeeding does not appear to confer a protective benefit against obesity. She mentioned that in her clinic she has observed many women who use both breastfeeding and bottle-feeding and often overfeed their infants, because they find it

difficult to distinguish between satiety and hunger cues. She encouraged an assessment of that behavior.

Matthew Gillman added that in the developing world it seems that 6 months of exclusive breastfeeding is a very good recommendation. In the developed world, on the other hand, he suggested some moderation of that recommendation, specifically 4 to 6 months. Based on his team's research results, the optimal time to introduce solid foods for obesity prevention is 4 to 6 months. He mentioned additional, emerging data from the allergy literature that also suggest that 4 to 6 months is the optimal time in Westernized countries to introduce solid foods. On the topic of infant feeding, Gillman encouraged moving the discussion beyond talking about breast versus bottle-feeding or breast versus formula feeding to talking about the different ways that people feed and other behaviors that affect infant growth trajectories and, later, metabolic function.

Famine Studies

There were several questions related to the famine studies described by Aryeh Stein. Andrea Baccarelli of the Harvard School of Public Health remarked that, much like the famine data suggest, prenatal exposure to endocrine-disrupting chemicals often has a more pronounced effect on females than on males, and he asked whether what he referred to as the "susceptibility window hypothesis" is enough to explain the difference. In the case of endocrine-disrupting chemicals, he said, he does not believe that it is. Stein replied that the differential susceptibility, or manifestation, of exposures seen in the two Dutch Hunger Winter studies were based on very small sample sizes in a very select population (Ravelli et al., 1999; Stein et al., 2007). During World War II, roughly two-thirds to three-quarters of all Dutch infants were delivered at home, yet the two studies that included both men and women were hospital-based prospective follow-up studies, so they represented a relatively low-risk group. He said that, yes, there is a signal, but it is unclear whether it is a strong signal.

Stein was also asked whether women who conceived during the studied famines, so the women who were not amenorrheic, might have had a greater body fat to begin with. "Absolutely," Stein replied, "but we don't know that." His assumption would be that because the ability to menstruate is physiologically regulated, then some of the amenorrhea was related to energy balance. But some of it might have been stress. Both of those factors were affected during the famine. In addition to physiological reasons for not conceiving, there were also behavioral reasons. For all of those reasons, comparisons between conceptions during famine and conceptions during any other period are confounded.

Animal Studies Versus Human Studies

The advantages, compared to the disadvantages, of using animal studies versus human studies was a topic that came up regularly over the course of the workshop discussion. In this session Kevin Grove of the Oregon National Primate Research Center challenged Gillman's call for consensus around an appropriate animal model. In Grove's opinion, there should be more variability in study designs, not less. There is no single great animal model for human health, he said. Not even humans are a great model, given that phase 1 and phase 2 trials are not very predictive of what happens in phase 3 outpatient trials. He suggested follow-up analyses of the variability among animal models to better understand how and why different models are relevant to human health (i.e., identify why one model predicted something relevant to human health while another did not, etc.).

Another workshop participant commented on Gillman's suggestion that animal studies be more like human randomized controlled trials. He said that even human randomized controlled trials are "so flawed" and the confounding factors are extraordinary, especially given that many people, particularly in desperate circumstances, are not willing to undergo randomization. Gillman agreed that even randomized controlled trials in humans tend not to be generalizable because they are among volunteers. But the same could be said of animals, he said. He shared an anecdote from a sheep researcher from New Zealand who said, "Well, we go up to the hill and choose the sheep that look the best." So, Gillman said, even animal experiments are not necessarily generalizable to the source population.

The Fetus and Placenta

An audience member commented that much of the information presented in this session on clinical applications had focused on how to monitor mothers or children, with little attention directed to how to monitor in utero development. In addition to monitoring gestational weight gain, the audience member said, researchers should also be thinking about the growth of the fetus and trying to identify in utero indicators that could be used to predict adiposity.

6

Data Gaps and Future Directions

OVERVIEW

The workshop concluded with two moderated discussions. First, Esa Davis of the University of Pittsburgh Medical Center moderated a discussion among all workshop speakers on possible future research directions, with an emphasis on expanding what scientists know about epigenetic-mediated associations between early developmental exposures and subsequent obesity-related health outcomes. Then Judith Hall of the University of British Columbia led a discussion on opportunities and challenges in epigenetics research that was open to all workshop participants.

FACILITATED DISCUSSION ON DATA GAPS AND FUTURE RESEARCH

Davis started the first discussion by listing what she observed as some consistent themes of the workshop discussion: the idea of permanency versus reversibility (i.e., with respect to epigenetic markers and their persistence over time), the need for predictive biomarkers, and efforts to understand causal mechanisms. She also observed that several speakers had called for efforts to collect prospective and longitudinal data. But, she asked, what kind of data? What are some existing cohorts or longitudinal data that could or should be used? What are some key data elements or features that any new cohorts should include?

How to Strengthen Human Observational Studies

Much of the discussion revolved around how to strengthen human observational studies in ways that will allow researchers to extract more types of useful information about the mediating role of epigenetics, especially given that, as Karen Lillycrop pointed out, many existing study cohorts were designed before epigenetics was on most researchers' minds.

For example, according to Lillycrop, it used to be that researchers thought that one biological sample was sufficient, that is, they would collect and look at DNA one time and that would be it. Even in cases when they collected repeated samples over time, they would pool the DNA because of the assumption that DNA remains stable over time. Obviously that is not the case with epigenetic markers, Lillycrop said. She suggested the collection of repeated biological samples over time so that epigenetic changes early in life, particularly during the first 1,000 days, can be monitored. Multiple sampling over time requires a source material. She suggested buccal cells (i.e., from the inside of the cheek) as a relatively noninvasive source, although collecting sufficient DNA from buccal cells from infants is difficult.

Matthew Gillman was of the opinion that, in fact, there are many cohorts, especially in the developed world, with repeated biological sampling as well as good phenotyping, and that many of those cohorts are being used to examine epigenetic and other biological mediators between exposures and outcomes. However, he agreed that the next generation of cohort studies could be improved. He encouraged more cohorts in countries in transition, especially given the increasing prevalence of obesity in the developing world. Additionally, while it is difficult to study preconception given that many pregnancies are unplanned and that there is no ready source of recruitment, designing new cohorts in ways that would allow for the study of preconception would be helpful in his opinion. Finally, he said, because most studies to date have limited information on fathers, new cohorts that provide repeated biological and behavioral data on fathers would also be very helpful.

Repeated sampling or not, Kevin Grove observed that, often, when he asks an investigator why he or she collected a particular sample, the investigator will reply that it was available or cost-effective. "That is not a way to do a study," Grove said, "especially when you get down to epigenetic analysis." He emphasized the need for rational tissue selection. While it may not be as easy to get a sample of the hypothalamus, for example, as it is the muscle, Grove stressed keeping in mind that, right now, most researchers are sampling what is easy to get, not what is relevant.

Rather than generating more epigenetic data as part of either existing or future cohort studies, Caroline Relton of Newcastle University and

the MRC Integrative Epidemiology Unit at the University of Bristol suggested that it would probably be better to collect genetic data on samples for which DNA methylation and other epigenetic data have already been collected. She emphasized the need to generate genetic and epigenetic data in tandem, especially given the extreme challenge of interpreting DNA methylation and other epigenetic signatures without an understanding of the underlying genetic architecture. Doing so would be especially helpful, she said, for cohorts of different ethnic groups. Without knowledge of the underlying genetic architecture, comparing and contrasting epigenetic data across different ethnic groups will be very challenging.

Relton also suggested that intensive sampling in early life, during critical windows of epigenomic plasticity, would be beneficial, as would synthesizing the information from differently aged cohorts as a way to examine epigenetic variation over time. She agreed with Gillman that there is huge potential for those carrying out family-based study designs to improve their collection of data on fathers and siblings in already existing cohorts.

Instead of measuring DNA methylation or RNA expression in samples, Andrea Baccarelli at the Harvard School of Public Health suggested that another option, especially if cost is a limiting factor, would be to obtain information from the many online databases that include those data. In some cases, even tissue-specific data are available. He also mentioned the recently evolving concept of poised genes, that is, genes that are poised to be more easily activated. In his opinion, information collected on poised genes would be very interesting in the context of longitudinal studies.

With respect to exposure data and echoing Gillman's call for more studies in countries in transition, Aryeh Stein of Emory University said that as an epidemiologist his interest is in the variance in prenatal, preconception, and childhood exposures. There is far more variance in the world than is concentrated in the Northern Hemisphere, yet virtually all existing cohort studies are in the north. He said, "What we need is a lot more investigation in the global south, where . . . all these problems are much larger."

During the later discussion moderated by Judith Hall, but of relevance here, Jacob Friedman of the University of Colorado, Denver, remarked that, as a geneticist, he works with colleagues who conduct genetic screens in infants. In Colorado, the goal is to screen every infant for rare disorder. What is missing, or what needs to be done in his opinion, is a screening of infants for biomolecules that are actually within the range of normal but can be predicted by changes in fetal growth, changes in maternal weight gain, or maternal exposures. Instead of core tissue blood samples, which contain a mix of fetal and maternal blood, heel-stick samples could be used to conduct the screenings within the first 24 to 48 hours of life. In response, Hall agreed that such data would be invaluable but noted that newborn

screening regulations vary by state with respect to which tissues can be used for what type of screening.

In addition to better epigenetic and exposure measures, Linda Adair of the University of North Carolina at Chapel Hill stressed the need for better outcome measures. She observed that several workshop speakers had critiqued body mass index (BMI) as an outcome measure. She called for more precise, but field-friendly (i.e., obtainable outside a laboratory or clinical setting), measures of body composition.

Interpreting Epigenetic Data

As scientists learn more about epigenetic mechanisms, they also learn more about their complexity. For example, as Robert Waterland of the Baylor College of Medicine had pointed out earlier during the workshop, not too many years ago it was believed that a methylated gene was a silent gene. Now, however, it appears that DNA methylation can either silence or activate a gene. In fact, as Baccarelli pointed out, DNA methylation is not even always associated with gene expression. It is only one of the layers that control gene expression. The same is true of histone modifications. The growing "morass of [epigenetic] data," as one participant described it, raised the question for Kevin Grove, does the knowledge exist and is there enough computing power to accurately predict likely biological outcomes based on those data?

Several participants agreed that computing power is not the limitation. Rather, the challenge will be to make sense of the data. Waterland pointed to the recent *Nature* paper summarizing the findings of the Roadmap Epigenomics Project (Kundaje et al., 2015), which, in Waterland's opinion, is an indication of the state of the art in the ability to link epigenome-wide annotation of various markers (i.e., not just DNA methylation markers, but also histone modifications and other markers) with gene expression. For Waterland, the question is whether the political will exists to make the investment necessary for understanding inter-individual variation in mechanism, cell type and tissue specificity, and other issues. As sequencing costs continue to decline, the greater expense will be data management and analysis. Relton agreed with Waterland that the computational power exists and that the greater challenge now is to knit the accruing data together and place it in context.

Referring to Baccarelli's statement that DNA methylation is only one of multiple layers of phenomena controlling gene expression, Marie-France Hivert of the Harvard Medical School remarked that, in her opinion, the next challenge is to interpret epigenetic data across those multiple layers. Additionally, she noted, not only are epigenetic markers tissue-specific, but they are also context-specific, with she and her research team having

observed signals present during pregnancy that do not exist outside of pregnancy. She suggested assembling teams of geneticists, physiologists, and other scientists to interpret the data.

During the second discussion, moderated by Hall, an audience member remarked that the complexity of the issues being discussed is going to increase as more types of data are collected in the future. The challenge of sorting through that complexity will be compounded by the fact that when a complex system is perturbed, the change is not necessarily linear. The system itself can change. He suggested that this is particularly true of developmentally plastic periods, when molecules develop new relationships with each other. He suggested that some of the new system science approaches may be helpful for identifying key mechanisms.

FACILITATED DISCUSSION ON OPPORTUNITIES AND CHALLENGES IN EPIGENETICS RESEARCH

As a prelude to the second discussion, Judith Hall remarked that a principle of clinical genetics is that the "really unusual case" is important. It is those cases, she said, teach researchers about pathways. For her, the *ob/ob* agouti mouse (agouti mice homozygous for the *ob/ob* mutation, which makes them completely leptin deficient and leads to extreme obesity) is an example of the really unusual case.

Hall presented a mammalian evolutionary perspective on the issues being discussed at the workshop. For her, thinking way back "in the sands of time," millions of years ago, the ability to be flexible and to have plasticity in response to environmental changes was how mammals evolved. While biologists have known this for some time, only recently have they begun to appreciate that flexibility and to study the multiple metabolic, psychological, and immunological pathways at play. When mammals first evolved, according to Hall, it was the placenta that allowed for mother–fetus communication. In Hall's opinion, that's what the Developmental Origins of Health and Disease (DOHaD), and epigenetics as part of that, is all about: the mother communicating with the fetus, via the placenta, to prepare the fetus to survive in the environment that it is about to enter.

Epigenetics is not a cool or mysterious thing, Hall said. Rather, it is simply that now that scientists have mapped the whole genome, they are "finally getting around to gene control," she said. They are finding that genes that are turned off have different markes than genes that are turned on and that there are several different types of such markers. "It's not magic," she said. "It is actually just kind of what one would expect."

Concerning the challenges in epigenetics research, Hall emphasized that there are many factors to consider when thinking about obesity, beginning with maternal birth weight. Most animal studies, she said, do not consider

maternal birth weight. The assumption is that maternal birth weights are identical. But in a litter of newborn rats, for example, not all eight rats are identical. Where they were located in the uterus may have had major effects. Hall encouraged researchers to be more careful about birth weight of the mother in both animal and human studies.

In addition to maternal birth weight, the field would benefit from greater collection of data on maternal health, stressors, diet, and related factors. The egg from which each of us was formed, Hall explained, was itself formed when our mothers were 6-week-old embryos. She disagreed with skeptics who think that maternal data are unobtainable. An estimate of birth weight, for example, can be derived simply from the mother knowing whether she was premature or not. Even better, researchers can find birth weights in hospital records where mothers were born. She herself has done that, Hall said; likewise with information about the father. Again, in her opinion, information on his birth weight, health, stressors, and diet is important. After all, sperm are formed 2 months before conception. She identified the diets of teenage boys and paternal exposures to endocrine disruptors (at any time before conception) as two areas of particular concern. For both fathers and mothers, she viewed "anything [in the environment] that is not natural," including plastics, insecticides, artificial hormones, and antibiotics and other drugs, as factors that could potentially affect epigenetic patterning. Socioeconomic stress also likely plays a role in her opinion.

Hall also called for greater consideration of age-specific expression. Humans go through many stages, including embryo, early fetus, later fetus, newborn, and infant, and the physiology of those different stages is not well understood. In Hall's opinion, a better understanding will come from examining which genes are turned on and off in every tissue at different stages of development. She suggested that in unfortunate situations where children in cohorts die, researchers could use tissues saved from autopsies to examine epigenetic markers. In order to do that, she added, pathologists need to be engaged in the effort.

In sum, without considering these many other factors, she said, "You don't get the whole picture." The challenge is, how? She sought answers from the audience.

Measuring Nutrition in Humans

An audience member asked how researchers can measure nutrition in humans, especially during pregnancy, and how they can link that information to epigenetic changes and phenotypic outcomes. Grove added that the challenge of measuring nutrition is made even more complicated by the reality that what is nutritious in one individual is not necessarily nutritious in another, depending on the body's biochemical and molecular

capabilities—for example, how someone's body handles different kinds of lipids or carbohydrates.

Another participant remarked that, as a nutritional epidemiologist, he has spent a fair amount of time thinking about how to measure what people eat. He has observed that researchers do not usually know what people eat; rather they know only what people say they eat, and the two are not the same. The question for him is, what is it that researchers want to know? Do they want to know what people are eating, what their microbiomes are eating, or their nutritional status? Those are different questions. If all that is sought is a measure of what people are eating, he speculated that in a few years researchers will be issuing Google glasses to study participants. That should provide a pretty good sense of what people are actually eating, he said. He agreed with Grove, however, that what that food does to the body is a different question.

The original thinking behind DOHaD, another participant said, emerged from studies relating lower birth weight to outcomes later in life, especially diabetes and cardiovascular disease. It was only later, over time, that the focus on lower birth weight shifted to a focus on maternal diet, with the term "under-nutrition" being, in the commenter's words, "thrown around." In the commenter's opinion, the real object of discussion should be nutrition of the fetus, with maternal diet being only one small piece of that. Other pieces, he said, include placental function, fetal physiology, and maternal nutritional and other exposures before pregnancy. While it is very important to consider maternal diet, both during and before pregnancy, he encouraged a broader view and greater consideration of growth and health of the fetus.

Hall appreciated mention of the original thinking being the developmental origins of health and disease paradigm and the way David Barker's focus on low birth weight infants triggered an "aha moment" for many researchers—that there was something very important about those infants. But what? In her opinion, good data are still needed.

Use of the Word "Epigenetics"

Waterland observed that much of the workshop discussion had focused on the word "epigenetics." He reminded the workshop audience that epigenetics is just one potential mechanism of developmental programming. He agreed with Sarah Richardson that scientists need to do a better job conveying the complexity of epigenetics when communicating about epigenetic science in the public sphere. "In order to convey the complexity," he said, "one thing we can do is not use 'epigenetics' and 'developmental programming' synonymously." He explained that epigenetics refers very specifically to a suite of cell autonomous molecular mechanisms that stably regulate

gene expression potential. Even though researchers know very well that developmental programming of body weight regulation occurs in humans, it is unclear whether it occurs via epigenetic mechanisms.

Sleep and Metabolic Dysfunction

Referring to Antonio Convit's discussion of sleep (see Chapter 4 for a summary of Convit's presentation), a workshop participant remarked that sleep is known to play a role in maintaining metabolic function. For example, poor sleep patterns have been associated with insulin resistance. Given that pregnant women experience varying abilities to sleep at different times during pregnancy and even postpartum, and that baby sleep patterns are also interrupted postnatally, the participant urged a better understanding of the role of sleep both during and after pregnancy and its effect on the fetus and infant. She expressed curiosity about what researchers have learned from animal studies about the effects of sleep on metabolic dysfunction.

The Persistence of Epigenetic Markers

While there was no extensive discussion during this final session on the temporary versus permanent nature of epigenetic patterning, as there had been at earlier times during the workshop, Friedman mentioned that based on human tissue cultures sampled from obese individuals, some epigenetic patterns are reproducible. The reproducibility of those patterns suggested to him that there is some persistence, even as exercise, diet, and other factors can impact the epigenome. He suspected that researchers will be learning a lot about that persistence as they continue to study human tissue cultures.

Pediatricians in the Clinic

An audience member who described himself as a pediatric weight management clinician observed that when he first started working with children with weight problems 12 years ago, he felt like he did not have the tools to help his patients. It seemed far too simplistic to tell a child and his or her parent that the child needed to eat less and exercise more. Now, 12 years later, this greater understanding of underlying mechanisms will help to empower not only parents of children with weight problems, but also primary care providers. By thinking about obesity as a disease of biochemistry and physiology, he said, "You will look at the child and the mother totally differently."

REFLECTIONS ON THE WORKSHOP DISCUSSION BY SHARI BARKIN

"In our beginning is our end."—T. S. Eliot

In her opening remarks on the second day of the workshop, Shari Barkin of the Vanderbilt University School of Medicine began her remarks by referring to Andrea Baccarelli's sheet music analogy, in which the sheet music is the genome, the composer's notes are epigenetic markers, and the music produced is the phenotype. The same sheet music is played differently by the cello than by the flute, for example, just as the same genome yields different phenotypes in different tissues because of tissue-specific epigenetic markers.

Barkin identified several lessons learned from the Day 1 presentations and discussion. First, DNA is a code that has context, and its context—that is, the environment in which an individual lives and where one's parents and grandparents lived—can affect modifiable epigenetic markers and, therefore, the way that DNA is expressed.

Second, exposure during pregnancy has phenotypic consequences for offspring. Maternal diet during pregnancy affects the patterning of the infant microbiome, which, in turn, affects infant metabolism; placental functioning affects infant inflammation; and leptin dysregulation affects appetite regulation and, later, adiposity. As Hivert discussed, just as genes are affected by context, so too are hormones, with either a negative or positive leptin regulation feedback loop operating, depending on maternal obesity.

A third point is that the paternal diet and fathers' contributions matter. Barkin urged researchers to measure those contributions.

Fourth is the mismatch hypothesis, as first mentioned by Linda Adair of the University of North Carolina at Chapel Hill and then discussed throughout the workshop. A difference in exposures in utero versus exposures ex utero creates a mismatch, where a fetus is primed while in utero for something different than what an individual experiences ex utero. Most of the discussion around the mismatch hypothesis focused on the mismatch created when an individual is exposed to a low-fat diet in utero but a normal or high-fat diet ex utero. Barkin raised the question of what happens when a phenotype is mismatched in the other direction, that is, when an individual is exposed to a high-fat diet in utero but either a normal or high-fat diet ex utero. It appears that sometimes when individuals are exposed to a high-fat diet in utero but a normal diet ex utero, the phenotype normalizes. It is unknown whether exposure to a high-fat diet in utero and a high-fat diet ex utero actually induces better health. For Barkin, the mismatch hypothesis again highlights the importance of context. It is

important to think not just about the present moment of time, she said, but the past as well. "We are the accumulation of everything that has come before," she said.

Another lesson learned is that the timing and duration of exposures matter. It is important to always ask the question, when did the exposure occur, and for how long?

A sixth lesson learned is what Barkin referred to as "the rule of Cs." As you are reading the science, she said, always ask, are the results showing a correlation, causation, or confounding? She referred to Caroline Relton's discussion of the multiple ways that this question can be examined and Relton's call for a triangulation of evidence to address and confirm causation.

Yet another lesson learned is that when considering the clinical application of obesity prevention strategies, the sex of the child matters. Boys and girls respond differently to the same exposures. Additionally, sometimes there may be a short-term payoff but with long-term consequences. A compelling way to think about the microbiome, Barkin observed, is not just that it weighs 3 pounds, but that the majority of genes in a human body are not human. Barkin referred to Meredith Hullar's presentation on the impact of the microbiome on human biological functioning, especially metabolism. Understanding the extraordinary symbiosis between the microbiome and human metabolism is important in Barkin's opinion.

Lastly, she referred to Antonio Convit's and Kevin Grove's focus on metabolic dysfunction more generally, not just BMI. BMI itself is a crude marker of obesity in her opinion. It is the underlying metabolic dysfunction reflected in obesity that creates a pro-inflammatory state and vascular dysregulation, whether in the placenta (as Grove discussed) or the brain (as Convit discussed), with significant downstream consequences (e.g., reduced hippocampal volume and placental insufficiency).

Closing Remarks

In her closing remarks to the workshop, Barkin reiterated that epigenetics is just one approach to understanding the dynamic interaction between genetics, environment, and childhood development that affects childhood obesity and one that potentially offers insights into periods of potential reversibility and prevention.

She also stressed the need, first, to assess epigenetic variation in the context of genetic variation, as Waterland had stated during his presentation and as Relton had echoed in her comments in one of the final workshop discussions (see above), and, second, to focus on regions in the genome with inter-individual variation.

Barkin urged clarity about how the science discussed at the workshop

can benefit society. Is the goal to shed light on causal mechanistic pathways? Or is the goal to develop predictive biomarkers for either diseases or intervention responses? Or is the goal a bit of both? Which tissues should be examined depends on the question. She said, "Our approach, depending on our goal, should be different." She also called attention to transgenerational influences and the need to consider what is meaningful and practical to include and assess.

Another issue to consider, she said, is technology. Barkin reiterated Waterland's remarks about DNA methylation being merely one way to understand epigenetics, and epigenetics being merely one way to understand developmental programming. She stressed the need to understand what the right technology is with regard to interpreting the data. For example, the current DNA methylation technology is predominantly focused on promoter regions, but enhancer and intragenic regions are just as important to consider. Furthermore, she suggested considering the possibility that when one is working with animal models, examining DNA methylation at any genomic region might not be the best approach. A better approach might be to examine microRNA. She urged the creation of animal models that will have the greatest opportunity for translation into people and, as Matthew Gillman had suggested during his presentation, translation from people back into an animal model.

Barkin underscored the need for developing more tools to understand the complexity of interactions discussed at the workshop. What is being seen is likely the result of a cascade of factors set in motion generations ago, she said. But which epigenetic markers are "in ink" (permanent) and which are "in pencil" (transient)? And when are those markers being made?

The opportunity is vast, Barkin said, especially given today's remarkable momentum behind big data. How then, she asked, can that momentum provide opportunities to better answer the questions posed at this workshop? The possibilities include developing systematic data elements, perhaps standard protocols, and ways to incorporate those elements or protocols across cohorts and longitudinal trials. She suggested starting not with what she called "the impossible things," but rather with data that are simple and compelling, such as maternal and paternal birth weight. Finally, she emphasized the importance of tissue specificity, particularly the placenta, given its role as "great communicator" between the mother and child. In closing, Barkin stated, "The power of epigenetic science is understanding what we can do today to affect the health of future generations, as well as what we can do today to mitigate or modify the effects on this generation."

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Appendix A

Workshop Agenda

Institute of Medicine and National Research Council
Food and Nutrition Board and Board on Children, Youth, and Families
Examining a Developmental Approach to Childhood Obesity:
The Fetal and Early Childhood Years
Planning Committee on Understanding the Dynamic Relationship Between Biology, Environment, and Early Childhood Development on Risk of Obesity

February 26–27, 2015
Keck Building, Room 100
The National Academies
500 Fifth Street, NW
Washington, DC

WORKSHOP OBJECTIVES

- Identify epigenetic-mediated relationships between exposure to risk factors during sensitive periods of development (gestation through age 3) and subsequent obesity-related health outcomes.
- Explore the science around periods of plasticity and potential reversibility of obesity risk in the context of early childhood development.
- Examine the translation of epigenetic science to guide early childhood obesity prevention and intervention to reduce obesity risk.

DAY 1

8:00–8:45 a.m. Registration

Introduction and Opening Remarks

8:50 a.m. **Welcome**
Shari Barkin, William K. Warren Family Foundation Chair in Medicine and Professor of Pediatrics, Monroe Carell Jr. Children's Hospital at Vanderbilt University

9:00 a.m. **Opening Remarks**
David M. Klurfeld, National Program Leader, Human Nutrition, Agricultural Research Service, U.S. Department of Agriculture
Sandra Hassink, Medical Director, Institute for Healthy Childhood Weight, American Academy of Pediatrics
Jamie Bussel, Program Officer, Robert Wood Johnson Foundation

Session 1: The Role of Epigenetics in Pediatric Obesity—Conceptual Overview

Moderated by Matthew Gillman, Harvard School of Public Health

9:30 a.m. **Fundamentals of Epigenetics**
Robert Waterland, Baylor College of Medicine

9:50 a.m. **Conceptual Model of Epigenetic Influence on Obesity Risk**
Andrea Baccarelli, Harvard School of Public Health

Session 2: Etiology and Causal Inference

Moderated by Karen Lillycrop, University of Southampton

10:10 a.m. **Epigenetic Mechanisms for Obesity Risk**
Jacob Friedman, University of Colorado, Denver

10:30 a.m. **The Role of Disparity in the Origins of Obesity Risk**
Linda Adair, University of North Carolina at Chapel Hill

10:50 a.m. **Fathers' Early Contribution to the Birth of the Child: The Role of Paternal RNAs**
Stephen Krawetz, Wayne State University

11:10 a.m. **Maternal Influences on Offspring's Epigenetics and Later Body Composition**
Caroline Relton, Newcastle University

11:30 a.m. **Q & A with Participants**
12:00 p.m. **Break for Lunch**

Session 3: Opportunities for Intervention and Prevention

Moderated by Leann Birch, University of Georgia

1:00 p.m. **Developmental Plasticity—Sensitive Periods and Risk of Obesity**
Karen Lillycrop, University of Southampton

1:20 p.m. **Maternal Health and Diet's Effect on Offspring's Metabolic Functioning**
Kevin Grove, Novo Nordisk

1:40 p.m. **Early Infant Rapid Weight Gain and the Epigenetics of Leptin**
Marie-France Hivert, Harvard Medical School

2:00 p.m. **Therapies to Reverse Metabolic Disturbances Arising as a Consequence of Developmental Programming**
Mark Vickers, University of Auckland

2:20 p.m. **Panel Discussion with Speakers**
Moderated by Leann Birch, University of Georgia

2:40 p.m. **Break**

3:00 p.m. **The Microbiome and Our Genome**
William Nierman, J. Craig Venter Institute

3:20 p.m. **The Epigenetics of the Microbiome**
Meredith Hullar, Fred Hutchinson Cancer Research Center

3:40 p.m. **Toxic Stress and Its Role in Childhood Obesity**
Antonio Convit, Nathan Kline Institute for Psychiatric Research

4:00 p.m. **Panel Discussion with Speakers**

4:30 p.m. **Concluding Remarks**
Shari Barkin, Monroe Carell Jr. Children's Hospital at Vanderbilt University

DAY 2

8:50 a.m. **Welcome and Summary from Day 1**
Shari Barkin, Monroe Carell Jr. Children's Hospital at Vanderbilt University

Session 4: Real-World Application

Moderated by Debra Haire-Joshu, Washington University in St. Louis

9:00 a.m. **Early Exposure Events and Obesity-Related Outcomes**
Aryeh Stein, Emory University

9:20 a.m. **Messages to Women About Epigenetics and Childhood Obesity**
Sarah Richardson, Harvard University

9:40 a.m. **Theory to Policy**
Matthew Gillman, Harvard Pilgrim Health Care Institute

10:00 a.m. **Theory to Clinical Practice**
Shari Barkin, Monroe Carell Jr. Children's Hospital at Vanderbilt University

10:20 a.m. **Panel Discussion with Speakers**

Session 5: Data Gaps and Future Directions

Moderated by Esa Davis, University of Pittsburgh Medical Center

10:40 a.m. **Facilitated Discussion on Data Gaps and Future Research**
Invited Speakers from Days 1 and 2

11:00 a.m. **Facilitated Discussion on Opportunities and Challenges in Epigenetics Research**
Judith Hall, University of British Columbia

11:30 a.m. **Chair's Summary and Final Thoughts**
Shari Barkin, Monroe Carell Jr. Children's Hospital at Vanderbilt University

12:00 p.m. **Adjourn Meeting**

Appendix B

Speaker Biographies

Linda S. Adair, Ph.D., is a professor of nutrition in the School of Public Health at the University of North Carolina. Dr. Adair is a biological anthropologist interested in maternal and child nutrition. Her theoretical orientation comes from human biology, and she is interested in how human populations respond to nutritional stresses. She is currently working on a large-scale longitudinal survey of women and children in the Philippines. This work involves an exploration of patterns and determinants of growth from infancy through young adulthood; the long-term consequences of fetal and early child-growth patterns; the development of chronic disease risk factors in adolescents and young adults; and determinants of women's nutritional status through the life cycle. She also collaborates with other Department of Nutrition faculty in the study of (1) gene–environment interactions as determinants of health and nutritional status; (2) feeding and parenting styles of African American parents and the growth of African American infants; and (3) factors affecting postpartum maternal-to-child transmission of HIV and maternal and child nutritional status in Malawi, as well as nutrition projects in rural South Africa and China. She teaches international nutrition, advanced methods of nutritional epidemiology, and the doctoral seminar. She received her B.S. in biological sciences in 1971 from the State University of New York at Stony Brook and her Ph.D. in biological anthropology from the University of Pennsylvania in 1980.

Andrea Baccarelli, M.D., M.P.H., Ph.D., is the Mark and Catherine Winkler Associate Professor of Environmental Epigenetics in the Department of Environmental Health and Department of Epidemiology at the Harvard

School of Public Health. His research focuses on identifying molecular and biological factors reflecting the impact of environmental exposures on cancer risk, with a particular interest in epigenetics. Epigenetic markers, including DNA methylation, histone modifications, and non-coding RNAs, modify chromatin structure and gene expression without changing the underlying DNA sequence. Unlike genetic mutations, which represent rare events with permanent consequences on genes, epigenetic changes are reversible and responsive to environmental influences. Using a highly quantitative pyrosequencing-based approach for DNA methylation analysis, Dr. Baccarelli has been examining the effects on DNA methylation of a variety of environmental carcinogens, including particulate air pollution, airborne benzene, metals, pesticides, dioxin-like compounds, and persistent organic pollutants, which are known to be relevant to cancer etiology.

Shari Barkin, M.D., M.S.H.S., is a professor of pediatrics and holds the William K. Warren Family Foundation Chair in Medicine at the Vanderbilt University School of Medicine, where she is also the director of the Division of General Pediatrics and the director of pediatric obesity research. The Barkin laboratory studies family-based, community-centered clinical interventions to improve health behaviors such as physical activity and nutrition in parent/young-child dyads. The lab is focused on changing early body mass index (BMI) trajectories in childhood to prevent childhood obesity and later related adult chronic conditions. The interventions developed and tested apply the ecologic model that considers the child in the context of his or her family, and the family in the context of its community, considering how to pragmatically transform scientific discovery into potentially sustainable interventions that can improve the public's health. A theme of the lab is the dynamic interaction among genetics, behavior, and environment at sensitive periods of childhood development. The lab applies a wide variety of techniques to address these complex problems, including qualitative and quantitative methodologies. The lab considers objective biologic measurements (such as fat mass and BMI), genetic measurements (genetic allelic risk scores, epigenetics), social measurements (social networks), and behavioral measurements (actigraphy changes over time in both parents and children, use of existing built environment to sustain healthy lifestyle behavior changes). Dr. Barkin serves as the principal investigator of the Growing Right Onto Wellness (GROW) Trial, a 7-year randomized controlled trial to prevent childhood obesity that is funded by the National Heart, Lung, and Blood Institute and the National Institute of Child Health and Human Development, and she serves on the steering committee for the Childhood Obesity Prevention and Treatment Research (COPTR) consortium of the National Institutes of Health. She is also in her second term on the Institute of Medicine and National Research Council's Board of Children, Youth,

and Families. Dr. Barkin received her medical degree from the University of Cincinnati Medical College and completed a Robert Wood Johnson Clinical Scholars fellowship at the University of California, Los Angeles.

Jamie B. Bussel, M.P.H., is a program officer in the area of childhood obesity and catalyzing demand for healthy practices and places with the Robert Wood Johnson Foundation. She directs initiatives that foster multidisciplinary partnerships and systems-level change strategies to transform the health of people and places. She leads the childhood obesity work focused on halting obesity from pregnancy through a child's fifth birthday. Previously, Ms. Bussel held research positions at the University of Medicine and Dentistry of the New Jersey School of Public Health and the University of Pennsylvania.

Antonio Convit, M.D., is the deputy director of the Nathan Kline Institute and a professor of psychiatry, medicine, and radiology at the New York University School of Medicine. Dr. Convit's work focuses on understanding the impact of obesity-mediated metabolic disease on the brain. He also created the Banishing Obesity and Diabetes in Youth (BODY) Project, a public health program to help obese adolescents reduce their risk of type 2 diabetes and early cardiovascular disease. Dr. Convit is a native of Venezuela. He obtained his M.D. from the University of Chicago, Pritzker School of Medicine, and trained in psychiatry at the New York University Medical Center.

Jacob E. Friedman, Ph.D., is a professor in the Department of Pediatrics at the University of Colorado, Denver. His primary focus is on understanding the role of early nutrition and the environment on molecular, endocrine, and epigenetic origins of childhood obesity and diabetes. This involved developing novel animal models of obesity (mouse, nonhuman primate) together with invasive human clinical investigation in vivo and in vitro, using human skeletal muscle, adipose tissue, and, more recently, umbilical-derived mesenchymal stem cells from infants born to obese women with and without gestational diabetes mellitus. Dr. Friedman is currently a principal investigator, a co–principal investigator, or a coinvestigator on multiple basic, clinical, and large-scale epidemiological studies of pregnancy and obesity and maternal–fetal outcomes that are funded by the National Institutes of Health, the American Diabetes Association, and The Bill & Melinda Gates Foundation.

Matthew Gillman, M.D., S.M., is a professor of medicine at the Harvard Medical School and the Harvard Pilgrim Health Care Institute, a professor of nutrition at the Harvard School of Public Health, and the director of the Obesity Prevention Program in the Harvard Pilgrim Health Care Institute's

Department of Population Medicine. His research interests include early life prevention of chronic disease, including obesity, diabetes, cardiovascular disease, and asthma; individual and policy-level interventions to prevent obesity and its consequences; and childhood cardiovascular risk factors. He directs Project Viva, a National Institutes of Health–funded cohort study of pregnant women and their offspring that focuses on effects of gestational diet and other factors on outcomes of pregnancy and childhood. Dr. Gillman also leads or participates in several other federally funded studies of diet, activity, obesity, and cardiovascular risk in children and adults. He has served in leadership roles in the U.S. National Children's Study, the International Society for Developmental Origins of Health and Disease, the American Heart Association, and the American Academy of Pediatrics. He was a member of the Institute of Medicine Committee to Reexamine IOM Pregnancy Weight Guidelines. He is an active teacher of medical students and mentor to research trainees. Formerly a primary care internist and pediatrician, Dr. Gillman's current clinical work is in preventive cardiology among children.

Kevin L. Grove, Ph.D., is a senior scientist in the Division of Diabetes, Obesity, & Metabolism and the Division of Reproductive & Developmental Sciences at the Oregon National Primate Research Center in Beaverton, Oregon. He is also the vice president of obesity research for Novo Nordisk in Seattle, Washington. In the past 20 years in Oregon, Dr. Grove has developed an internationally recognized research program focused on how poor pregnancy health and nutrition place offspring at a higher risk of metabolic and psychiatric diseases. His group also focuses on dietary and nutrient supplements that may prevent these health complications. Both of these programs extensively use nonhuman primate (NHP) models. Using these highly relevant and translational research models, Dr. Grove has built international collaborations to understand the critical aspects of malnutrition during pregnancy, including both consumption of Western-style diets and the impact of under-nutrition. Dr. Grove received his B.S. in the Department of Animal Science at Washington State University in 1990 and his Ph.D. in neuroscience from the College of Veterinary Medicine at the same university in 1994. He did his postdoctoral work at the Institute of Clinical Research of Montreal.

Judith G. Hall, M.D., M.Sc., is a clinical geneticist and pediatrician. She is currently a professor emerita of pediatrics and medical genetics at the University of British Columbia. Her research interests are human congenital anomalies, including neural tube defects, the genetics of short stature, mechanisms of disease such as mosaicism and imprinting, the natural history of genetic disorders, the genetics of connective tissue disorders such

as arthrogryposis, and dwarfism and monozygotic twins. She has contributed in many leadership roles, including the presidency of the American Society of Human Genetics and the American Pediatrics Society. Dr. Hall has served on numerous national and international committees and boards and has received many honors for her scientific contributions and lifetime achievements. Among her publications are summary reviews and articles that are considered classics, having introduced aspects of the new genetics. Dr. Hall advocated for folic acid supplementation, pediatric physician resources, the development of specific disease health guidelines, and research on rare genetic disorders and natural history. She trained at Wellesley College, the University of Washington School of Medicine, and Johns Hopkins Hospital.

Sandra G. Hassink, M.D., F.A.A.P., is the president of the American Academy of Pediatrics (AAP) and chair of the AAP Institute for Healthy Childhood Weight advisory board and steering committee. In addition, she chairs the ethics committee at Alfred I. duPont Hospital for Children and cochairs the Delaware State ethics committee. She is a member of the institutional review board and has a master's degree in pastoral care and counseling. Dr. Hassink is an author of the obesity prevention segment of the Expert Committee recommendations, senior editor of *A Parent's Guide to Childhood Obesity*, author of *Pediatric Obesity: Prevention, Intervention, and Treatment Strategies for Primary Care*, and author of *Clinical Guide to Pediatric Weight Management*. She worked on the GLIDES project funded by the Agency for Healthcare Research and Quality to embed the Expert Committee recommendation on obesity into the emergency health record at Nemours and is currently the principal investigator on an obesity cluster grant developing population health management systems for children with obesity. She has collaborated in basic research efforts to identify pathophysiologic mechanisms of obesity, centering on the role of leptin, and has lectured widely in the field of pediatric obesity.

Marie-France Hivert, M.D., M.M.Sc., is an assistant professor in the Department of Population Medicine at Harvard Pilgrim Health Care Institute at Harvard Medical School. Dr. Hivert is a clinical investigator with a primary focus on the etiology and primordial prevention of obesity and related comorbidities, particularly type 2 diabetes and gestational diabetes. Her interests also include fetal metabolic programming mechanisms and the integration of genetics, epigenetics, and environmental factors contributing to obesity and related disorders. She is currently involved in many international consortia investigating the genetic determinants of glycemic regulation during and outside of pregnancy. Dr. Hivert completed her clinical training as an endocrinologist in 2007

at the Université de Sherbrooke (Quebec, Canada). She was awarded a scholar research award from the Fonds de Recherche du Québec—Santé, a clinical scientist award from the Canadian Diabetes Association, and the New Investigator Award from the Canadian Institutes of Health Research (CIHR). From CIHR, she also received the Maud Menten New Principal Investigator Award from the Institute of Genetics in 2011. She has initiated her research in primary prevention by conducting a trial of lifestyle intervention to prevent weight gain in young adults, and her work led to upgrading the medical school curriculum at Université de Sherbrooke to allow better training in lifestyle counseling of future physicians. Related to this expertise, Dr. Hivert is involved in the Physical Activity Committee at the American Heart Association. She completed her postdoctoral fellowship at Massachusetts General Hospital and her master's degree in medical sciences in the Scholars in Clinical Sciences Program at Harvard Medical School.

Meredith A. J. Hullar, Ph.D., is a senior staff scientist at the Fred Hutchinson Cancer Research Center. Her research interests include the role of the microbiome and diet in human health. Her research focuses on how the gut microbiome metabolizes dietary constituents and alters exposures that may influence health outcomes related to cancer. She uses a combination of dietary interventions and cross-sectional human population designs to study changes in the microbial community composition and functional genes associated with health outcomes. More specifically, she is interested in the role of the gut microbiome in obesity, how the metabolism of diet by microbiota may influence host epigenetics, and intermediary mechanisms of inflammation modulated by the gut microbiome. Dr. Hullar received her Ph.D. from Harvard University in 2000.

David Klurfeld, Ph.D., is the national program leader for human nutrition in the Agricultural Research Service of the U.S. Department of Agriculture (USDA). He has responsibility for the scientific direction of the intramural human nutrition research conducted by USDA laboratories. Prior to government service he was a professor in and the chair of the Department of Nutrition and Food Sciences at Wayne State University in Detroit, Michigan, and before that was on the faculty of The Wistar Institute and the University of Pennsylvania School of Medicine. Dr. Klurfeld has published more than 185 peer-reviewed articles and book chapters. He was editor-in-chief of the *Journal of the American College of Nutrition* for 6 years and is currently associate editor of the *American Journal of Clinical Nutrition*. He is a member of the National Institute for Diabetes, Digestive, and Kidney Diseases Council.

Stephen Krawetz, Ph.D., is the Charlotte B. Failing Professor of Fetal Therapy and Diagnosis, the associate director of the C. S. Mott Center for Human Growth and Development, and the director of the Center of Excellence: Paternal Impact of Toxicological Exposure at Wayne State University School of Medicine, Department of Obstetrics and Gynecology and Center for Molecular Medicine and Genetics. Dr. Krawetz is well recognized in the fields of reproductive genetics and bioinformatics. Using human spermatogenesis as a model system, his primary research focus is directed toward understanding the long-range genetic mechanisms that dictate cell fate. His laboratory continues to implement and develop state-of-the-art technologies to determine how RNA feeds back to the genome to modulate the system. The spermatozoal RNAs delivered at fertilization may provide an essential component in early paternal genome reprogramming, acting as genetic and epigenetic effectors. Dr. Krawetz received his Ph.D. in biochemistry from the University of Toronto in 1983 and trained with Gordon Dixon at the University of Calgary as an Alberta Heritage Foundation for Medical Research postdoctoral fellow.

Karen A. Lillycrop, Ph.D., is a professor of epigenetics in the Centre for Biological Sciences at the University of Southampton, United Kingdom. Dr. Lillycrop's research focuses on the effect of early life environment on the epigenome and long consequences for disease susceptibility. In collaboration with Dr. Graham Burdge (Faculty of Medicine), she showed for the first time that maternal nutritional constraint induces long-term epigenetic changes in the regulation of key metabolic genes leading to persistent changes in phenotype. She is a founding member of the Epigen consortium, an international consortium investigating the role of epigenetic processes in the developmental origins of disease.

William Nierman, Ph.D., is a professor in and the director of the Infectious Diseases Program at the J. Craig Venter Institute. He has extensive experience in infectious disease genomics, with particular expertise in genomic studies of bacterial and fungal pathogens. Dr. Nierman led the projects that resulted in the first genome sequences for the fungal pathogen *Aspergillus fumigatus* and the select agent bacterial pathogen *Burkholderia mallei*. Infections by these pathogens are or can be initiated in the lungs after inhaling airborne particles containing the bacteria or fungal spores. He is studying the significance of the lung microbiome, biofilms, and antimicrobial resistance in the context of these diseases. He received his B.S. in chemistry from the U.S. Naval Academy and his Ph.D. in biochemistry from the University of California, Berkeley.

Caroline Relton, P.G.C.E., Ph.D., is a professor of genetics and epigenetic epidemiology at Newcastle University and the MRC Integrative Epidemiology Unit at the University of Bristol. Her primary research interest is the application of epidemiological approaches to improve our understanding of the role that epigenetic patterns may play in health and development. Ongoing work in Dr. Relton's laboratory includes projects focusing on the role of epigenetic variation in obesity, type 2 diabetes, and related comorbidities; the role of epigenetic variation in women's health through the menopause; determinants of DNA methylation variation in infants and children; the identification of epigenetic biomarkers of cognitive function; the role of DNA methylation in the pathogenesis of lung cancer; and variation in epigenetic signatures during fetal development. Underpinning these projects is the methodological development of epidemiological tools to strengthen causal inference in the context of epigenetic studies.

Sarah S. Richardson, M.A., Ph.D., is the John L. Loeb Associate Professor of the Social Sciences at Harvard University. She is jointly appointed in the Department of the History of Science and the Committee on Degrees in Studies of Women, Gender, and Sexuality. A historian and philosopher of science, her research focuses on race and gender in the biosciences and on the social dimensions of scientific knowledge. Dr. Richardson's research presses for scholarly reflection on the many developments under way in the present post-genomic moment. Her essay, "Maternal Bodies in the Post-genomic Order," discussed the implications of a prominent post-genomic research stream that situates the maternal body as a central site of epigenetic programming and transmission and as a significant locus of medical and public health intervention.

Aryeh D. Stein, M.P.H., Ph.D., is a professor in the Hubert Department of Global Health of the Rollins School of Public Health, Emory University, with a joint appointment in the Department of Epidemiology. He is a member of the faculty of the Nutrition and Health Sciences program of the Division of Biological and Biomedical Sciences in the Laney Graduate School of Arts and Sciences. In his research Dr. Stein uses critical periods of susceptibility to nutritional deficits and surfeits (such as war-induced famine or migration) to study the role of nutrition over the life course (prenatal, childhood, adulthood) on the development of adult chronic disease. He has secondary interests in the methods of dietary assessment and program evaluation. He is currently working with Cooperative for Assistance and Relief Everywhere (CARE) and International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B in the design and implementation of a novel approach to program evaluation in Bangladesh; with the Consortium for Health Oriented Research in Transitional Societies (COHORTS) inves-

tigative team on the analysis of data from birth cohort studies in Brazil, Guatemala, India, the Philippines, and South Africa; with investigators from South Africa on the extension of the Birth to Twenty study to the next generation; and with the Young Lives investigators to study the consequences through adolescence of variation in growth in childhood.

Mark H. Vickers, M.Sc., Ph.D., is an associate professor and senior research fellow in the Liggins Institute at the University of Auckland. Dr. Vickers's research focus is on the effect of alterations in early life nutrition on the later health and well-being of offspring with a particular focus on the development of obesity and the metabolic syndrome. Dr. Vickers has established a number of preclinical models using the paradigm of altered early-life nutrition to examine the mechanistic basis of programming during critical periods of developmental plasticity. He also investigates the potential for the reversibility of developmental programming via both nutritional and pharmacologic interventions and was one of the first to show that developmental programming was potentially reversible with interventions in the early life period via the adipokine leptin. Dr. Vickers's original work on developmental programming was named the most cited paper of the decade 2001–2011 in the *American Journal of Physiology: Endocrinology and Metabolism*. He has published more than 90 peer-reviewed papers and 6 book chapters in the field of early life origins of adult disease and is on the editorial board of a number of journals in this area.

Robert Waterland, Ph.D., is an associate professor of pediatrics and molecular and human genetics at Baylor College of Medicine. His research is aimed at understanding how nutrition during prenatal and early postnatal development affects individual susceptibility to various adult-onset chronic diseases. Dr. Waterland's group focuses on nutritional influences on developmental epigenetics as a likely mediating mechanism. The Waterland group is increasingly interested in whether a mother's obesity and nutrition before and during pregnancy affect developmental epigenetics in the hypothalamus and, consequently, body weight regulation in her offspring.