The Interplay Between Environmental Chemical Exposures and Obesity: Proceedings of a Workshop

DETAILS

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THE INTERPLAY BETWEEN ENVIRONMENTAL CHEMICAL EXPOSURES AND OBESITY

Proceedings of a Workshop

Robert Pool, *Rapporteur*

Roundtable on Environmental Health Sciences, Research, and Medicine

Board on Population Health and Public Health Practice

Health and Medicine Division

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This Proceedings of a Workshop has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published Proceedings of a Workshop as sound as possible and to ensure that the Proceedings of a Workshop 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 Proceedings of a Workshop:

Marie Capdevielle, Colgate-Palmolive Company
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Linda D. Meyers, Independent Nutrition Consultant
Winston F. Wong, Kaiser Permanente

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the Proceedings of a Workshop before its release. The review of this Proceedings of a Workshop was overseen by **Elena Nightingale.** She was responsible for making certain that an independent examination of this Proceedings of a Workshop was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this Proceedings of a Workshop rests entirely with the rapporteur and the institution.

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ACRONYMS AND ABBREVIATIONS

ACOG	American Congress of Obstetricians and Gynecologists
Ad36	adenovirus type 36
ADI	accepted daily intake
AhR	aryl hydrocarbon receptor
ATP	adenosine triphosphate
BCERP	Breast Cancer and Environmental Research Program
BDE 47	brominated diphenyl ether 47
BMI	body mass index
BPA	bisphenol A
cAMP	cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CREBP	cAMP-responsive element-binding protein
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DEHP	diethylhexyl phthalate
DES	diethylstilbestrol
EDC	endocrine-disrupting chemical
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GLP1	glucagon-like peptide 1
HMD	Health and Medicine Division
IOM	Institute of Medicine
NCCG	National Center for Chemical Genomics
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program (of the NIEHS)
OB-GYN	obstetrician-gynecologist

PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PFOA	perfluorooctanoic acid
POP	persistent organic pollutant
PPARγ	peroxisome proliferator-activated receptor γ
PVC	polyvinyl chloride
ROS	reactive oxygen species
RXR	retinoid X receptor
TCA	tricarboxylic acid (cycle)
TCDD	tetrachlorodibenzodioxin
Tox21	Toxicology in the 21st Century (project)
TSCA	Toxic Substances Control Act
USGS	U.S. Geological Survey

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Introduction[1]

On March 2 and 3, 2015, the National Academies of Sciences, Engineering, and Medicine's Roundtable on Environmental Health Sciences, Research, and Medicine held a workshop to explore the role that chemical exposures may play in the development of obesity. The obesity epidemic that has gripped the United States and much of the developed world for the past several decades has proved remarkably resistant to the various approaches tried by clinicians and public health officials to fight it. This raises the possibility that, in addition to the continued exploration of consumer understanding and behavior, new approaches that go beyond the standard focus on energy intake and expenditure may also be needed to combat the multifactorial problem of obesity. The workshop statement of task is provided in Box 1-1.

The speakers at the workshop discussed evidence from both studies with animal models and human epidemiological studies that exposure to environmental chemicals is linked both to weight gain and to glucose tolerance, insulin sensitivity, inflammation, and other aspects of the metabolic syndrome. In addition to conventional environmental chemical exposures, the planning committee for this workshop included one panel to discuss the potential role of other exposures, including sugar, artificial sweeteners, and antibiotics, in aiding or causing obesity. The speakers also examined possible biological pathways and mechanisms underlying the potential linkages.

[1] The planning committee's role was limited to planning the workshop, and this Proceedings of a Workshop 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 National Academies of Sciences, Engineering, and Medicine, and they should not be construed as reflecting any group consensus.

BOX 1-1
Statement of Task

An ad hoc committee will plan and conduct a public workshop featuring presentations and discussions to outline current scientific understanding of the effect of environmental chemical exposures on the development of obesity and of potential preventive interventions. The committee will identify specific topics to be addressed, develop the agenda, select and invite speakers and other participants, and moderate the discussions. An individually authored summary of the presentations and discussions at the workshop will be prepared by a designated rapporteur in accordance with institutional guidelines.

After hearing about the present state of the science of environmental exposures and obesity, the speakers discussed future research needs and offered suggestions for policies that could reduce the health and human costs of the current epidemic of obesity. The workshop did not focus broadly on public health interventions to treat or prevent obesity. The workshop audience, which took part in the discussions, included both attendees at the workshop, which was held at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, and also people who watched via a webcast and who were able to submit questions to the speakers through the workshop's website.

This workshop, The Interplay Between Environmental Exposures and Obesity, was one in a series of workshops focused on current and emerging environmental issues and their impacts on human health. These workshops are sponsored by the Roundtable on Environmental Health Sciences, Research, and Medicine. The Roundtable was established in 1998 to provide a mechanism for parties from academic programs, consumer interest groups, government agencies, and industry to convene and discuss sensitive and difficult environmental public health issues. The purpose is to share perspectives and foster rigorous dialogue but not to provide recommendations.

The following is a summary and synthesis of the presentations and discussions that took place during the 2 days of the workshop. When reading this Proceedings of a Workshop, it is important to keep in mind that the opinions expressed and any recommendations made are those of the individual speakers themselves and do not represent the position of the Academies. Indeed, the purpose of the Roundtable is to provide a mechanism for interested parties in environmental health to meet and discuss sensitive and difficult environmental issues in a neutral setting. The

Roundtable fosters dialogue about these issues, but it does not provide recommendations or even try to find a consensus on these issues.

ORGANIZATION OF THIS PROCEEDINGS OF A WORKSHOP

The organization of this Proceedings of a Workshop follows the structure of the workshop's proceedings. Chapter 2 describes the presentations and discussions during Session 1 of the workshop that focused on framing the issue of obesity from both the public health and the environmental health perspectives. Chapter 3 summarizes the presentations and discussions during Session 2 of the workshop, which explored the role of chemical exposures and obesity over the life span. Chapter 4 covers presentations on the biological pathways and environmental influences as well as the subsequent discussions that occurred in the workshop's third session. Chapter 5 summarizes the presentations and discussions from Session 4 of the workshop on emerging evidence on other exposures that may play a role in the development of obesity. Chapter 6 includes the presentations and discussions from the workshop's fifth session discussing future research needs. Finally, Chapter 7 recaps the discussions from the workshop's final session, Session 6, which covered potential policy solutions to obesity discussed at the workshop. The workshop agenda is found in Appendix A, and biographical sketches of the workshop speakers are included in Appendix B.

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Framing the Problem

The workshop's first session was dedicated to framing the problem of environmental exposures and obesity. To do that, two speakers described the issue from two different perspectives: the public health perspective and the environmental health perspective.

PUBLIC HEALTH OVERVIEW

The first speaker was William Dietz, the director of the Sumner Redstone Global Center for Prevention and Wellness at the Milken Institute of Public Health at George Washington University. He presented his observations via the telephone.

Background on Obesity

Dietz began by describing the standard criteria for obesity, which is based on body mass index (BMI). BMI is defined as weight in kilograms divided by height in meters squared. For example, a 6-foot man (or woman) who weighs 184 pounds has a BMI of 25, as does a woman (or man) who is 5 feet 4 inches and 145 pounds. A BMI of 25 is at the lower end of being overweight, which is defined as having a BMI from 25 to less than 30. A person with a BMI of 30 or above is said to have obesity. The classifications are not perfect, and a number of men are classified as overweight when in reality they simply have more muscle mass than normal. Thus, there is a lot of misclassification, particularly for men with BMIs between 25 and 30. However, "For both men and women," Dietz said, "a BMI greater than 30 is invariably associated with increased body fat unless you play linebacker for the New England Patriots."

The criteria for children are different because they are growing. A child who is above the 85th percentile in BMI for his or her age is said to be overweight, while those whose BMI is above the 95th percentile are said to have obesity. However, Dietz emphasized, these standards are based

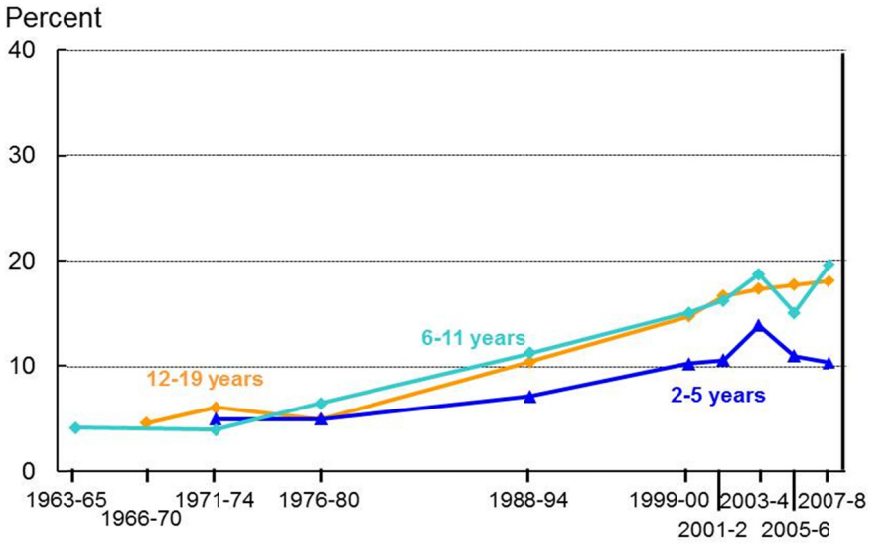

FIGURE 2-1 Obesity trends in 2- to 19-year-old U.S. children.
NOTE: Obesity is defined as a BMI greater than or equal to the gender- and weight-specific 95th percentile BMI from the 2000 Centers for Disease Control and Prevention (CDC) growth charts (CDC, 2000).
SOURCES: Dietz presentation to workshop, March 2, 2015, from CDC, 2014.

on national surveys and historical data that were collected before the rapid increase in obesity. Thus, it is possible for 17 percent of children to have obesity, because 17 percent of a national sample of children from several decades ago have a BMI at or above the 95th percentile.

The percentage of children in the United States who have obesity has been increasing steadily since the late 1970s (see Figure 2-1). Currently, nearly 20 percent of children between 6 and 19 years old have obesity.

There have been similar increases in obesity among children in countries around the world. The worst problems have been in the developed world, but even in developing countries the prevalence of obesity has been increasing, although the prevalence still remains much lower than that in the developed world.

Obesity has also increased in the adult population. For instance, the prevalence of obesity among U.S. men doubled between 1976–1980 and 2003–2004. The increase was approximately uniform across Caucasians, African Americans, and Mexican Americans. There was a similar approximate doubling of the obesity rates among U.S. women during that same time, although the overall prevalence of obesity was significantly higher among women than among men (Flegal et al., 2010).

One of the prevalent misconceptions about obesity concerns its relationship with poverty, Dietz said. Among men, the prevalence of obesity differs very little among the various socioeconomic classes or among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans. Interestingly, the only statistically significant relationship between socioeconomic class and obesity appears in African-American men and Mexican-American men, where upper-income men are significantly more likely to have obesity than those in other socioeconomic classes (Ogden et al., 2010).

The story among U.S. women is different. According to data from the 2005 to 2008 National Health and Nutrition Examination Survey, women in lower socioeconomic brackets are significantly more likely to have obesity than those in the middle or upper socioeconomic brackets. However, when broken into racial/ethnic categories, the relationship is significant only among non-Hispanic whites. There are no significant socioeconomic gradients for obesity among African-American women or Mexican-American women (Ogden et al., 2010).

Factors Leading to Obesity

The rapid increases in obesity seen over the past decade cannot be explained by genetics, Dietz said. More than 100 genes have been shown to be related to obesity, but those genes have always been present in the population in approximately the same proportions that exist today. Dietz explained that genes may affect susceptibility to obesity through their impact on the individual's energy balance either by calorie intake or by calorie expenditure.

It does not take much of a shift in energy balance to produce obesity, Dietz said. For example, the shift in mean body weight of 2- to 5-year-olds since the 1970s can be accounted for by an excess of approximately 30 calories per day. However, the change in the energy balance necessary to reduce obesity is much greater than that necessary to produce it. Physical activity can play a significant role in changing body composition, but it is a poor way to lose weight because it is hard to achieve the major caloric deficits necessary for weight loss through physical activity.

Although on one level obesity is simply the product of an energy imbalance, it is actually extremely complex. Dietz illustrated this point with a slide showing various pathways inside and outside the body related to obesity. At the center of the illustration were the critical interactions between energy intake and energy expenditure, the

imbalance of which accounts for obesity. But many factors influence energy intake and expenditure, and these are loosely grouped into seven categories: food production, food consumption, societal influences, individual psychology, individual activity, the activity environment, and biology. Dietz said that he expected that much of the focus of the workshop would be on biological factors, which are various mechanisms that affect the pathways that regulate energy intake and expenditure. The nonbiological factors, in contrast, affect the susceptibility of individuals to an energy imbalance. They do not cause obesity in the traditional sense of biological agents, but they make it more or less likely.

Social and Behavioral Influences

For the rest of his presentation, Dietz focused on how the macro-environment and behavior influence susceptibility to obesity.

In the 1950s, he said, the typical diet consisted of milk and other dairy products, meat and eggs, potatoes, fruits, and vegetables—in short, mostly unprocessed foods that were prepared at home. Today, a much larger portion of the average American diet is highly processed: pizza, sodas, canned foods, and so on. The shifts in food practices from then until now have been enormous. There are a variety of reasons for this shift, Dietz said, including the increased availability and lower cost of highly processed foods as well as increased portion sizes.

"All of these factors promote increased food intake," Dietz said. "The more variety an individual is exposed to, the more likely [he or she is] to overconsume foods. The greater the portion size, the more likely we are to overconsume," Dietz said. Because of the reduced consumption of unprocessed foods like fruits and vegetables, higher-calorie foods account for a greater part of the diet.

There have been comparable changes in physical activity, Dietz said. They may be less quantifiable, but they are nonetheless important. Physical education and recess have been eliminated or reduced in schools. The time spent in front of television and computer screens has increased, particularly in children. The use of appliances has displaced what used to be household activities, like washing the dishes and hanging up the clothes to dry. The movement of large numbers of people to suburbs means that children are less likely to walk to school because many suburbs lack sidewalks, and even if they have sidewalks, they do not connect people with places where people want to go. As a result, people are increasingly reliant on cars. Research has shown that the more

time that someone spends in a car, the more likely he or she is to have obesity. Finally, because society is now in a postindustrial era, there has been a shift from manufacturing to services, which has led to a decrease in the amount of energy that people spend on performing physical activities.

Maternal behavioral factors associated with obesity in a child include a higher prepregnancy weight, excessive weight gain during pregnancy, gestational diabetes, and tobacco use during pregnancy.

Finally, early exposure to various adverse experiences—including physical and verbal abuse, family incarcerations, divorce, poverty, drug use, and alcohol use—is associated with an increased prevalence of severe obesity in adulthood. The connection between these early experiences and obesity in adulthood is in part due to the effects of the experiences on brain development.

Recent Progress

There have recently been some encouraging data about obesity, Dietz said. For example, over the past decade or so there has been a plateau in the prevalence of obesity in both boys and girls 2 to 19 years old. Similarly, over the past decade there have been no significant increases in obesity among adults, either men or women. What is particularly encouraging, he said, is that recent data indicate that the obesity rate among 2- to 5-year-old U.S. children has actually started to drop, after nearly three decades of increases.

Local data have shown a similar trend. Although the quality of the local data is much more variable, six states and 16 communities have reported that the rates of childhood obesity have dropped. "There is still work that needs to be done to validate the samples to assure that the samples themselves are comparable," Dietz said, "but I think we can say in some of the states and communities which have been examined pretty intensively … there are significant decreases in the prevalence of childhood obesity."

Why have the rates started to decrease? Researchers are beginning to look into that question, Dietz said. One factor seems to be that nationally there have been substantial changes in food consumption. Between 1999–2000 and 2009–2010, the average consumption of sugar drinks in the United States dropped by 68 calories per day among 2- to 19-year-olds and by 45 calories per day among adults (Kit et al., 2013). Between 2003–2004 and 2007–2008, fast food consumption in the United States

dropped by 64 calories per day among 2- to 11-year-olds, by 14 calories per day among 12- to 19-year-olds, and by 33 calories per day among adults (Powell et al., 2012). An agreement between the companies that supply 25 percent of the calories in the United States and the Healthy Weight Commitment Foundation pledged in 2010 to reduce the number of calories in the U.S. food supply by 1.5 trillion calories; the actual reduction achieved in 2014 was 6.4 trillion calories, or 78 calories per person per day.

These changes in consumption could clearly account for flattening of the obesity prevalence curve, Dietz said. However, he said, "that is no cause for complacency because we still have a prevalence of about 20 percent obesity in 6- to 11-year-olds and about the same, maybe a little more, in 12- to 19-year-olds, and about 34 percent of the adult population is obese." Thus, there is still work to do.

ENVIRONMENTAL HEALTH OVERVIEW

The next speaker was Jerry Heindel, a health science administrator in the Division of Extramural Research and Training at the National Institute of Environmental Health Sciences (NIEHS). He provided an environmental health perspective on the current obesity epidemic.

A Brief History of the Field

Heindel began by offering a brief history of the field of environmental exposures and obesity. The field got its start in 2002, he said, with a review article by Paula Baillie-Hamilton, "Chemical toxins: A hypothesis to explain the global obesity epidemic" (Baillie-Hamilton, 2002). In that article, Baillie-Hamilton offered a compelling chart showing how closely the rise in the rates of obesity correlated with the increase in chemical production, with a certain lag time (see Figure 2-2). "Of course, it was just a correlation," Heindel said, "but it pulled together a lot of data and got people thinking."

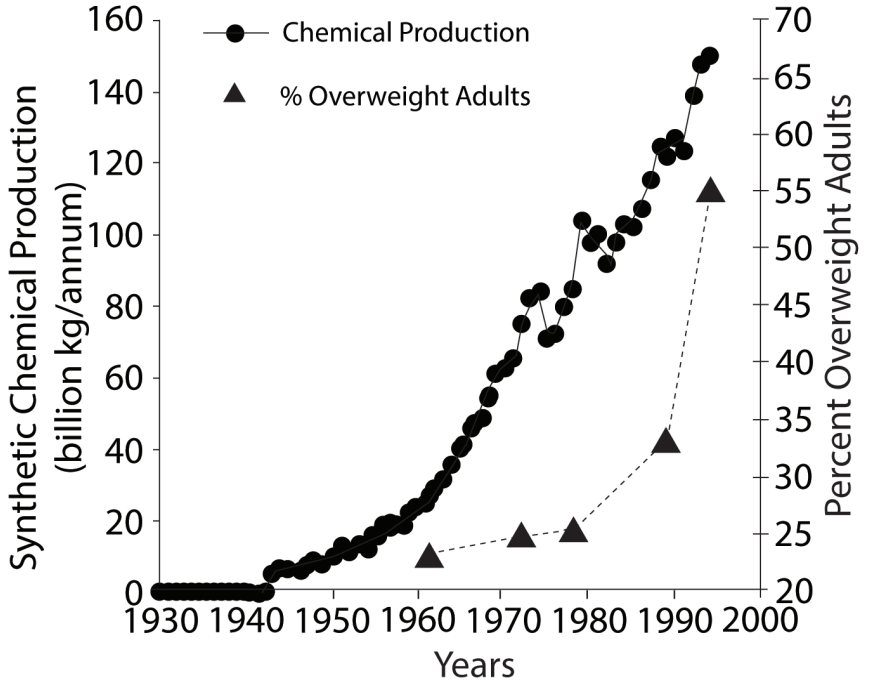

FIGURE 2-2 Correlation between rise in chemical production and increase in obesity rates.
SOURCES: Heindel presentation to workshop, March 2, 2015, from Baillie-Hamilton, 2002. The publisher for this copyrighted material is Mary Ann Liebert, Inc., Publishers.

Even more compelling than the chart, however, was the literature search that Baillie-Hamilton had carried out. In that search she identified a number of toxicological studies going back to the 1970s and 1980s that had shown that various chemicals increased weight in experimental subjects. The types of chemicals on her list included pesticides, such as organophosphates and carbamates; polychlorinated biphenyls (PCBs); polybrominated biphenyls and fire retardants; heavy metals; solvents; and plastics, such as phthalates and bisphenol A (BPA). At the time that the studies had been done, Heindel said, no one was paying attention to increased weight in the subjects because the focus was on the decreases in weight and the general toxicity caused by high doses of the substances.

Then, over the next few years there were several commentaries on the subject published, and NIEHS funded an initiative to understand the fetal basis of disease, which included the fetal origins of obesity in its

purview. In 2004, Heindel and Ed Levin of Duke University held the first symposium on the fetal origins of and environmental influences on obesity. The biggest change occurred in 2006, when Bruce Blumberg of the University of California, Irvine, wrote a review article and coined the term "obesogen,"[1] Heindel said. "I think that really stimulated the field because it caught on in the press."

In just the past few years, NIEHS has funded another initiative on the role of environmental chemicals in the development of obesity, type 2 diabetes, and the metabolic syndrome with the goal of stimulating new research in the field. That initiative is ongoing.

Background Information on Obesity: Setting the Stage

Next, Heindel offered some background on obesity and its causes. A number of factors are involved in the development of obesity, he said, including one's genetic background, congenital illness, drug use, viruses, antibiotics, and various environmental factors, including a lack of exercise, stress, a lack of sleep, and nutrition. The focus of his talk, he said, was on one particular environmental factor that leads to obesity: exposure to environmental chemicals.

Body weight is controlled by the endocrine system, Heindel explained. The endocrine system is highly complex and interrelated. There are hormones that dictate appetite and satiety as well as the development of adipose tissue. Because it is a finely tuned system, endocrine-disrupting chemicals can throw off its operation and lead to weight gain.

An endocrine disrupter, Heindel explained, is defined as an exogenous chemical or mixture of chemicals that interferes with any aspect of hormone action. More than 800 chemicals are now known to have some endocrine-disrupting activity. These chemicals fall into more than a dozen different classes, according to their intended uses, including pesticides, herbicides, flame retardants, plastics, plasticizers, surfactants, solvents, heavy metals, personal care products, sunscreens, and cosmetics. The point, Heindel said, is that these chemicals were designed for a specific purpose, but they also have the side effect that they can interfere with some aspect of the endocrine system.

[1] "Obesogen" refers to chemical compounds that may have an impact on metabolic processes or may increase individuals' susceptibility to obesity, or both. The term is used in this Proceedings of a Workshop in the manner in which the researchers used it at the workshop.

Do these chemicals in the environment make it into the human body, and is there enough exposure to these chemicals that some effects could be expected? The data say yes, Heindel said. He mentioned in particular a study from the Centers for Disease Control and Prevention (CDC) that found measurable amounts of nearly 300 different chemicals in cord blood from babies. In addition, a small study of 50 pregnant women found 47 chemicals in every one of the women tested. Further, some chemicals were found in every one of thousands of people tested by CDC. "Certainly," he said, "there is significant exposure to these endocrine-disrupting chemicals."

Heindel cautioned that the presence of these chemicals in the womb does not mean that they are causing any harm. However, he added, "it does mean we have accepted a strategy whereby every pregnant woman is contaminated with a variety of chemicals without her knowledge with the potential for harm to either her or the baby."

The Obesogen Hypothesis

Data collected over the past 10 or 12 years clearly show that developmental exposures to environmental chemicals can lead to a variety of diseases and dysfunctions later in life, Heindel said. In particular, the period of development that takes place in utero and early in childhood is the period when the human body is most sensitive to exposure to environmental chemicals, and such exposures can disrupt development in ways that cause problems long after the chemicals are gone.

A variety of diseases are caused by such developmental exposures, Heindel said, and he believes that obesity is clearly one of them. He pointed out that although there are chemicals that will cause weight gain in adults, it is believed that the developmental stage is much more sensitive to metabolic disruptions and the development of obesity, and as a result, the field has been focused on developmental exposures to chemicals linked to an increased likelihood of obesity later in life.

This is the obesogen hypothesis: that the obesity epidemic is due, in part, to environmental exposures during development. In particular, the hypothesis is that a subset of endocrine-disrupting chemicals, which are called obesogens, act during development and disrupt adipose tissue development in such a way that the disruption alters the number of fat cells. The chemicals can also alter subsequent food intake and metabolism by having effects on the pancreas, adipose tissue, liver,

gastrointestinal tract, brain, or muscle. The ultimate result is that these environmental endocrine-disrupting chemicals alter the programming of the body's set point or its sensitivity to the development of obesity later in life.

This is a very important point, Heindel said. The chemicals are not causing obesity per se, but rather they play a role increasing the body's sensitivity to the development of obesity. "It is very important that you all realize that we who are working in this field understand that food intake and exercise are very important and that they are certainly key to the obesity epidemic," Heindel said. "But we believe that environmental chemicals are altering the set point or sensitivity for gaining weight—that is, how much food does it take to put on weight and how much exercise does it take to reduce weight. Those effects are occurring via alterations in this developmental programming of this endocrine system that controls weight gain."

Examples of Obesogens

There are a number of examples of such obesogens, Heindel said, with clear data showing a connection between environmental exposures and obesity. For example, more than 20 different epidemiological studies have shown that cigarette smoking by a mother during pregnancy results in her child having an increased likelihood of being obese. The obesity generally shows up at about the time that the child starts school, he said.

There is some interesting evidence related to prenatal exposure to diethylstilbestrol (DES). This is a drug that was given to millions of women to prevent miscarriage. It did not actually help with that, but it did cause a number of different diseases and dysfunctions, including some very rare cancers, in the children of the mothers who took it.

Although not demonstrated in humans, animal models have demonstrated that one of the possible effects of prenatal exposure to DES is obesity. In one experiment, newborn mice were given DES for 5 days beginning at birth. Once the exposed mice hit puberty, they began gaining weight significantly faster than control animals that did not get the drug. Then, by the time the exposed mice were 9 months old, they were morbidly obese. Interestingly, they got fat without eating any more or exercising any less than the control mice.

Other animal studies have contributed to a growing body of evidence indicating that environmental exposures can increase susceptibility to obesity. BPA, a chemical used to make various plastics such as the ones

used in water bottles, has shown a slightly different effect in mice. In one series of experiments, BPA exposure did not lead to weight gain but, rather, led to an increase in the percentage of body fat and a decrease in the percentage of lean body mass.

Another study of BPA looked for the mechanisms behind the chemical's effect. In this case, the researchers used a different animal model, and they did see increased weight in the animals exposed to BPA as well as increased food intake. When the researchers examined the brains of the animals, they found an increased number of appetite neurons and a decreased number of satiety neurons, indicating that changes to the numbers of appetite and satiety neurons may have been how BPA exerts its influence.

Yet another study looked at exposure to diethylhexyl phthalate (DEHP), a plasticizer found in various plastics, including plastic toys. Developmental exposure to the chemical increased visceral fat tissue and also the number of fat cells in an animal model. The increase in weight in the exposed animals was relatively minor, but they had huge amounts of fat filling up their abdomens.

That experiment illustrated an important point about environmental exposures. The development of increased fat actually occurred at the lowest dose tested. At the highest dose—500 milligrams per kilogram, which is the usual dose that toxicologists use to look for effects of these chemicals on different systems—there was no increase in fat. The lesson, Heindel said, is that the experimenter must pay attention to the effects of very low doses because in many cases the dose–response curves are not linear.

In addition to animal models, Heindel said that about 33 human epidemiology studies have now linked developmental exposure to environmental chemicals to weight gains in children later in life. The chemicals that have exhibited such effects include PCBs, BPA, hexachlorobenzene, polycyclic aromatic hydrocarbons, and the chemicals produced by maternal smoking.

Recently, some troubling data in lab animals indicate that it is possible to have transgenerational inheritance of obesity—that is, that the chemically produced obesity can be passed along to subsequent generations. In these experiments, a pregnant female is exposed to an environmental chemical, and the offspring are examined for effects. Then, the male offspring are mated with females that have had no such exposure to get a third generation, and the process is repeated. Obesity from the second generation shows up again in the third and fourth

generations in animals that were never exposed to the chemical at all, Heindel explained. Some studies have shown such an effect with tributyltin, the pesticide dichlorodiphenyltrichloroethane (DDT), jet fuel, and a mixture of BPA and two phthalates.

This is very troubling, Heindel said, because it indicates that if a pregnant mother is exposed to a chemical, it may affect not only her children but also her grandchildren and her great grandchildren as well.

At this point there is a large list of chemicals for which data for either humans or animals suggest a metabolic disruption or an obesogenic effect, and it seems to be just the tip of the iceberg, Heindel said, because it seems that a new chemical is being added to the list every month or two.

Data Gaps and Needs

NIEHS is now funding 57 grants in the area of obesity and diabetes, Heindel said. Of those, 32 are in humans, 20 are in animals, and 5 are basic cellular and molecular studies. In the 32 studies with human birth cohorts, developmental exposures to various chemicals are assessed, and the children are followed later in life to see if they become overweight or obese. Thus, in the next 4 or 5 years, he said, there will be a huge increase in the amount of data available on this issue both from the human studies and from the animal studies, plus all of the other studies being funded around the globe.

Still, he added, the field is still young—only about 10 years old—and there are many data gaps and needs and many questions to be answered: screens need to be developed to determine which chemicals have the ability to cause weight gain, for instance; dose–responses need to be determined; the sites at which the chemicals act and their mechanisms need to be discovered; the animal studies need to be coordinated with the human studies so that their insights can be compared and combined; and so on.

Heindel closed by noting that because the field is so new, there are many opportunities to help direct the research. That is why meetings like this workshop are so valuable, he said, so that it is sooner, rather than later, that the field is able to understand the importance of environmental chemicals in the obesity epidemic.

DISCUSSION

Lynn Goldman of the Milken Institute School of Public Health at George Washington University opened the discussion session with a

comment about the relatively modest changes in caloric consumption or energy use that are necessary to either go from a normal weight to obese or move from obese to a normal weight. This would seem to have profound implications for policy, she said, and she asked both speakers to comment.

Heindel answered that the fact that it takes only a small increase in calories to result in weight gain over time helps people to accept the idea that environmental chemicals can play a role. For the most part, he said, the effects of environmental chemicals are not large; they are just increasing the susceptibility or altering the set point. But that has the ability to play a large role if only a small change in calorie intake is necessary to lead to obesity.

Dietz said that it is important to distinguish between levels of obesity. A person with a BMI of 32 is obese and could have gotten there with a daily excess of only 200 calories or so, but for a person with a BMI of, say, 44, the caloric gap would have to have been much greater—perhaps an additional 700 or 800 calories a day. This is also what they will need to cut from their diets to eventually return to a normal weight. It is a mistake to assume that all obesity is the same, for example, that a person with a BMI of 32 is the same as one with a BMI of 44, in terms of either the factors that got them there or the metabolic consequences. "I think we need to be much more sophisticated about understanding the different phenotypes of obesity and what the contributing factors are," he said.

Bernie Goldstein of the University of Pittsburgh asked about the effects of DES on the children of mothers who took it. Is there any evidence that they were more likely to become obese?

Heindel answered that several researchers have been trying for many years to link DES exposure in women to weight gain in the offspring, but they have not been able to pin that down. The problem, he suggested, is that there is little information about the doses that these women received, and there was a huge variation in both the dosing and the timing of the dosing from woman to woman. Some received DES in the first trimester, some in their second, and some in their third, so no one has been able to prove a link. However, he noted that there are many anecdotal, but interesting, data about people who are morbidly obese, have been for their whole life, and did not know why. It turns out that they were the daughters of mothers who had taken DES.

Linda Birnbaum of NIEHS commented that, historically, one of the problems with developmental toxicology studies is that the animals were

not held long enough to see obesity develop. They were sacrificed just before birth, soon after birth, at weaning, or maybe even at puberty, but they were not kept until the time that a big difference in weight gain would become apparent. But, she said, with some of the new paradigms that are being used, researchers are doing long-term studies, starting with developmental in utero exposure and holding the animals until they are 2 years of age. Given that, researchers should start seeing some things that have been difficult to see before.

Birnbaum then asked Heindel whether preconceptional exposures might play a role in obesity. She also asked if exposures at puberty might have an effect, because that seems to be another time when there is increased susceptibility to endocrine-disrupting chemicals.

Heindel said that although the field began with a focus on developmental exposure in utero or neonatally, in the past 2 or 3 years researchers have begun to realize that there are probably other sensitive windows, such as paternal exposure and maternal exposure before pregnancy or prepuberty. "Any time there is a huge change in hormone levels," he said, "there are going to be major changes in epigenetic regulation. If environmental chemicals can perturb that process, then the end result will be some problem later on. As we move forward, we are certainly going to look at other windows of exposure."

Barbara Corkey of Boston University commented that the epidemiological studies that had been discussed during the session could form the basis for some interesting hypotheses, but they did not actually prove causation. Heindel responded that she was correct, that the field is very descriptive at this point. The hope is that researchers will work from the correlations in the epidemiological studies and test those in animal models in an attempt to show causality between the particular chemical exposure and weight gain. Once that happens, there will a much better understanding of exactly what is going on.

Birnbaum added that one of the most important directions in this area is the move toward looking at multiple chemical exposures. "None of us are exposed to one potential obesogen by itself," she noted. "With some of the Tox21 [the Toxicology Testing in the 21st Century project] approaches that are being used by EPA [the U.S. Environmental Protection Agency] and NIEHS—and FDA [the U.S. Food and Drug Administration] is partnering with us as well—we are not testing 1 or 10 or 100 chemicals, but we are testing thousands and thousands of chemicals through large numbers of assays. And in fact, within the past couple of years, we have added assays that are involved in the integrated

pathways that are associated with obesity. I think that will at least provide us a great deal of screening prioritization, but eventually actual understanding."

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3

Developmental View of the Role of Chemical Exposures and Obesity

In the workshop's second session, three speakers discussed what is known about the relationship between obesity and exposure to various chemicals. In particular, the session was devoted to epidemiological studies that have examined that relationship. Gwen Collman, the director of the extramural program at the National Institute of Environmental Health Sciences (NIEHS), chaired the session.

EFFECT OF PRENATAL EXPOSURE TO ORGANOCHLORINES ON CHILDHOOD OBESITY

The first speaker was Dania Valvi, a postdoctoral research fellow at the Harvard T.H. Chan School of Public Health and the Center for Research in Environmental Epidemiology in Barcelona, Spain. In her research, Valvi focuses on whether early life exposures to environmental chemicals influence children, with a special interest in studying obesity and metabolic diseases.

Valvi's presentation detailed evidence from birth cohort studies evaluating the impact of prenatal and postnatal exposures to persistent organic pollutants (POPs) on the development of childhood obesity. In particular, she presented evidence from two studies: the INMA birth cohort

study from Spain[1] and the Children's Health and the Environment in the Faroes study.[2]

Persistent Organic Pollutants

One group of potentially obesogenic chemical substances that Valvi's research focuses on is POPs. This group of chemicals includes pesticides, such as dichlorodiphenyltrichloroethane (DDT) and its prime metabolite, dichlorodiphenyldichloroethylene (DDE); hexachlorobenzene; industrial chemicals, including polychlorinated biphenyls (PCBs); and their by-products. The use of POPs was gradually restricted until they were finally banned in developed countries beginning in the 1970s. However, widespread exposure to POPs is still of interest because of their characteristics. In particular, they have a slow biodegradation rate of decades, so they are highly persistent in the environment; they can be transported over long distances in the environment through air and water; and they are highly lipophilic, that is, they have an affinity to and tend to dissolve in fats and therefore can accumulate in animal and human fat tissues and remain there for years. Biomonitoring studies are still reporting detectable concentrations of most of these compounds in the blood of a high percentage of the population—more than 90 percent of subjects examined, including pregnant women and children.

The main route by which people are exposed to POPs nowadays is through the food that they eat, particularly foods with high fat contents, such as fatty fish, meat, and dairy products. Furthermore, children are exposed to these chemicals very early in life: before birth through the maternal bloodstream via the placenta and after birth through breast milk.

[1] INMA (Infancia y Medio Ambiente) is a Spanish research network focused on studying environmental pollutants in the air, water, and diets of children and how these pollutants affect children's health, starting during pregnancy and continuing through childhood development until the end of adolescence. For more information, see http://www.proyectoinma.org/presentacion-inma/en_index.html (accessed August 3, 2015).

[2] The Children's Health and the Environment in the Faroes study focuses on the health of children and adults in the Faroe Islands, focusing specifically on the impact of marine contaminants on a population with a seafood-heavy diet. For more information, see http://www.chef-project.dk (accessed March 23, 2016).

POPs are one of the chemical groups that have been hypothesized to cause obesity in humans. Their obesogenic effects have been considered in a growing number of human studies; however, few animal studies aiming to elucidate the effects of these chemicals on obesity are currently being conducted; there is much less evidence from experimental studies for these chemicals than for other emerging chemicals, such as the plasticizers bisphenol A (BPA) and phthalates.

Although the mechanisms by which POPs may cause obesity remain unknown, animal studies support their role in weight gain. As an example, Valvi described a recent study on DDT carried out in mice by La Merrill and colleagues (2014). This study found that developmental exposures to DDT increased fat mass and that the effects were mediated through decreases of energy expenditures in females but not in males. The reductions in energy expenditure also became worse after the animals were placed on a high-fat diet.

Summarizing evidence from animal studies, Valvi said that even though more work is needed to elucidate the underlying mechanisms, the available evidence seems to suggest that susceptibility may depend on sex and other factors, such as diet. Animal studies have further shown that POPs, like other endocrine disrupters, may exhibit different effects at lower and higher doses of exposure.

From the human studies available, most studies are birth cohort studies that evaluated exposures during pregnancy and looked for associations with childhood obesity; almost none of these studies evaluated postnatal exposure. The best studied of the POPs so far are DDE and PCBs. Almost all of the studies have assessed obesity using body mass index (BMI), which is just an indirect measure of adiposity; however, it is of interest to see whether chemical exposures increase fat mass and not just BMI. Overall findings for the association between low-dose DDE exposure and increased BMI are fairly consistent, while the associations for other POPs are less consistent across studies.

INMA Birth Cohort Studies

Valvi then turned to a description of the INMA birth cohort studies in Spain.[3] That country, she noted, has rates of overweight and obesity in children that are among the highest in Europe.

[3] For more information, see http://www.proyectoinma.org/en_index.html (accessed March 23, 2016).

INMA is a network of seven birth cohort studies: the oldest three cohorts in the geographical regions of Granada, Menorca, and Ribera D'Ebre were started between 1997 and 2000, and the more recent ones in the regions of Asturias, Guipuzkoa, Sabadell, and Valencia were begun between 2004 and 2007. Exposure levels in the oldest cohorts are higher because the environmental levels of the chemicals were higher at that time.

The concentrations of POPs in expectant mothers were measured using serum samples collected in pregnancy and cord blood samples collected at birth. Exposure to a wide list of other environmental pollutants was also measured by analyzing biological samples (blood, urine, hair) collected from the mothers during pregnancy. The heights and weights of the children from birth onward were taken from their medical records and also directly measured by the researchers at various ages. The researchers also used questionnaires to collect extensive data on demographics and lifestyle factors.

Valvi first described the results from the newer cohorts, where the levels of exposure were lower (Mendez et al., 2011; Valvi et al., 2014). Both DDE exposure and hexachlorobenzene exposure were associated with the rapid growth of an infant in the first 6 months of life and a subsequent increase in the risk of being overweight at 1 year of age. Findings from these studies further suggested that the associations may be influenced by the child's sex and, less definitively, by the maternal prepregnancy weight and the duration of exclusive breast-feeding.

There was no evidence that prenatal exposure to PCBs was associated with either rapid growth or the likelihood of being overweight at age 1 year.

Next Valvi described the results from the older Menorca birth cohort (Smink et al., 2008; Valvi et al., 2012). They found that prenatal exposure to hexachlorobenzene increased the risk for both being overweight and having obesity when the child was 7 years of age. There was also some suggestion of an association between prenatal exposure to DDE and being overweight at age 7 years, but the association was stronger for the second tertile than for the third tertile, that is, when the exposure levels were medium rather than high.

Because the exposure levels were higher in the Menorca study, DDT was detectable in the vast majority of the cord blood samples analyzed; in contrast, in the later cohorts, most of the mothers had DDT concentrations below the limits of detection. Prenatal DDT exposure was nonlinearly associated with being overweight at age 7 years, but only in

boys and in children who had higher levels of consumption of total fats in their diets and not in children with lower fat intakes.

In contrast, prenatal exposure to PCBs was associated with a higher risk for being overweight at age 7 years in girls but not in boys.

The INMA researchers further evaluated the associations between exposures to multiple chemicals, including 27 different endocrine disrupters whose levels were measured in maternal biological samples collected in pregnancy, and child BMI at age 7 years using principal component analyses. The findings from this multipollutant approach showed that associations between POPs and childhood obesity remain robust after accounting in the models for exposure to other chemicals thought to be linked to childhood obesity, including BPA (Valvi et al., 2013), phthalates (Valvi et al., 2015), and polybrominated diphenyl ethers (PBDEs) and metals (Agay-Shay et al., 2015).

Summarizing the INMA birth cohort studies, Valvi said that exposures to DDE and hexachlorobenzene were associated with obesity-related outcomes both very early in life, in the first year of age, and later, at the age of 7 years. Exposure to PCBs was not linked to growth outcomes early in life, but there was a suggestion that prenatal exposure may increase weight only in girls, manifesting around the age of 7 years. For DDT there was a nonlinear association between exposure and weight, but only in boys and in children with high fat intakes. There was little evidence that these associations may be influenced by other potential modifiers: maternal prepregnancy weight and/or exclusive breast-feeding duration.

Children's Health and the Environment in the Faroes Study

Valvi also discussed findings from the birth cohort studies in the Faroe Islands, which are situated between the Norwegian Sea and the northern Atlantic Ocean.[4] The prevalence of overweight in Faroese children is much lower than that in Spanish children: just 22 percent of 5- to 7-year-olds but 37 percent of Spanish 6-year-olds were classified as overweight by use of the 2007 World Health Organization growth reference (WHO, 2007).

The population of the Faroe Islands is very homogeneous in terms of demographics and lifestyle characteristics, Valvi said. Most of the exposure to POPs is due to the consumption of whale meat, because these islands are inhabited by whale hunters.

[4] For more information, see http://www.chef-project.dk.

Since 1986, five birth cohorts have been recruited and are being followed up periodically. Valvi showed results from the third cohort, which was recruited at about the same time that the INMA Menorca cohort was recruited. The researchers collected blood samples from the mothers during pregnancy and then from the children at 5, 7, and 13 years of age. They also measured the children's weights and heights at the same ages. An advantage of this study is that exposure to POPs as well as other environmental contaminants was measured both prenatally and postnatally by the use of serum samples collected from mothers and children.

The study found some evidence that prenatal exposure to POPs is associated with an increased risk of obesity at 7 years of age (Tang-Péronard et al., 2014). However, following up on these findings Valvi examined the growth of the children between the ages of 7 and 13 years and found that the children who were more highly exposed to both DDE and PCBs during pregnancy had a reduced BMI gain compared with the less exposed children; there was no such association for the serum concentrations of POPs of the children at 5 years of age, and no clear association with the risk for being overweight at 13 years of age was shown for either prenatal or postnatal exposures. Valvi speculated that the negative associations between prenatal exposures and gain in BMI could be due to puberty status, because at age 13 years some of the children had already entered puberty, while others had not. Furthermore, there is some evidence from other studies that prenatal exposure to chemicals may influence the time of puberty. One interesting finding of this study, Valvi said, is that prenatal exposure to these chemicals may be more critical than postnatal exposure.

Conclusions of Valvi's Presentation

Finally, Valvi showed a couple of slides that summarized the current state of the evidence concerning prenatal exposure to POPs and obesity measures, mainly the BMI of the child. She drew several conclusions from these studies and the literature review:

- Low-level exposure to POPs is associated with childhood growth and obesity.
- Studies evaluating the persistence of the associations later in adolescence and adult life are currently lacking.

- The prenatal period (and perhaps the early postnatal period, as prenatal and postnatal exposures in the first years of life are highly correlated and hard to disentangle) may be the most critical window of exposure.
- Susceptibility may vary according to sex and perhaps also according to breast-feeding duration, maternal weight before pregnancy, and the child's consumption of fat.

Future research, Valvi said, will focus on continuation of the cohorts' follow-up, evaluation of the persistence of the associations at later ages, and identification of windows of exposure and susceptibility. It is also important to improve the assessments of obesity and use more direct adiposity measurements than just BMI. More work is also needed to identify the most susceptible groups and to evaluate the overall obesogenic effects of mixtures of various chemicals and not just POPs.

Finally, she said, further research is needed to better elucidate the mechanistic pathways by which these chemicals may influence growth. A way to do this is to integrate biomarker data into the human studies. This is not that simple, she said, because the most relevant biomarkers to be measured are not yet known. "But it is something that we are working on," she said, "and the collaboration between experimental and epidemiological studies will help to elucidate which is the best way to study mechanistic effects."

Discussion

Linda Birnbaum of NIEHS asked Valvi if her studies had examined exposures to dioxin-like PCBs, because mechanistic studies suggest that these can affect adipocyte growth and differentiation. Valvi answered that they have data on aryl hydrocarbon receptor activity but have not yet finalized the analysis. She also suggested that the effects of dioxin-like compounds may be more complex than what people may think because they act through a number of receptors, not just the aryl hydrocarbon receptor, which has so far been the main focus in human studies.

Birnbaum also commented that she appreciated Valvi's discussion of how the effects of exposure to POPs may differ between the sexes. This has too often been ignored in the past, she said. Valvi agreed and added that the effective study of such differences in the effects between sex will require larger studies that are costly and wider collaboration among existing cohorts.

Sheela Sathyanarayana from the University of Washington asked Valvi how she handled the fact that the results of these sorts of epidemiological exposure studies can be confounded by the children's diets, particularly their fat intake. The problem of such confounding is a major issue, Valvi acknowledged. Because diet is the main source of exposure to these chemicals, it is difficult to know which part of the effect is due to the chemicals and which part is due to high fat intake. Therefore, she said, in their studies she and her colleagues have accounted for dietary factors, such as fat or carbohydrate intakes. Estimates of the effect of the associations with prenatal exposures do not usually change when such dietary factors are taken into account, she said, but she indicated that they assessed the diet using food frequency questionnaires, which is not the most accurate method to use for dietary assessment.

ENDOCRINE-DISRUPTING CHEMICALS, ONSET OF PUBERTY, AND OBESITY

The second speaker was Frank Biro, the director of research in adolescent and transition medicine at the Cincinnati Children's Hospital Medical Center and a professor in the Department of Pediatrics at the University of Cincinnati. He spoke about the effects of endocrine-disrupting chemicals on the onset of puberty and obesity.

Puberty

Biro began by defining puberty. It is a series of interrelated changes involving just about every system in the human body. There is a pubertal growth spurt that is the only time in postnatal life that there is an acceleration in the rate of growth. There are profound changes in body composition. There is a maturation of the adrenal axis and a reactivation of the hypothalamic-pituitary-gonadal axis, which is fully functioning in a full-term infant but which gets turned off in the first 6 months of life. There is achievement of the ability to reproduce. Puberty can be considered a window of susceptibility in two ways. First, it can be a sensitive window to environmental exposures, so the timing of puberty is affected by what is going on outside the body. Second, puberty can serve as a special window of susceptibility to later adult morbidity and mortality, such as breast cancer.

Several physiological changes are associated with puberty. Adrenarche is the activation of the adrenal cortex for the production of adrenal

androgens. Pubarche is the appearance of pubic hair. Thelarche is the appearance of breast tissue. Gonadarche is the appearance of secondary sexual characteristics and has traditionally been defined to be the gonadal production of sex steroids. Menarche is the age of the first menstrual period.

Biro then described the sequence of events associated with puberty in girls determined from data from the Growth and Health Study carried out by the National Heart, Lung, and Blood Institute in the 1980s. It begins with adrenarche, the appearance of adrenal hormones from the adrenal gland. The first sign is an increase in ovarian volume, which is something that clinicians cannot see. With this comes the beginning of the pubertal growth spurt, as the girl begins to shoot up in height. After that comes the appearance of breast tissue, which is typically what clinicians use to define the onset of puberty, and the appearance of pubic hair. A year and a half or so after the appearance of breast tissue is the peak growth rate, and 6 to 12 months after that is the first menstrual period. The completion of puberty occurs with full development of the breasts and pubic hair, but a bit of growth still occurs even after the attainment of what appears to be full pubertal maturation.

Relationship Between Obesity and Puberty

A number of studies have reported an association between obesity and earlier breast maturation in girls. A recent study by Biro and colleagues (Biro et al., 2013) carried out with a group of 1,239 girls determined that BMI accounted for 14 percent of the variance in the age of onset of pubertal maturation, while race and ethnicity accounted for only 4 percent. This was the first study to find BMI to be a more important contributor to the onset of puberty than race and ethnicity, Biro said.

In 1974, Frisch and McArthur proposed that puberty would start a year or two after a child reached a certain critical level of body fat (Frisch and McArthur, 1974). Their work would be validated much later. In 1997, Matkovic and colleagues pointed out that the relationship was actually between leptin, a hormone produced by fat cells, and earlier menarche. They reported that for every increase in the serum leptin concentration of 1 nanogram per milliliter, the age of menarche dropped by 1 month (Matkovic et al., 1997).

In 2002, Grumbach argued that the studies with leptin clearly showed that leptin is necessary for kids to go into puberty but that it is

not sufficient and that there must be an additional mechanism. That mechanism was proposed to be the gonadotropin-releasing hormone pulse generator, a group of neurons located near the hypothalamus (Grumbach, 2002). In 2012, Bianco (Biro, 2015) reported that increased adiposity was associated with the earlier activation of the luteinizing hormone pulse generator. This, Biro said, could be considered the gonadostat, if you will.

However, potential mechanisms in addition to obesity impact the onset of breast development in girls, Biro said. He showed a graph that plotted the percentage of girls who have started breast development by a certain age (see Figure 3-1). For this graph, only the development of white girls was plotted.

The solid blue line describes data published in 1997 by Herman-Giddens and colleagues (Herman-Giddens et al., 1997). At the time many researchers doubted their results because a significant percentage of girls were starting breast development much earlier than expected—about 5 percent by the age of 7 years, for instance. But, Biro said, later studies confirmed their data indicating the onset of breast development in girls earlier than had previously been thought.

Then, in late 2013 Biro and colleagues published the results of a similar analysis done with girls who participated in the Breast Cancer and Environmental Research Program (BCERP). He showed curves representing the onset of breast development in white girls in that study (see Figure 3-1). Not surprisingly, breast development in the overweight and obese girls—those whose BMIs were above the 85th percentile—came much earlier than that in the girls that Herman-Giddens and colleagues had reported on. However, the girls whose BMIs were below the 85th percentile and whose average BMI was very similar to that of the girls in the study of Herman-Giddens and colleagues also showed an earlier onset of breast development: an average of about 8 months earlier than had been the case in 1997. What happened between 1997 and 2013? Biro and his colleagues have proposed that the earlier age of onset of breast development may be due to some environmental exposures that they are now evaluating.

The relationship between adiposity and the onset of puberty in boys is a little bit less clear-cut, Biro said. Of more than a dozen papers that have examined this issue, several report that a higher BMI leads to an earlier onset of puberty, several note that a higher BMI leads to a later onset of pubertal maturation, and a few state that the relationship is not

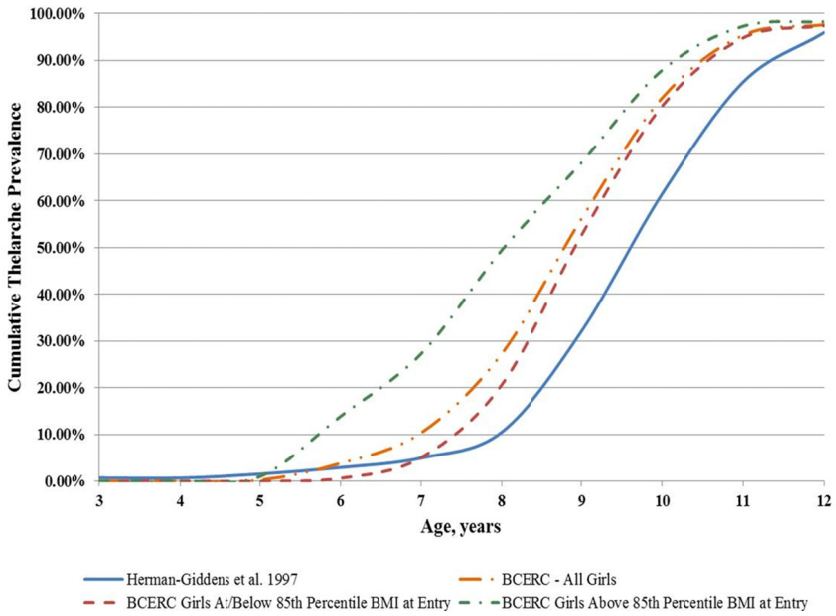

FIGURE 3-1 Onset of breast development.
NOTE: BCERC = Breast Cancer and the Environment Research Centers; BMI = body mass index.
SOURCES: Biro presentation to workshop, March 2, 2015, from Biro et al., 2013. Reproduced with permission from *Pediatrics*, vol. 132, pages 1019–1027, copyright 2013 by the American Academy of Pediatrics.

clear. Typically, he said, the European studies suggest that a higher BMI is associated with puberty arriving a little bit earlier, while the American studies suggest that a higher BMI is associated with puberty arriving a little bit later.

Biro suggested that what may be happening is that there is a J-shaped or U-shaped curve that describes the relationship between BMI and pubertal onset in boys. That is, an increasing BMI leads to an earlier onset of puberty until the BMI reaches obesity levels, as which point there is a delay in the onset of pubertal maturation.

Putting the various studies together, Biro suggested how various mechanisms could play a role in the relationship between BMI or the amount of body fat and the onset of puberty. He noted that his model was adapted from a 2008 paper by Ahmed and colleagues (Ahmed et al., 2008). In the model, various exposures could lead to increased obesity and increased amounts of visceral fat, including exposures consisting of a consistent energy imbalance, endocrine-disrupting chemicals, and inade-

quate prenatal growth. As Biro noted, it is the babies who are small for their gestational age who have a higher propensity toward obesity. The larger amount of fat has various consequences: higher levels of leptin; increased levels of aromatase, which is the enzyme that converts androgens into estrogens; and insulin resistance and elevated insulin levels. The insulin resistance and elevated insulin levels in turn act directly on the adrenals and increase the production of the adrenal androgens, which leads to earlier adrenarche. Insulin also acts on the liver to lower the levels of sex hormone-binding globulin, which means that there is a greater bioavailability of the sex steroids. Elevated levels of insulin also act on the ovaries and lead to increased androgen production.

After this, Biro spoke about some recent results showing an intriguing relationship between obesity and puberty. Using a sensitive method to measure serum estradiol levels, he and his colleagues examined those levels in a group of girls at various points in time as the girls approached and entered puberty. For the girls in the study who had a BMI below the median, the change in estradiol levels was as expected: a slow increase as they approached puberty and then a sharp increase as they entered puberty. But for the girls with a BMI above the median—and these were mostly overweight or obese girls—the estradiol levels barely increased as they went through pubertal maturation.

At first, Biro said, he thought the results could not be true. How could these overweight and obese girls even be going through puberty? Then he recalled that obese women have higher rates of breast cancer and that the mechanism that has been proposed to explain that is that in these women's fat cells, aromatase is converting adrenal androgens into estrogen, leading to higher levels of estrogens and an increased risk of breast cancer.

So, Biro said, what he believes that this study shows is that while the overweight and obese girls are not getting a big increase in estradiol levels, they do have high local levels of estrogen because of the conversion of adrenal androgens in their fat cells without elevated serum estrogen levels. Thus, they are able to go through puberty but without the extra estradiol.

Environmental Influences on Puberty

Biro then described the study on the environmental and genetic determinants of puberty, BCERP. The study began 13 or 14 years ago with the goal of collecting markers of breast development and other

physiological changes of sexual maturation to look at environmental stressors and see if they might be leading to future breast cancer risks. The stressors included lifestyle, nutrition, body size, and exposures.

There were three sites for the BCERP: in East Harlem in New York City through the Mount Sinai School of Medicine, in the Bay Area of California through Kaiser Permanente of Northern California, and schools in the greater Cincinnati, Ohio, area supplemented with the daughters and granddaughters of women enrolled in the Breast Cancer Registry of Greater Cincinnati. The sites recruited girls from 6 to 8 years old and saw them yearly or every 6 months. Over 2.5 years they recruited more than 1,200 girls about evenly divided among black non-Hispanic, white non-Hispanic, and Hispanic girls.

They were looking for the effects of endocrine-disrupting chemicals (EDCs), which interfere with how hormones are synthesized, how they are broken down, or how they act on the hormone receptor, sometimes by increasing the signal and sometimes by decreasing it.

The specific effect of one of these chemicals may depend on the timing of exposure, Biro said. For example, one study found that soy formula consumption during infancy leads to earlier menarche, while two others found that soy consumption in childhood led to a delay in the onset of breast tissue development. The timing may be the critical piece, he said.

EDCs have been found to act through a variety of molecular mechanisms. For example, BPA has been associated with increased aromatase activity, while phthalates and perfluorinated chemicals have been associated with decreased activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase; 3β-hydroxysteroid dehydrogenase is involved in converting estrone to estradiol and androstenediol to testosterone, and 17β-hydroxysteroid dehydrogenase is associated with the production of the sex steroids. In animal experiments, it has been shown that BPA stimulates gene transcription for kisspeptin 1 (KiSS1), which has been proposed to be the hormone leading to the onset of puberty.

The studies with the girls participating in BCERP did indeed find a number of environmental influences on puberty (Wolff et al., 2014). Biro first spoke about the results of studies with phthalates. Phthalates fall into two general categories, he explained: low-molecular-weight phthalates, which are found in fragrances and personal care products, and high-molecular-weight phthalates, which appear in soft plastics, sealants, and flooring. The researchers found an earlier onset of breast development in

girls exposed to low-molecular-weight phthalates, but with additional analysis they found that the effect seemed to work via an increase in BMI: girls exposed to the phthalates had higher average BMIs, which in turn led to an earlier onset of puberty. In contrast, exposure to high-molecular-weight phthalates was associated with a later appearance of pubic hair, but the effect was greater in normal-weight girls than in overweight girls, whose greater BMI may have had a mediating effect (see Figure 3-2).

The group has also looked at the effects of phenols, which are used for a variety of applications: antiseptics, sunscreen, mothballs, hand sanitizers, and more. The chemistry of the phenols is similar to that of sex hormones. For example, one of the phenols used in sunscreen, BPA, has also been used in the past as a pharmacological estrogen, so it has estrogenic properties. According to a study whose results Biro showed at the workshop but which has not been published, various phenols have been linked with effects on breast development. Both the sunscreen agent benzophenone-3 and enterolactone were associated with later maturation, while both 2,5-dichlorophenol, a chemical found in mothballs, and triclosan led to an earlier onset of breast development.

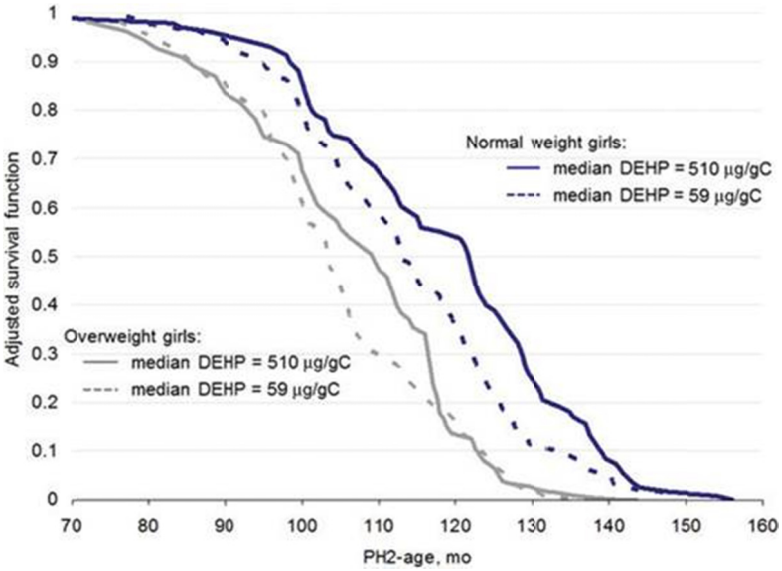

FIGURE 3-2 Delayed appearance of pubic hair in girls exposed to high-molecular-weight phthalates.
NOTE: DEHP = bis(2-ethylhexyl) phthalate; gC = creatinine.
SOURCES: Biro presentation to workshop, March 2, 2015, from Wolff et al., 2014.

Like phenols, perfluorinated chemicals have widespread applications in consumer products, such as in grease repellents and water stain repellents, and also industrial applications, such as in the production of Teflon, and research has shown that exposure to these chemicals is widespread in the population. Studies have shown various effects, including exposure to perfluorooctanoic acid being linked with shifts in the onset of breast development, especially among normal-weight girls.

In conclusion, Biro said that it will be important to examine the effects of exposure to some of the replacement chemicals now being used, such as bisphenol S, which is being used in place of BPA in a number of applications. It will also be important to begin looking at the effects of mixtures of compounds and not just single chemicals. "For better or worse, we live in an ocean of compounds," he said. "It is much more difficult to try to sort through what is happening with a mixture of compounds."

In the brief question-and-answer period after the presentation, an audience participant asked if Biro and his colleagues had considered alternative approaches to modeling, such as structural equation modeling, to disentangle the effects of the multiple chemicals that people are exposed to.

Biro responded that he and his colleagues have collected a vast amount of data on the girls in their studies: dietary patterns, anthropometric examinations, urine and serum biomarkers, sex steroids, fasting insulin, and glucose, among others. "Right now, we are dealing with two and three parameters at a time," he said. "After we have sorted through those, then we will start looking at these much more complicated models…. I think that after we control for some of these other mechanisms and start exploring how these mechanisms are related to each other, we will start getting a better story about these exposures."

OBELIX

The third presenter was Juliette Legler, a professor of toxicology and environmental health and the deputy head of the Department of Chemistry and Biology in the Institute of Environmental Studies at the Vrije University Amsterdam in the Netherlands. She was the director of the OBELIX project, which studied possible links between early life exposure to endocrine-disrupting chemicals and the development of obesity later in life.

OBELIX, Legler explained, was named for a famous French cartoon character, a very large, strong man who got his strength and his huge size

as a young boy by falling into a cauldron of magic potion. The question is, What was in that cauldron? That, in a sense, is what OBELIX is trying to find out for real people.

The project involved seven institutes in five European countries. It lasted 4.5 years and finished at the end of 2013. At the time of the workshop, Legler said, researchers were still busy getting all the resulting papers published and working on a large integrated review paper that was to be submitted soon.

The basic hypothesis behind OBELIX, Legler said, was that perinatal exposure to EDCs plays a role in the development of obesity later in life, and the project examined various mechanisms that could explain that connection. EDCs could, for example, cause changes in adipocyte differentiation. They could also cause a change in birth weight that would lead to changes in a child's long-term growth trajectories. They could affect early growth and BMI in children, or they might cause changes in hormone and lipid metabolism.

OBELIX involved a collaboration between epidemiologists and toxicologists who informed each other's work. The epidemiologists carried out studies on four mother-and-child cohorts from Belgium, the Netherlands, Norway, and Slovakia, while the toxicologists performed animal studies and in vitro mechanistic studies.

The epidemiologists and toxicologists studied four classes of compounds in common: non-dioxin-like PCBs, perfluorinated compounds, dioxin-like compounds, and phthalates. The epidemiologists also carried out studies on brominated flame retardants and organochlorine pesticides, while the toxicologists carried out an additional study on BPA. "We selected these compounds because we were very interested in different types of endocrine-disrupting chemicals," Legler explained. "It was a fact-finding mission looking at different endocrine mechanisms that they could disrupt and if this could perhaps be linked to the potential obesogenic effects of these compounds."

The epidemiologists carrying out the studies determined exposures to the various compounds by examining cord blood or the mother's milk, or both. The children were up to 6 or 7 years of age at follow-up.

The OBELIX epidemiologists worked with researchers across Europe who were carrying out studies on their own birth cohorts to expand the number and breadth of studies. In one of the first such collaborative studies, the researchers performed a detailed exposure assessment of PCB and DDE levels in cord blood in approximately 8,000 children in various cohorts throughout Europe. When they compared

exposure to birth weight, they found that PCB exposure was significantly associated with a decreased birth weight (Govarts et al., 2012). It was one of the largest studies of its kind indicating an association between PCBs and lower birth weight, Legler said.

The OBELIX team wanted to know if this lower birth weight would translate into changes in growth and weight in these children as they got older, she said. So they followed the children in their studies up to 7 years of age and measured growth, BMI, and the levels of certain metabolic hormones, such as leptin, adiponectin, and insulin.

The complementary animal studies were designed to be very similar in design to the human studies. The mice were exposed to environmental chemicals through their mothers' blood supply during gestation and for another 3 weeks after birth through breast-feeding until the animals were weaned. The mice were given one of eight different doses, all of which were below the level at which developmental toxicities are observed. In other words, Legler said, they were low, nontoxic doses. The animals then grew to adulthood, and some of them were given a high-fat diet. The various endpoints measured included body weight; fat pad weight; histopathology; food consumption; physical activity; serum lipid and insulin levels; as well as leptin, adiponectin, and glucagon levels.

Legler provided a brief look at the results of the OBELIX studies, many of which are still under review at different journals. In one case, for instance, there was a significant increase in growth in early life related to perinatal exposure to dioxin-like compounds, with exposed children being about 350 grams heavier at 2 years of age than unexposed children. However, when the children were followed to 7 years of age, there was a positive association only in girls and not in boys. A separate study with a smaller cohort looked at the levels of leptin, adiponectin, and insulin in the blood and found a significant negative association between exposure and levels of serum adiponectin in both boys and girls. Serum adiponectin, Legler noted, is an important hormone involved in regulating glucose levels and fatty acid breakdown.

In the related animal studies in which mice were exposed to dioxin-like compounds before and after birth, after 1 year the exposed female mice were significantly heavier than the unexposed female mice, particularly when they were given a high-fat diet. And not only were the mice heavier, but they also developed more fat tissue.

In studies with perfluorooctanoic acid (PFOA), exposed children grew more quickly up to 24 months of age, but there was no difference in growth between exposed and unexposed children at 7 years of age. In the

animal studies, there was actually a decrease in weight among exposed female mice. Thus, in contrast to the dioxin-like chemicals, which had an obesogenic effect, PFOA did the opposite in females and led to weight loss.

In their in vitro studies the OBELIX researchers studied, among other things, the effects of EDCs on the differentiation of preadipocytes into mature fat cells. Some EDCs, such as tributyltin and brominated diphenyl ether 47 (BDE 47), a flame retardant, increased differentiation. However, other chemicals, such as tetrachlorodibenzodioxin (TCDD), actually inhibited fat cell differentiation. The researchers also found dose–response relationships for some of the chemicals, with the amount of adipocyte differentiation increasing as the dose increased. This is strong evidence that some of these compounds—including tributyltin, BPA, and BDE 47—may induce fat cell differentiation.

Further investigation showed that several of these chemicals affected global DNA methylation in fat cells. DNA methylation, the biochemical process of adding methyl groups to certain sites on a DNA molecule, can reduce the expression of a gene to which the methyl group has been attached. The researchers found that some chemicals, such as tributyltin, led to decreased methylation, while others, such as BPA, increased the level of methylation. "This gave us some indication that … the stimulation of adipocyte differentiation is accompanied by changes in DNA methylation," Legler said. "We were interested to know what genes were involved here."

One of the genes involved, she said, is PPARγ2, the so-called master regulator of fat cell differentiation. The researchers showed that BDE 47 causes a decrease in the methylation of the promoter sequence of the PPARγ2 gene, which leads to increased expression of the gene and increased fat cell differentiation, so this appears to be the mechanism by which BDE 47 leads to an increased number of fat cells.

In conclusion, Legler said that the OBELIX project provided evidence that endocrine-disrupting chemicals do indeed play a role in obesity, affecting growth and metabolic pathways. Not all of the chemicals led to heavier phenotypes, she noted. Some of them did, but some of them actually led to leaner phenotypes. In particular, there was evidence from both epidemiological and animal studies that DDE and dioxin-like compounds lead to heavier phenotypes, while there was evidence that PCB 153 inhibits growth in children.

"We do believe that the current levels of EDCs may pose a risk for metabolic disruption," Legler said, but the effects vary by chemical, by

sex, and by time of exposure. There are clear differences between the effects on males and those on females and between prenatal and postnatal effects.

It will be important, she added, to see if these short-term effects on adiposity and BMI have long-term consequences. She said that it is known that obesity in early childhood is a predictor of long-term obesity, so it seems likely that there will indeed be long-term effects, but the data are lacking. "We really would like to follow up with these kids much longer," she said.

In the future, she said, her lab will be following up on the OBELIX cohorts as part of another European project that is focused on neuro-development. In particular, researchers in her lab have been working for the past 3 or 4 years on an alternative model of obesity using the zebrafish model. One of the advantages of using zebrafish is that they are transparent when young. It is possible to stain a young fish with a lipid stain that causes fat cells to stand out and then watch as the fat tissue develops.

The research on the zebrafish has shown that certain environmental chemicals, such as flame retardants and UV filters, disturb both the metabolism and the circadian rhythms in the exposed fish. The fish "show absolutely no circadian rhythm anymore when they are exposed to the UV filter," Legler said. "We are really wondering what is the chicken and what is the egg. Are these chemicals affecting circadian rhythm and that is affecting metabolism, or the other way around? Is metabolism affected by obesogenic chemicals that disrupt circadian cycling?" The research may eventually point to alternative mechanisms by which chemical exposures can affect metabolism, she said.

DISCUSSION

In the general discussion session following the three presentations described above, Kristina Rother from the National Institutes of Health asked whether the various epidemiological studies discussed in the session had taken socioeconomic status into account. Legler answered that the epidemiologist on her team did see differences according to socioeconomic status and used socioeconomic classes as a covariate in the models. In particular, in the Slovakian cohort, about two-thirds of the children were of Roma origin. Legler noted that the greatest effects on obesity as well as the highest exposure levels were in this class and that it was certainly an important factor included in the analyses.

Biro added that not only socioeconomic status but also race/ethnicity and the specific environments in which people live must be taken into account. All these factors are interrelated, and it can be difficult to tease apart their influences.

Rother suggested that one connection between obesity and circadian rhythms is that the effect of a particular chemical may vary according to when in a circadian cycle one is exposed. Insulin is known to have a circadian cycle, for instance, with insulin responses to eating being higher in the morning than in the evening. So perhaps the body's responses to obesogens might vary by time of day as well. Legler responded that her group has not looked at that particular issue and that, indeed, the animals are exposed to chemicals continuously through the 24-hour cycle. This may have masked a specific daytime- or nighttime-related effect, she speculated. She also noted that circadian rhythms do not appear until a certain point in development when the brain is far enough along in development that the circadian cycle is established. Thus, whether a chemical affects the development of the circadian rhythm would depend on the timing of the exposure.

Judy LaKind from the University of Maryland School of Medicine, who was watching via the webcast, asked if the models for postnatal exposure and BMI took into account the fact that breast-fed children often have a growth rate lower than that of formula-fed children. Legler responded that in the European studies there were essentially no women who did not breast-feed, so the only related factor that could be examined was the amount of time that the children were breast-fed.

Legler added that there was a close relationship between the amount of prenatal exposure to a particular chemical and the amount of postnatal exposure through breast-feeding, so it was impossible to completely isolate the effects of the two types of exposures. "But I think the important point we wanted to make here," she said, "is you cannot neglect how important postnatal exposure is for some of these exposures, certainly for women who have a longer breast-feeding duration."

Valvi added that in her studies she has included detailed information on the duration of breast-feeding and also on exclusive breast-feeding that further accounted for the age of introduction of solid foods and formula milk and that when she adjusted for the duration of breast-feeding, the associations were attenuated but remained significant.

Sandra Haslam of Michigan State University asked Legler whether she fed some of the animals in her study a diet of regular chow, in addition to giving some animals a high-fat diet. Then she asked whether

Legler's group had taken into account the differences in diet among the subjects of their studies.

Legler answered that in most cases she saw no significant effects on body weight in animals fed a normal chow diet, which is why in most of the studies the animals were given a high-fat challenge at the end of the follow-up period. The one exception was that BPA-exposed male mice on the normal chow diet did show a gain in body weight beginning in early development. In humans, she said, she and her colleagues found it difficult to get reliable information on the mothers' diets when they were pregnant and breast-feeding, and they saw no correlation between a mother's diet and her child's weight in the future.

Sally Darney from the U.S. Environmental Protection Agency asked if anyone had looked at the possible effects of geographical location on obesity. She pointed out that the data that Valvi presented indicated a higher prevalence of obesity in the southern European countries and that the southeastern United States also has a higher rate of obesity. So could latitude make a difference? Could growing up in a warm climate instead of a cold one have long-term effects on weight?

Valvi said that she does not think that there is "some weird confounding" related to latitude that affects the relationships between environmental chemical exposures and obesity, because pooled analysis in recent collaborative European projects have shown associations to be in the same direction across cohorts. Furthermore, she said, she thought that genetic differences or perhaps differences in diet or physical activity between the northern and the southern European countries was a more likely explanation for the differences in obesity prevalence than latitude. Still, she said, there are data indicating that climate conditions influence birth weight, which is an important determinant for obesity later in life. Thus, she said, it might also be that there is a relationship between climate or geographical latitude and obesity.

Archana Lamichhane from the University of North Carolina at Chapel Hill asked about what effects a mother's gestational diabetes might have on her child's birth weight and later risks of obesity and other metabolic diseases.

Valvi responded that a number of studies indicate that prenatal exposure to various environmental chemicals may increase the child's risk for diabetes later in life. "And if prenatal exposure can increase the risk during postnatal life," she said, "why would not exposing the mother increase the risk for diabetes appearing during pregnancy? That is a possible scenario." Thus, she continued, it is possible that gestational

diabetes mediates some of the effects that they see between prenatal chemical exposures and childhood obesity. It is also possible that gestational diabetes serves as a confounder because diabetes during pregnancy could change the metabolism of chemicals in the mothers and may explain the associations with child obesity shown later in life. Either way, she concluded, it is clearly an important factor to consider.

Sangeeta Khare of the U.S. Food and Drug Administration asked if the method of delivery—naturally versus by cesarean section—has any effects on obesity. Valvi responded that the type of delivery has been associated with both birth weight and later metabolic risk. However, controlling for the type of delivery in the models was not shown to influence the associations. Legler said that it is possible that children delivered by cesarean section may be exposed to certain chemicals, such as BPA, because of the medical care associated with such a delivery. In particular, she said, premature babies are generally attached to various sorts of tubes—feeding tubes, breathing tubes, and so on—and that could result in an exposure to certain chemicals in the period just after birth. Valvi then commented that their studies would probably not have captured exposures to the variety of nonpersistent chemicals that are used as part of medical practice and care at the time of or immediately after birth.

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4

Biological Pathways and Environmental Influences

Given the evidence from epidemiological and animal studies that exposure to various environmental chemicals in utero or shortly after birth can affect metabolism, body composition, and weight later in life, the obvious question is, How? The workshop's third session was devoted to exploring the various biological pathways and mechanisms by which environmental influences may have their effects.

HIGH-THROUGHPUT SCREENING OF ENVIRONMENTAL CHEMICALS

The first speaker in the session was Scott Auerbach, a molecular toxicologist with the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS).

Tox21 and ToxCast

Auerbach began by describing Toxicology in the 21st Century (Tox21) and ToxCast, two complementary and collaborative projects aimed at generating a community data resource on compounds of concern to the public health community. Both use high-throughput data screening and are essentially data-mining efforts. They are carried out by various contractors or through a collaboration between NTP and the National Center for Chemical Genomics (NCCG). The result will be a public resource.

Auerbach explains that these projects did not begin as an effort to identify chemicals that caused obesity. Instead, they began because no toxicity data are available for many of the chemicals used. Indeed, he said that for the tens of thousands of chemicals in use at significant levels, there is little or no safety or toxicological information that can be used to evaluate the health risks that they pose.

In response to concerns about this situation, NTP began a collaboration with NCCG in 2005 to screen chemicals. In 2007, the U.S. Environmental Protection Agency (EPA) started screening chemicals through its program called ToxCast. The Tox21 program was formed as a collaboration among EPA, the National Institutes of Health, and the U.S. Food and Drug Administration (FDA). Then, in 2008, Tox21 and ToxCast joined forces to form the "Tox21 community."

The Tox21 community had several goals: to identify patterns of compound-induced biological responses to characterize toxicity and disease pathways, facilitate cross-species extrapolation, and model low-dose extrapolation; to prioritize compounds for more extensive toxicological evaluation; and to develop models that could predict the biological response in humans.

In Phase I, which ran from 2005 to 2010, the collaboration screened more than 3,000 compounds using a variety of in vitro assays. In Phase II, which started in 2011, ToxCast screened about 700 compounds in about 700 assays plus about 1,000 compounds in endocrine activity assays. Meanwhile, Tox21 screened the chemicals in a 10,000-compound library that was created as a part of the interagency collaboration. The 10,000-compound library includes drugs, drug-like compounds, and active pharmaceutical ingredients from FDA; ToxCast Phase I and II compounds and compounds from such programs as the Antimicrobial Registration Program and the Endocrine Disruptor Screening Program from EPA; and a wide variety of compounds supplied by NTP. The diversity of chemical structures found in the library is quite large, Auerbach said.

These 10,000 compounds were screened three times at 15 concentrations in a variety of quantitative high-throughput screening assays, such as assays for nuclear receptor activation or inhibition and assays for cellular stress response pathways.

A main difference between Tox21 and ToxCast, he noted, is that Tox21 covers a lot more chemicals, but ToxCast has a lot more assays. There is an overlap between the two data sets, with certain chemicals being included in both Tox21 and ToxCast.

Mining of the Tox21 Data

Auerbach's main focus in his presentation was on what is being done to mine the data from the ToxCast and Tox21 programs to find chemicals that might pose an obesogenic or diabetogenic risk.

Auerbach described the analysis that he presented as a hypothesis-generating exercise. The goal is to find chemicals that might activate certain biological pathways that may lead to obesity or diabetes and that can be further assessed in complex assay systems.

They are doing this in two ways. The first approach Auerbach described employs the ToxPi ranking system. This approach starts by defining key biological processes associated with obesity or diabetes (e.g., adipocyte differentiation). Expert input and literature mining are then used to match ToxCast and Tox21 assays to the biological processes (e.g., peroxisome proliferator-activated receptor γ [PPARγ] would be associated with a biological process related to adipocyte differentiation). The selected assays are then used to build a ToxPi (i.e., a toxicity pie) for the biological process of interest, where each assay represents a slice of the ToxPi. The more potent the effect of the chemical on the assay is, the larger the slice is. The ToxPis are then ranked by their area, with the ToxPis with the larger areas being ranked higher.

When the process was tested on the original 309 chemicals in ToxCast Phase I to identify chemicals involved in such biological processes as adipocyte differentiation and feeding behavior, it did indeed pull out the appropriate chemicals. For example, when ToxPi was applied to feeding behavior—which is particularly interesting, because it has a large number of neurological functions associated with it—the top eight compounds identified by the tool were all known to be associated with obesity in humans. This success offers confidence that ToxPi can effectively screen for chemicals from the much larger 10,000-compound library.

The second approach being used to mine the Tox21 data is what Auerbach called the "sentinel chemical correlation." This approach is purely data driven, he said, and does not involve input from experts.

The approach is based on a particular premise: chemicals that exhibit similar biological properties across high-throughput screening assays will likely exhibit similar biological properties in vivo. Thus, for example, one can start with a known obesogen and look for chemicals in the database that have similar results by the collection of assays; such chemicals are likely candidate obesogens themselves.

One of the challenges is to determine how to measure the similarity of the findings from the two assays. After all, the results of the assays are not numbers but rather are functions that map such things as the size of a response to various concentrations of a chemical. The details are somewhat technical, but these mappings can be characterized in various

ways that make it easier to compare different assays. One can look at certain thresholds, for example. Once this is done, it is possible to run correlation software on the data set, looking for assays with similar responses to the same chemical. From there, one builds relationship networks that reflect how closely correlated various chemicals are. The result is a map of the relational space for the biological activity of the different chemicals, and one can use that map to pick out chemicals with biological activity similar to that of a target chemical.

As an example, Auerbach showed the network for a PPARγ activator called rosiglitazone, which triggers adipocyte differentiation. The chemicals in the network that are closest to rosiglitazone are all pharmacologically related to rosiglitazone. This shows that the approach can be used to identify chemicals that are likely candidates for having particular properties.

The tools are publicly available and a browser allows users to input a chemical and quickly find other chemicals in the 10,000-compound library with similar properties. Thus, a researcher can, for instance, input a chemical that is a prototype agent causing adipocyte differentiation and find other chemicals in the data set that behave very similarly to it to test those other chemicals.

To make the tool even more valuable, the developers created a collection of chemical-to-biological annotations for the various chemicals in the data set. This makes it possible to do "chemical annotation enrichment analysis," which is similar in spirit to the commonly used gene annotation enrichment analysis. It offers a way of judging the value of the chemicals that one identifies through the sentinel chemical correlation. As Auerbach described it, "I go fishing. I throw out that line and I pull in a bunch of chemicals. How do I know if I have … any truth in that bag? Am I just getting random association?" The chemical annotation enrichment analysis helps evaluate the plausibility that the prioritized chemicals have an effect.

Discussion

In the brief question-and-answer session following Auerbach's presentation, Bruce Blumberg of the University of California, Irvine, said that his group had done a great deal of validation testing of the sorts of predictions that Auerbach had described and that the results had not been as good as they had hoped. The problem lies, he suggested, in the disparity between the activity and the different assays. "For example, if

you take the PPARγ assay, you add a gene assay that ToxCast uses and the original NCCG assays that ToxCast used and the NovaScreen," he said. "There is absolutely no correlation among those three. If you now plug in the GeneBLAzer assays that NCCG is doing for Tox21, those do not correlate either." Given that the same receptor can have such a disparate set of results, he asked, what does that imply about the quality of the results that a researcher can get from the approach that Auerbach described?

Auerbach acknowledged that this is a significant challenge. "Part of the challenge that we are dealing with is the lower-potency environmental chemicals," he said. "What I can tell you from reviewing the Tox21 data is that pharmacology works. It works very well. The positive controls run very well. When you start getting up into the higher-micromolar dosing and start looking at activity there, there is some variability." And that variability can affect the predictions made by ToxPi, he said.

Another workshop participant commented that the ToxCast and Tox21 assays are mostly for gene expression. There are no good assays to determine whether a particular chemical has obesogenic-type activity, for example—no good assays for lipid accumulation, no metabolic competence assays, and so on. This lack, the participant suggested, may be part of the reason for the problems in the results, and it makes it more difficult to identify chemicals that have some sort of obesogenic effect.

Auerbach agreed and said that the next generation of Tox21 would include more complex emergent cellular phenotypes, such as lipid accumulation. In general, he said, the commenter was correct. "We are typically measuring the point of contact between chemical and biology, which is the receptor," and the assays use things like transformed cell lines that do not have metabolic activation. "The next generation of assays that we will be working on will have those capabilities."

EFFECTS OF PERSISTENT ORGANIC POLLUTANTS ON ADIPOSE TISSUE

The next speaker was Robert Barouki, a biochemist and a molecular biologist at Paris Descartes University who studies the toxicity mechanisms of environmental pollutants, with a particular focus on the dioxin receptor aryl hydrocarbon (AhR). He discussed the effects of persistent organic pollutants (POPs) on the function of adipose tissue.

While scientists used to consider adipose tissue little more than a storage organ, Barouki said, in recent years researchers have learned that adipose tissue has a number of very interesting physical, metabolic,

endocrine, and maybe even toxicological functions. Thus, the consequences can be serious if the functioning of adipose tissue is altered or interrupted by environmental chemicals.

Barouki's research comes at the relationship between adipose tissue and pollutants from two different angles. First, he seeks to understand how the presence of increased adipose tissue mass affects the uptake and kinetics of pollutants and the toxicity of pollutants. Second, he examines how pollutants affect adipose tissue, including endocrine disruption, metabolic disruption, and inflammation and oxidative stress. In particular, he said, his talk would focus on the effects of POPs on inflammation and inflammatory processes.

Protective Function of Adipose Tissue

Adipose tissue plays a number of roles in toxicology, Barouki said. One of them is a protective function, as some POPs have an affinity for adipose tissue, which holds them and protects other organs from their toxic effects. This would be helpful mostly for acute exposure, he noted.

The evidence for this role of adipose tissue comes from various studies in which aquatic or terrestrial animals were exposed to dioxins or other POPs. Those animals with the largest amount of fat mass had the highest levels of protection from the exposures, which led one research to term the phenomenon "survival of the fattest" (Lassiter and Hallam, 1990).

Recent evidence indicates that the same effect may be seen in humans. In particular, epidemiological studies have found that if you examine death rates among people with very high levels of POPs in their blood, people who are obese have a significantly lower risk of death than thin people. Of course, among those with low levels of POPs in their blood, the obese are at a high risk of dying, but having large amounts of fat tissue does seem to have a protective effect against the toxicity of POPs (see Figure 4-1).

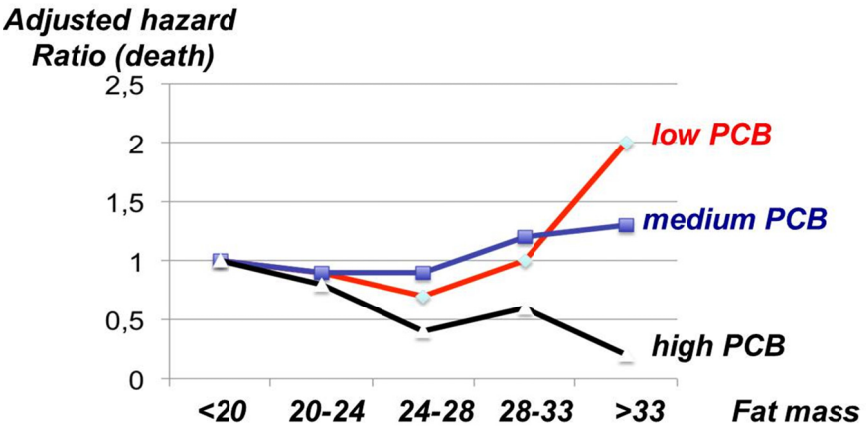

FIGURE 4-1 Protective effect of fat mass in humans when serum POP levels are high.
NOTE: PCB = polychlorinated biphenyl.
SOURCES: Barouki presentation to workshop, March 2, 2015. Data from Hong et al., 2012.

But what happens in the long run to obese people whose adipose tissue has stored toxic chemicals? They may be protected from acute exposures, but what happens if the chemicals stored in the fat tissue are later released? Barouki set out to find out.

One approach is to study obese individuals who have undergone or who are undergoing massive weight loss, such as those who have had bariatric surgery, so Barouki and his colleagues recruited 86 patients who were undergoing this surgery along with 23 lean controls. They first measured the POPs in their subjects before the surgery. When the concentration of the chemicals in adipose tissue was measured in terms of the amount of POPs per gram of lipid, Barouki and colleagues found that obese subjects had significantly lower concentrations than the controls. In short, the POPs were distributed more diffusely in their fat tissue. However, when the total amount of POPs that an individual was carrying was calculated, the obese individuals had a significantly higher burden because they had so much more fat tissue.

When the researchers examined the correlations between the amount of POPs present in serum or adipose tissue versus the various phenotypes of the individuals in the study, they found that the higher that the amount of serum POPs was, the higher that the levels of cholesterol and triglycerides

were. Furthermore, liver toxicity was positively correlated with higher levels of POPs in the blood.

Gene expression studies showed that the levels of expression of some of the genes that are the classical targets of POPs were increased in the adipose tissue of the obese subjects. This may indicate that the POPs were having some effect on the adipose tissue, Barouki said.

Barouki's team then examined the obese subjects after their bariatric surgery. They found that the amount of POPs in the blood increased steadily over time in the year after the surgery. "These people lose 30 or 40 kilograms of weight during the few weeks and few months [after surgery]," he said. "There is an increase in POPs in serum, which suggests that when you lose weight, you probably release these pollutants in your serum."

Ideally, once the pollutants were released into the blood, they would be eliminated from the body, Barouki said, but this did not happen, at least to a large degree. At most they saw a decrease in the total burden of some pollutants of about 4 percent. What probably happens, he said, is that the POPs released into the blood are taken back up by the adipose tissue because these people still have a lot of fat, even after the weight loss following the surgery.

In general, the obese subjects saw improvements in their serum lipid levels and liver parameters after the surgery, but the improvement was significantly smaller in those with higher levels of POPs in their blood. The one unexpected, even paradoxical effect that Barouki and his colleagues observed was related to sensitivity to insulin, Barouki said. Those subjects who had higher levels of POPs in their blood had greater improvement in their insulin sensitivity than those with lower levels of POPs. "It is not so easy to explain," Barouki said. "We have an idea about the effect on gluconeogenesis [that is, production of glucose in the liver, which could be decreased by certain pollutants] that could explain part of it, but this is still a paradox, I have to say."

Barouki also noted that other researchers have also observed higher levels of POPs in the blood of people who have experienced long-term weight loss.

The bottom line, he said, is that adipose tissue plays an important role in protecting against acute exposure to environmental chemicals. However, the fact that fat tissue takes up these chemicals and releases them over time—following weight loss or in other situations—means that this process can lead to toxicity in the long run.

Inflammatory Effects of Pollutants on Adipocytes

Barouki then turned to the issue of how POPs affect adipocytes. The adipose tissue of obese individuals is different from that of lean individuals in a variety of ways. There are more cells, and the cells are larger. There are differences in the presence of inflammatory cells, in the presence of fibrosis, in the vascularization of the fat tissue, and so on. Barouki's focus is on differences in inflammation.

To study this, he uses a human cell line descended from an adipose tissue-derived stem cell. The cells can be differentiated, so it is possible to examine both the preadipocyte stage and the fully differentiated adipocyte. The adipocytes in the cell line accumulate fat over time as they age and grow.

After the addition of dioxin or polychlorinated biphenyls (PCBs) to some of the cells, it is possible to use large-scale transcriptomic studies to see what pathways are modified in the cells in response to the pollutants. Research has shown that the pathway most affected is the inflammatory pathway, with its cytokines, chemokines, and so forth.

Repeating these in vitro studies in mice, the researchers treated a group of mice with 10 micrograms per kilogram of dioxin and assayed them for cytokines. What they found was that there was an increase in the adipose tissue cytokine levels. This was not observed, however, in AhR-knockout mice, in which the gene for the AhR dioxin receptor had been removed. This implies that the response to dioxin was through the AhR pathway.

Another important observation, Barouki said, was that when the adipose tissue in these dioxin-challenged mice was examined, it was possible to see macrophages accumulating in the adipose tissue. Thus, exposure to dioxin leads to an increase in the number of macrophages in the adipose tissue, indicative of an inflammatory response. In general, Barouki said, the more inflammation in the adipose tissue that there is, the more metabolic consequences that there are.

It is very important to note that the pollutants can provoke this effect in an acute way, Barouki said. "This is not a chronic or obesogenic effect," he said. "This is acute. It can provoke an increase in the inflammation of the adipose tissue and the invasion by macrophages."

Barouki also mentioned recent work by a colleague, Jerome Ruzzin, who has studied the levels of POPs in metabolically abnormal versus metabolically healthy obese individuals (Gauthier et al., 2014). Many obese individuals are actually metabolically healthy, he noted, while

others are metabolically abnormal. Ruzzin found that higher POP levels made it more likely for an individual to be metabolically abnormal. In terms of inflammation, this makes sense. "If the mechanism is inflammation," Barouki said, "then it will have more metabolic consequences."

Summing up his presentation, Barouki said that it seems likely that adipose tissue has a protective effect under acute conditions. It binds the POPs and keeps them from going to other places in the body. "It is better to have them in the adipose tissue than in your brain," he said. But over time the adipose tissue can release the POPs into the bloodstream, potentially leading to chronic problems elsewhere in the body.

On the flip side—talking about how POPs affect adipose tissue rather than how adipose tissue affects POPs—Barouki said that the data indicate that POPs result in a relatively acute effect on the inflammation of the adipose tissue and probably have some metabolic effects as well. They can also affect the programming of the individual and of obesogens.

Discussion

In the question-and-answer session following Barouki's presentation, Sarah Rothenberg of the University of South Carolina asked for more details on when chemicals are released from adipocytes. What are the life stages at which they are released? Are they only released when you lose weight? Are they released during pregnancy? Are they released during old age?

There has been little research on that topic, Barouki said. It is not known, for instance, exactly where the POPs are stored inside the adipocytes. He said he suspects that the release of POPs from the adipocytes is simply a matter of physical chemistry, with adipocytes constantly releasing and taking up the chemicals, and that when a person loses weight and the adipocytes shrink, they release some of the pollutants they have been holding onto. He also noted that during breast-feeding, some of the POPs go into the milk, so that the mother loses some of them then, with the child gaining them.

TRANSGENERATIONAL EFFECTS OF OBESOGENS: TRIBUTYLTIN

The session's third speaker was Bruce Blumberg, a professor of developmental and cell biology and pharmaceutical sciences at the University of California, Irvine. He discussed how the effects of

obesogens can become permanent and heritable by reprogramming a set of stem cells in the body.

To set the stage, Blumberg spoke in general about why people become obese. He indicated that the prevailing wisdom is simply that people consume more calories than they burn, and while that is a major part of it, he explained that is not all there is to it. In one set of experiments, for example, two sets of mice were fed the same number of calories per day, but one set could eat whenever they wanted, while the other was given their food in three pulses. The first group of mice—which could eat whenever they wanted—got fatter than the ones who ate in three pulses.

Indeed, it is well recognized that there are other factors than simply the energy balance, Blumberg said. These include stress, inadequate sleep, prenatal nutrition, "thrifty" genes that have evolved to make the most of scarce calories, and exposure to various chemicals in the environment.

Tributyltin

It is this last factor that Blumberg and his colleagues have been studying in recent years—in particular, whether disturbances in signaling pathways caused by endocrine-disrupting chemicals play a role in adipogenesis and obesity. The specific obesogen that they have been focusing on is tributyltin, a chemical known primarily for its effect on snails. It causes imposex, which is the imposition of male sexual characteristics on female snails.

Blumberg said he first became interested in tributyltin a dozen years ago when he heard that it could genetically reverse female fish into male fish. He set out to determine which hormone receptors might be targets for tributyltin, expecting a steroid receptor to be involved. Instead, he found that tributyltin activates two nuclear receptors, retinoid X receptor (RXR) and PPARγ, which are critical in adipogenesis, or the formation of fat cells. It turns out that if you expose mice to tributyltin while they are still in the womb, they grow up to be fatter than normal by about 15 percent. Strangely enough, the mice are no heavier than the control animals and actually weigh a little less. What happens is that the amount of fat increases at the expense of other types of body tissue, so that the overall weight of the animal does not increase.

"What we have been trying to understand for probably almost 10 years now is how does this [tributyltin] exposure cause weight gain," he

said. "Does it change the hormonal control of appetite and satiety? Are these extrahungry mice? Do they eat more? We have not noticed any big changes." Indeed, other studies have shown that these mice actually consume less food than the controls. So perhaps the adipocytes are doing a better job of storing lipids. Or perhaps there are more fat cells or more pre-fat cells.

The latter is the favored model, Blumberg said. The reason that he and his colleagues favor this model is because of the existence of a particular type of cell called a mesenchymal stem cell, which can be induced to become an adipocyte by exposure to rosiglitazone, a PPARγ antagonist.

Mesenchymal stem cells, he explained, can follow many different paths. They can make muscle, cartilage, fat, or bone. PPARγ controls the choice between becoming a fat cell or becoming a bone cell. PPARγ expression in a mesenchymal stem cell commits it to the adipocyte lineage, Blumberg said, while PPARγ knockout commits it to the bone lineage.

Tributyltin's Transgenerational Effects

The first experiments that Blumberg's group carried out examined what happened to mesenchymal stem cells when mice were exposed prenatally to tributyltin or to rosiglitazone. They found that exposure to either chemical caused a significant increase—about twofold—in the ability of those stem cells to become fat cells. This was reflected by an increase in such fat markers as fatty acid binding protein 4, leptin, and a half a dozen others, he said. At the same time, the ability of the cells to differentiate into bone was inhibited. This was indicated by the downregulation of a variety of bone markers, such as osteopontin, osteocalcin, and Runx2, by the prenatal treatment. Even when the cells were induced to become bone cells, they still expressed fat markers. "These cells are predisposed to become fat cells after this prenatal exposure to tributyltin," Blumberg said.

Perhaps the most surprising finding, though, was that some of the effects of tributyltin exposure were passed down to succeeding generations. Blumberg's team exposed pregnant mice to tributyltin throughout their pregnancies by giving it to them in their drinking water. The doses were very low, about 5 to 50 times less than the reported no-observed-adverse-effects level. They raised the mice born to those mothers, bred them to

create a second generation, and bred those mice to create a third generation.

An effect in either the first generation (F1) or the second generation (F2) is called a multigenerational effect, reflecting the fact that these animals themselves were exposed to the tributyltin: the first-generation mice as embryos inside the mothers and the second-generation mice as germ cells inside the first-generation mice. An effect in the third generation (F3) is called transgenerational because there is no such exposure.

What Blumberg found was that the tributyltin had transgenerational effects. For example, perirenal and interscapular fat deposits were substantially larger in the F2 and F3 mice than in the original mice or in the F1 mice. Furthermore, all three generations of mice, but particularly the third-generation mice, had substantially higher levels of expression of fat-related genes in the mesenchymal stem cells than control mice. In other words, the genes related to the production of fat cells were much more active in the exposed mice and their descendants than in unexposed mice.

Blumberg has since been exploring the mechanism by which the effects on the mesenchymal stem cells are passed from generation to generation. He said that he suspects that it is an epigenetic effect, and he has been studying the methylation of the DNA in the stem cells. In some cases, he said, genes in the stem cells are more highly methylated in exposed mice and their descendants—and, thus, the expression of the genes is decreased—while other genes are less methylated in the exposed mice and their descendants. This indicates, Blumberg said, that there are indeed epigenetic effects resulting from the original exposure of pregnant mice to tributyltin.

His group is now looking very deeply into what is going on, he said, and is carrying out a full genomic analysis of the mesenchymal stem cells in the sperm from four generations of mice. “The goal,” he said, “is to link changes in transcription with changes in DNA and histone methylation to identify epimutations responsible for this transgenerational inheritance. As you probably know, the existence of DNA methylation changes that persist from generation to generation is quite controversial. There are many people who simply do not believe that. We would like to know if it is true or if we are seeing perhaps the effects of a long noncoding RNA or histone methylation that is being transmitted. We really do not know the answer.”

Recently, Blumberg's postdoctoral student, Raquel Chamorro-Garcia, discovered a surprising effect in fourth-generation (F4) mice in the tributyltin experiment. The mice were raised on a normal chow diet, which is about 13 percent fat by calories, for the first 19 weeks of life. They were then switched to a breeder chow, which has 21 percent fat by calories, for 6 weeks, and then they were switched back to the normal chow.

The body weight of these mice did nothing surprising, but the effects of the slightly higher-fat diet on the fat mass in the male F4 mice were striking. Immediately after the diet was switched, the male descendants of the tributyltin-exposed mice got fatter, and they continued to get fatter than the controls for the entire 6 weeks, and then they stayed fatter after the switch back to the normal diet. A much higher percentage of the body weight of fourth-generation male mice was fat tissue and a much lower percentage was lean tissue. The effect was much less striking in females. There was a small effect, but it was just barely statistically significant.

Mechanisms

Blumberg and his team also looked for genes whose expression was affected by tributyltin, which should offer an indication of which genes are responsible for the effects that they were seeing. Many of them were involved in lipid storage and lipid transport, which was to be expected.

What is important to keep in mind, Blumberg said, is that there are actually two processes at work. One is the commitment of the mesenchymal cells to the adipogenic pathway, that is, to becoming fat cells. The second is the actual differentiation of the stem cells into fat cells. In terms of the genes involved, those are two separate pathways. What Blumberg's experiments have shown is that multiple generations of mice exposed to tributyltin all have undifferentiated mesenchymal stem cells with adipogenic gene expression profiles, meaning that they are poised to become fat cells.

Other work has shown that rosiglitazone does very little to cause such differentiation: the highest dose of rosiglitazone can just barely push these cells down that pathway. On the other hand, tributyltin is very effective in inducing these cells toward the adipogenic pathway. Indeed, 50 nanomolar tributyltin is vastly more potent than 500 nanomolar rosiglitazone.

In terms of the actions of individual genes, PPARγ does not push mesenchymal stem cells to commit to becoming fat cells. It can differen-

tiate cells that are already committed, but it cannot drive the process itself. Other genes, however, are induced by tributyltin and are involved in inducing these cells to commit to the adipogenic lineage.

Are Environmental Chemicals Making Us Fat?

Given everything that is now known about the effects of tributyltin, could it and other organotins be contributing to today's obesity epidemic? It is known, Blumberg said, that adult exposure to tributyltin rapidly induces adipogenic genes. It is also known that drugs that activate PPARγ increase obesity. Furthermore, prenatal exposure to tributyltin permanently alters the adult phenotype, and it recruits mesenchymal stem cells to the adipocyte lineage and diverts them away from the bone lineage.

However, Blumberg continued, a key question is whether humans are exposed to sufficient levels of tributyltin to lead to these effects, and that is controversial. There are few data on the subject, and the data that exist are conflicting. It is clear, however, that tributyltin does exist in the environment. He mentioned that the New York Department of Public Health has found it in house dust, so it is likely that humans are exposed to it. Polyvinyl chloride (PVC) plastic also has a certain amount of tributyltin in it.

"I would like to argue that human exposure to organotins might reach levels sufficient to activate these high-affinity receptors, RXR and PPARγ," he said. "Although I cannot say that organotins are making us fat, I think it is probably a fair statement to say that there are such things as obesogens that modify our response to calories such that we are more likely to get fat than we would be if we were not exposed."

There is a great deal that is not known about environmental exposures and obesity, Blumberg said. It is not known how many obesogens there are, although he thinks that it could be many hundreds. "We do not know what the body burdens and the population are in most of these chemicals," he said. "We do not know what all the molecular targets are.…We do not know a lot about critical windows of exposure.…We do not know how prenatal exposure alters the adult phenotype."

It is pretty clear, Blumberg said, that diet and exercise by themselves are not sufficient to explain the obesity epidemic, particularly given the example of the obesity epidemic in 6-month-old infants studied by Rob Lustig at the University of California, San Francisco. So what else is going on?

The research clearly indicates that obesogens exist and that certain chemicals make people fat or increase susceptibility to weight gain, and research has identified a number of candidates. Prenatal exposure to tributyltin and another chemical that Blumberg has tested, triflumizole, reprograms exposed animals to be fat. The existence of these obesogens implies, Blumberg said, that scientists and clinicians need to shift the paradigm from trying to treat obese adults to preventing children from becoming obese in the first place. This can be done by optimizing their nutrition, making sure that they get enough exercise and sleep, and all the standard things, but reducing their exposure to obesogens will be an important component, he said.

A growing number of chemicals have been shown to have trans-generational effects. Blumberg's lab has shown it for tributyltin, and other researchers have shown it for such chemicals as vinclozolin, certain plastics, jet fuel, and dichlorodiphenyltrichloroethane. The existence of transgenerational effects raises the stakes for the argument concerning which chemicals should be regulated and which standard should be used to determine which chemicals should be taken off the market, Blumberg said. "That is not a decision for me to make, but that is a discussion that we need to have."

Discussion

In the question-and-answer session following Blumberg's presentation, Robert Barouki asked Blumberg about the body composition of the lab animals that gained body fat without gaining weight. What was lost? Blumberg answered that it was lean mass, not water, and it was probably mostly muscle because it would be unlikely that the animals lost bone mass so quickly.

Another participant asked that if mice were treated with different obesogens, would some common genes be upregulated or downregulated for every chemical while the expression of other genes would be affected differently for different individual obesogens and, if so, if that would be a way to get insights into the mechanisms. Blumberg answered that in treating with different chemicals one would expect to see some common and some distinct effects and added that the experiment would more likely be carried out in mesenchymal stem cells than animals.

Al McGartland of EPA commented that until the mid-2000s, when this use was banned, tributyltin was used on the bottom of boats, and he asked if this might be one source of exposure, perhaps from people

eating shrimp or other seafood that had been exposed. It is important to determine the source of exposure, Blumberg said, but he suspects that seafood is a relatively small contributor to human exposure compared with PVC plastic.

Jerry Heindel of NIEHS commented that the key element in the obesogen hypothesis is that exposure to a chemical must alter a person's set point or sensitivity for gaining weight. Up to now, he said, no one had demonstrated a potential obesogen that changed the set point in this way, but Blumberg's experiments with the higher-fat diet pushing tributyltin-exposed mice, even in the fourth generation, to gain weight when controls did not was just such a demonstration, Heindel said. "I think that is really a proof of principle for the obesogen hypothesis," he said. "It is really a key piece of data."

Scott Auerbach pointed out that one of the known properties of organometallics such as tributyltin is that they tend to be toxic to mitochondria, the energy sources of the cell. One way that they exert their obesogenic effect, he speculated, could be by damaging the body's ability to burn calories. Blumberg responded that this was a good suggestion and that he will soon be able to test such ideas by putting the F4 mice in metabolic cages and testing their metabolism.

EFFECTS OF PERINATAL EXPOSURE TO BISPHENOL A ON OBESITY AND METABOLIC DISEASE LATER IN LIFE

The next speaker was Beverly Rubin, an associate professor of integrative physiology and pathobiology at the Tufts University School of Medicine and the Sackler School of Graduate Biomedical Sciences. She discussed the mechanisms by which perinatal exposure to the chemical bisphenol A (BPA) can lead to obesity and metabolic disease later in life.

Rubin began with some basic information about BPA. It is ubiquitous in the environment, and 93 percent of the population has detectable levels of BPA in their urine. The chemical is found in cash register receipts, in the resin lining tin cans, in dental materials, in polycarbonate plastic often used in food and beverage storage containers, and in many other products that humans come into contact with daily.

BPA is also an endocrine disrupter that has been documented to have a large number of effects on humans, including reproductive effects, development of neoplasias, and disruption of brain development. The ones of most relevance to the workshop are increased adiposity, changes in glucose homeostasis, and an increased risk of diabetes and liver pathology.

Some years back, Rubin said, there was some controversy regarding BPA's effects on body weight, with some research showing that it led to weight gain, some showing that it led to weight loss, and some showing that it led to no change. At present, there are many more papers reporting weight gain in animals exposed to BPA, but there are still some conflicting reports.

Human studies have reported an association between exposure to BPA and increased body weight, increased waist circumference, and increased body mass index (BMI). Studies have also found an association between urine BPA levels and obesity-associated metabolic changes, including insulin resistance and diabetes.

So in carrying out their research, Rubin and her colleagues had a series of questions that they wished to answer: Does developmental exposure to BPA alter body weight and body composition and contribute to other associated components of metabolic disease? What BPA doses cause weight gain and increased fat mass? What is the critical window for BPA exposure? Are there different effects in males versus females? And does early exposure interact with the Western diet in adulthood?

Part 1: Effects of Perinatal and Peripubertal Doses of BPA

In the first part of the study, Rubin's group examined the dose–response using four different doses of BPA and a control in mice. They also looked at two different exposure windows: a perinatal exposure window that went from day 8 of gestation through lactational day 16 and a perinatal plus peripubertal exposure window that took the perinatal exposure window and added an additional exposure after the animals were weaned, with the animals being given drinking water with BPA from postnatal day 20 to postnatal day 35. They looked at males and females separately, and they measured internal dose levels to understand what kinds of exposures that the animals were actually getting.

Three of the BPA doses were below the tolerable daily intake level of 50 micrograms per kilogram of body weight, and one was a bit higher but still well below the lowest observed adverse effect level of 50 milligrams per kilogram of body weight.

For male mice exposed perinatally to BPA, the researchers measured the percentage of body fat and that of lean tissue at 50, 90, and 130 days after birth. The control mice, which had not been exposed to BPA, had the lowest percentage of body fat and the highest percentage of lean tissue. With increasing doses of BPA—0.25, 2.5, and 25 micrograms per

kilogram of body weight—the mice had increasing percentages of body fat and decreasing percentages of lean tissue. But beyond that, the effect turned around, so that the mice given a dose of 25 micrograms per kilogram of body weight had the highest percentage of body fat and the lowest percentage of lean tissue, while mice given 250 micrograms per kilogram of body weight actually had less body fat and more lean tissue that all of the other mice except the controls.

Something very similar was seen in females exposed perinatally and peripubertally. The females exposed to 2.5 micrograms per kilogram of body weight had the largest amount of fat mass, while those given doses of 250 micrograms per kilogram of body weight had noticeably less fat mass, and, indeed, there was little difference between the control animals and the mice given 250 micrograms per kilogram of body weight. Looking at just the animals given 250 micrograms per kilogram of body weight, the difference was not noticeable, Rubin commented.

Early into the data collection for this part of the experiment, she said, it seemed that the animals exposed perinatally (the P group) and those exposed both perinatally and peripubertally (the P+P group) were developing in a very similar way. In both groups, the midrange exposures were leading to increased body fat. However, she said, as the animals got older, it became apparent that that second exposure peripubertally had exacerbated the adverse effects in the females but not in the males.

For example, at 40 weeks the P+P females were significantly more insulin resistant than those exposed just perinatally. Furthermore, data from serum assays suggested that the P+P females had more pronounced metabolic issues than the P females. In particular, the serum triglyceride levels were significantly higher in the P+P females than in the P females.

These data suggest that the peripubertal period may be another critical window for the effects of BPA exposure, at least in females. Although the effects during the peripubertal period have been poorly studied to date, Rubin said that her studies suggest that it is an important time during which BPA has an influence in females. Furthermore, she said, in a recent study by Kim and colleagues, a methylation analysis in 10- to 13-year-old girls suggested that BPA exposure during preadolescent development may affect the specific epigenomic modification of genes in pathways relevant to human health (Kim et al., 2013).

Summing up the results from the first part of her study, Rubin said that the key points were that BPA leads to changes in body weight and body composition in lab animals, that these changes depend on the sex of

the animal, and that although the P and the P+P groups at first appeared to be similarly affected, as the animals aged, both the body composition and the metabolic parameters of the P+P females were more affected. Furthermore, she added, internal BPA doses that were measured at the Centers for Disease Control and Prevention in collaboration with Antonio Calafat suggested that the doses of BPA used in the studies were environmentally relevant, that is, that they were within the range that has been observed in humans.

Part 2: Effects of BPA Combined with a High-Fat Diet

In the second part of the study, Rubin and her colleagues looked at the effects of early BPA exposure in mice combined with a high-fat diet (in which 45 percent of calories were from fat) in adulthood. They did not use the lowest or the highest dose from Part 1 and instead gave the animals either 2.5 or 25 micrograms per kilogram of body weight (and included controls that did not receive BPA). "The exposure window we chose was the perinatal window," she said. "It was most effective for the males, probably not the best choice for the females, but we had to make a decision."

They took littermates matched for body weight and body composition at 8 weeks of age. One male and one female from each litter were on the high-fat diet, and one male and one female from the same litter were placed on the normal chow diet for the remainder of the study.

For the male mice fed normal chow, the animals that were exposed to either dose of BPA were significantly heavier than the control animals. The same was true for fat mass: the exposed animals had more fat mass than the controls.

All the males on the high-fat diet weighed much more and had more fat than the ones on the usual chow. The BPA-exposed animals were heavier, but the difference was not as large as that for the chow diet. "We were really disappointed when we saw [these] data," Rubin said, "because we thought that the high-fat diet would really make them fat. But then again, when you take a look at how fat these animals already are, I am wondering if we have maxed out their capacity because they are up there. The mean weight here is 73 grams for a CD1 male. That is pretty big."

In the females, on the other hand, there did seem to be some synergy between the BPA exposure and the high-fat diet, and the females given the dose of 25 micrograms per kilogram of body weight and fed the high-

fat diet were significantly fatter than unexposed females on a high-fat diet.

When her team looked at the efficiency with which the animals gained weight, they found that, for a normal chow diet, the males exposed to BPA gained more weight by eating the same amount of food.

Although there was no synergy between BPA exposure and a high-fat diet in males in terms of weight gain, the researchers did see such an effect when they looked for evidence of altered glucose homeostasis. In particular, the high-fat diet appeared to exacerbate the effects of BPA on glucose homeostasis. Furthermore, perinatal exposure to BPA in the male mice resulted in increased fasting insulin levels in the chow-fed animals when measured at 13 to 15 weeks of age and at 22 weeks of age. The glucose levels that they measured were similar across the various groups, but the amount of insulin that it took to maintain these levels was a lot higher in the BPA-exposed mice than in the controls.

In the animals fed a high-fat diet, the glucose levels were, again, similar across the three groups at 13 to 15 weeks of age, but the exposed animals required higher levels of insulin to maintain their glucose levels. And by 22 weeks of age, the glucose levels in the exposed animals fed a high-fat diet were no longer being maintained and they were developing severe hyperglycemia. The hyperglycemia was strikingly bad, Rubin said, and many of the animals became diabetic.

The BPA-exposed animals also had increased inflammation in their adipose tissue, which was exacerbated by the high-fat diet. The livers of the exposed animals had higher lipid levels. There was also increased expression of lipogenic and adipogenic genes in the livers of the BPA-exposed animals as well as very significant increases in the levels of expression of cholesterol-synthesizing genes.

All these effects of BPA on body weight, body composition, and elements of metabolic disease differed by BPA dose, sex, exposure window, and diet, Rubin said.

Future Work

Since she and her colleagues began this work, Rubin said, there has been a growing body of data from studies with mice and rats that corroborate their findings that early BPA exposure may act as an obesogen, may alter body composition and glucose homeostasis, and may affect liver function. But, she said, many important questions remain.

Probably the most important question is what happens with humans. Epidemiological studies have found associations between levels of BPA and various parameters of metabolic disease in adults. In children and teens there is a correlation between BPA levels and body weight. A correlation between maternal BPA levels and children's BMI at 4 years of age has been reported. And a recent report has described an association between early BPA exposure and increased leptin levels in 9-year-old boys. Still, few clear conclusions can be drawn from the currently available data.

It is probably too early to understand all of the potential consequences of prenatal or perinatal exposure to BPA in humans, Rubin said. Those studies are being done now, and it will take a while to see effects, particularly if the changes are occurring in adulthood, because that will require waiting a long time between exposure and the appearance of effects.

Another important question, she said, is what are the mechanisms through which BPA has these various effects in animals? BPA is known to have estrogenic actions, and other estrogen-related chemicals, such as diethylstilbestrol, are known to increase body weight when given perinatally. BPA can directly affect adipose tissue and the pancreas and liver. It has effects on various endocrine components, including the thyroid and adrenal glands, and these are very important in weight regulation. The circuits that control food intake and metabolism are developing during the period in which the laboratory animals were exposed in the studies. "We really want to take a look at this," Rubin said, "not so much for food intake, but for metabolism."

BPA has also been observed to cause epigenetic changes. It is an open question whether BPA affects the microbiome, but the animals in the studies were exposed during periods that are very important for microbiome development. Preliminary data from a pilot study suggest that good bacteria are decreased in 5-month-old female animals, and the bacteria seen in intestinal pathologies are increased, but Rubin and colleagues would like to explore that finding further.

Finally, she said, BPA seems to alter metabolic pathways. Rubin concluded by saying that she and her colleagues are working to determine exactly what kinds of changes are occurring in metabolic pathways that influence body weight.

Discussion

During the short question-and-answer period that followed Rubin's presentation, Melissa Perry of the Milken Institute School of Public Health at George Washington University asked for details about the effects of the different doses. Rubin responded that she had initially expected the 250-microgram-per-kilogram dose to give better effects than the 25-microgram-per-kilogram dose, but that was not the case at all. "It is very clear that the low doses of BPA are the effective ones here," she said. "Once you get across a certain border, it no longer has the effect, which may explain why there is controversy in the literature. I think it really depends on strain, dose, time of exposure, and lots of different factors that can all enter into this picture."

She added that her team had tried to maintain stable levels of BPA in their animals and eventually used osmotic minipumps to accomplish that.

Liza Makowski from the University of North Carolina at Chapel Hill asked about the effects of BPA on macrophages and whether that seemed to be a direct effect of the BPA on the macrophages themselves or if it was an indirect effect through adipocytes. Rubin said that based on the observations from her studies, she believes that the BPA makes the adipose tissue dysfunctional. The adipocytes in the exposed animals become much larger than those in control animals because they pack in a lot of lipids, and eventually they just give out. "I believe what we are looking at is the macrophages coming in to clean up the mess," she said.

EFFECTS OF ENVIRONMENTAL CHEMICALS ON ENERGY METABOLISM AND INSULIN SECRETION

The final speaker in this session was Barbara Corkey, the Zoltan Kohn Professor of Medicine and Biochemistry at the Boston University School of Medicine.

Corkey began by saying that her research is rooted in a specific problem: that there is neither a cure for obesity or diabetes nor an understanding of the molecular basis for these diseases. The majority of people in the field focus on insulin resistance as the primary pathology in the development of diabetes, she said, but she and her colleagues started to question that because it was not leading to answers. Perhaps, she said, that focus is wrong.

Instead, she suggested, hyperinsulinemia rather than insulin resistance might be the problem—in other words, a defect at the level of the beta cell. And if that is the case, then it is a natural question to ask whether there are

changes in our environment that affect basal insulin secretion, although, as Corkey pointed out, almost no studies have been done to examine what regulates basal insulin secretion.

Is Hyperinsulinemia the Problem?

In exploring whether hyperinsulinemia might underlie diabetes, Corkey began by describing data from studies of patients who undergo bariatric surgery. One of the things that the data showed is that subjects with severe obesity and type 2 diabetes had basal fasting insulin secretion levels that were nine times greater than those of lean subjects (Pories et al., 1992).

To see what would happen if insulin levels were artificially raised, researchers implanted insulin pumps in rats for a 10-day period and afterward gave the rats a glucose tolerance test. It showed that the higher insulin levels were associated with impaired glucose tolerance—just the opposite of what one might expect according to the usual understanding (Juan et al., 1999).

In a test with human subjects who were put on a weight loss regimen, half were also given diazoxide, which is an inhibitor of insulin secretion. The weight loss during the diet was much greater when insulin secretion was inhibited (Alemzadeh et al., 1998). Again, Corkey said, this is not what one would expect.

In reality, Corkey said, whenever either insulin resistance or hypersecretion is present, so is the other, but if one wishes to find a solution to diabetes, it is important to know which comes first. So, she said, she and her colleagues began to look at a model in which beta cell hypersecretion was the problem. This led to hyperinsulinemia, which in turn led to obesity, diabetes, and insulin resistance.

"That is an okay hypothesis," she said, "because what we know is that obesity, diabetes, and elevated fat all cause hypersecretion and insulin resistance. As far as we know, they all occur together. Any one of these could be primary."

It is actually not a surprise that insulin infusion should cause insulin resistance, she said. It has been known for a long time that insulin downregulates its receptor. Inhibition of the secretion of insulin improves insulin resistance and increases weight loss, and under all of these different manipulations of insulin, which is supposed to be controlling glucose, normal glucose levels are maintained.

"That got us to think even further," she said, "that maybe insulin resistance is beneficial." It may be an adaptive response aimed at maintaining normal levels of glucose in the presence of high levels of insulin, and so improving insulin sensitivity, without doing something about the hypersecretion, might lead to the problem of hypoglycemia.

Possible Causes of Hyperinsulinemia

If hyperinsulinemia is the problem, Corkey asked, what might cause insulin secretion in the absence of stimulatory fuel? One possibility was suggested by an experiment in which she and her colleagues incubated rat islets in the presence of fatty acid and compared their response to glucose to the response of cells incubated without the fatty acids present. What they found was that the islets incubated with fatty acid ramped up their insulin response much faster so that their half-maximal response occurred at much lower levels of glucose. There occurred hypersecretion where normally none would occur, Corkey said. Work done by one of her students has shown a similar effect when incubating cells are exposed to high levels of glucose: the insulin response ramps up faster than normal. And in cells incubated with both high levels of fat and high levels of glucose, the ramp-up is even faster.

Could something in the modern human diet be having a similar effect on human islet cells? Certainly, the modern diet has high fat and high sugar contents, Corkey noted, but there are many other aspects to be considered. "There are processed foods. There are thousands of new agents. The interesting thing, which I am sure everyone in this room is clearly aware of, is that almost none of these have been evaluated as [a] potential cause of metabolic disease."

To discover what environmental agents might be causing beta cell hypersecretion of insulin, which would then lead to hyperinsulinemia, obesity, diabetes, and insulin resistance, Corkey and her colleagues began to do high-throughput screening of a variety of agents. "It did not last very long because the hit rate was so high," she said.

To offer some examples, she described the first three chemicals that her screening identified to lead to hypersecretion. The first was mono-acylglycerides, which are commonly added to food products as emulsifiers and preservatives. Many dairy products contain them, for instance. They are very effective in elevating insulin secretion without any stimulatory glucose, she said.

The second group they looked at was artificial sweeteners. Tests showed that sucralose, aspartame, and saccharin all increase basal insulin secretion in the absence of stimulatory glucose.

Then they looked at iron. There are many reports in the literature that people with elevated levels of iron are more prone to diabetes, Corkey said, and the modern diet has more iron than historic diets. "Our red meats have more iron than they ever did before because our food animals have been modified so that they have more lean, which means they have more iron," she said. Again, her team found that iron induced insulin stimulation and more iron stimulated more insulin. It also had an effect in the presence of glucose, causing the production of much more insulin than normal.

So what are these various agents doing to stimulate insulin secretion? Corkey and her colleagues examined various known molecular steps involved in glucose-induced insulin secretion and did not find any that were triggered by these various agents. What they did find, however, was that both monoglycerides and glucose lead to a change in redox state in rat islets. The fact that glucose causes this change in redox state in islet cells was well known, but they discovered that monoglycerides had almost exactly the same effect.

The apparent mechanism is that the monoglycerides are causing the generation of reactive oxygen species (ROS) in the islet cells. Experiments showed that both iron and saccharin similarly led to the production of ROS. Another experiment showed that the addition of ROS scavengers, which removed the ROS, prevented monoglycerides from triggering insulin secretion. Thus, Corkey said, it seems that the agents that they were studying trigger insulin secretion through changes in the redox state and the production of ROS in the islets. This insulin production can take place in the absence of glucose or any other of the usual fuels that signal the islets to produce insulin.

Effects on Other Systems in the Body

There is no reason to believe that the agents that increase the level of ROS in the islets do not have effects elsewhere in the body, Corkey noted. After all, they are transported around the body through the circulatory system and thus interact with all of the body's organs. Therefore, she and her colleagues asked whether such agents might have effects elsewhere in the body.

In experiments with hepatocytes, she and her colleagues varied the redox state outside the cells and found that as it went to a more oxidized condition, there was an increase in ROS production inside the cells. "This implies—it does not prove—that changes in the circulating redox state can be communicated to the inside of a cell," Corkey said.

Furthermore, there is significant evidence that changes in redox state and ROS can alter function. In has been shown in hepatocytes, for instance, that decreases in the redox state increase ROS and inhibit hepatic glucose production, adipocyte lipolysis, and triglyceride synthesis. They have also shown that changes in the redox state alter function in fat cells and, as described above, beta cells.

The bottom line, she said, "is that there is a circulating system that is a reflection of the metabolic state that informs all the cells in the body of what this situation is." Although she has not yet done the experiments, she said that she expects that when she tests toxic or obesogenic agents in the same way, they will change the parameters in the bloodstream, which will then have an effect on all the various cells that are sensitive to such changes.

Summing up, Corkey said that the current hypothesis that eating too much and exercising too little causes obesity has not worked very well in the sense that it has not led to successful solutions to the current obesity epidemic. "I think we forgot the most important variable," she said, "which is involuntary control of energy metabolism." Extreme examples of such involuntary control of metabolism include hibernating mammals, which decrease their energy expenditure fourfold but not volitionally, and migrating birds, which have a sevenfold increase in their energy expenditure.

In the well-known Vermont prisoner study, lean subjects worked to increase their body weight (Salans et al., 1971). Although it was not the point of the study, an interesting finding was that the prisoners required between 6,000 and 8,000 calories per day to gain 20 percent excess weight, Corkey mentioned. The prisoners required so many more calories because they were wasting a lot of energy. On the other hand, dieters are known to decrease their energy expenditure quite dramatically so that they do not require as many calories to maintain their weight.

It is possible to show the same sort of energy variation in cells. In one experiment, Corkey said, she and her colleagues were able to measure the "leak" in beta cells—the oxygen that was being consumed but that was not used to make adenosine triphosphate (ATP), the body's main source of energy. By adding a combination of oleate and palmitate

to the cells, they were able to double the leak. Similarly, the provision of excess fuel to a variety of types of cells has been shown to cause the cells to become less efficient and to waste energy. In short, it is possible to vary cells' energy efficiency by various means.

"Our current hypothesis," Corkey said, "is that ROS can control both energy efficiency and respiration" and that it is the dysregulation of energy efficiency rather than overeating that causes obesity. "We should at least consider it."

DISCUSSION

After the presentations, there was a wide-ranging discussion involving all of the panelists, the Roundtable members, and the members of the audience, both those who were physically at the workshop and those who were attending via the webcast.

Barbara Corkey began the discussion by asking her fellow panelists if they had heard any data that contradicted their own data or if there seemed to be relative consistency. Beverly Rubin responded that she was struck by how all of the presenters had similarities in their data. Robert Barouki said that one problem is that the body has many targets for pollutants, including the estrogen receptors, adipocytes, the pancreas, and others. What is not clear, he said, is whether there really are so many different targets or whether there are just a few targets that are influencing various things in the body so that, depending on the experimental setting, some researchers see one thing and others see another. "In Tox21," he said, "you have so many pathways that you are testing. And sometimes it does not fit with the biological experiment that you do afterwards. The question is, Are we really looking at all the relevant pathways? How many are still lacking?"

Corkey replied that it would be useful to get input from a systems biologist. One major problem, she said, is that single signals can affect many different systems. She said that she expects that obesogens generate some general signaling molecules that affect many different types of cells. "These are not simple one target diseases," she said. "There have been many targets identified. None of them has worked."

Xiaoyan Pang from the University of Illinois suggested that there is a good reason why a single environmental agent circulating through the body would affect different tissues or cell types differently. The various epigenomes among individuals may explain different tissue responses, Pang said.

"I don't do epigenetics," Corkey responded, "but it certainly makes a lot of sense to me that that is yet another factor that has to play in. It is sort of in the same category as the microbiome." In all of these things, she said, it is important to determine whether the differences that are observed are a cause or a consequence, but researchers generally do not spend a lot of time on that.

A webcast audience member asked Scott Auerbach if there were ever any pathways not picked up by the chemical enrichment analysis that he had expected would be picked up, such as pathways identified in the expert analysis. Auerbach said that there were. "One of the things about the chemical enrichment analysis or the sentinel chemical analysis is that it goes after one specific mechanism typically because you pick a prototype chemical," he said. "I picked rosiglitazone, which is a well-known PPARγ activator, … whereas with the ToxPi approach, we take into account all the assays that the experts have recommended. These assays are diverse. You get a different set of chemicals because you are looking at a diversity of assays as opposed to using just correlation and biological activity with one chemical with one very specific or a pattern of activity."

Nik Dhurandhar of Texas Tech University asked the presenters if they had any suggestions for how to reduce the contribution of these various chemicals to obesity. Bruce Blumberg suggested prevention: "Avoid them as much as you are able." Barouki said that because it appears that inflammation may play a role, anti-inflammatory agents might be useful, but ultimately, nothing equals prevention in terms of public health issues. Corkey said that the biggest challenge is how to communicate these findings and provoke the appropriate changes in policy.

Henry Anderson of the Wisconsin Division of Public Health asked about the nature of obesity as a disease. Is it, for instance, really a collection of different diseases?

Barouki said that clinicians clearly see it as different diseases. Some obese individuals, for example, do not have metabolic issues, while others, even those with a lower BMI, do have metabolic problems.

Frank Biro of the University of Cincinnati mentioned a paper published in the *New England Journal of Medicine* that examined adiponectin levels in a group of young boys in India. Those who had higher levels of adiponectin were far less likely to develop insulin resistance and type 2 diabetes even if they attained higher BMIs.

A workshop participant noted that most of the discussion about the factors leading to obesity had ignored behavioral issues. Are there environmental chemicals that have a direct effect on the brain?

Auerbach commented that when he searched through the side effects of various pharmaceutical drugs to look for drugs known to cause weight gain and obesity, he found that 90 percent of such drugs were neuroactive substances. So, yes, some environmental chemicals are likely to have direct effects on the brain, but it is harder to study such effects, he said, because it is difficult to characterize behavior, he said.

Beverly Rubin said that, having been trained as a neuroendocrinologist, she thinks about the potential effects of chemicals (particularly endocrine-disrupting chemicals) on the developing brain. She is convinced, she said, that BPA is having effects because the developing brain is very sensitive to it. "We have seen reports of changes in the hippocampus, changes in cortical development, and changes in neurotransmitter development," she said, "and, actually, in the metabolic fingerprinting we have been doing on our animals, we do see at postnatal day 2 differences in neurotransmitters in the brain." In short, she said that the brain is likely to be one of the major targets of environmental chemicals. The problem will be to determine exactly what those chemicals are doing to the brain. "These are very complex pathways that still aren't completely worked out," she said. "If they were, the drug companies would be doing really well at getting us a drug that works for weight loss."

Corkey asked the other presenters if anyone has looked to see if any of the obesogens act on the parts of the brain that play a role in appetite or in satiety.

Jerry Heindel of NIEHS responded that BPA exposure during development increases food intake and that this has been correlated with increases in the number of appetite neurons and decreases in the number of satiety neurons. He also commented that one place where people are not looking is the hedonic pathway, the part of the brain that is involved with food cravings. "A lot of people think overweight is emotional eating," he said. "It is like a food addiction. There [are] a lot of data on addiction and changes in dopamine receptors in the brain and all of that. I think that is a whole field that people doing obesogen research need to get up to speed on and to look for effects in those areas."

Rubin said that there are some data suggesting that early exposure to BPA does change dopamine levels in the brain. This would also fit with the reports of hyperactivity, she said, because that could also be caused by changes in the dopaminergic system.

Dhurandhar asked the speakers if any of their research had found any of the chemicals to cross the blood–brain barrier. Rubin replied that BPA, particularly during development, can easily cross the blood–brain barrier. Blumberg noted that at least some of the organotins are neurotoxins and thus must cross the blood–brain barrier. Corkey said that some of the monoglyceride-type compounds resemble 2-arachidonoylglycerol or the endocannabinoids and that those chemicals do enter the brain. Auerbach also said that small (that is, low-molecular-weight) molecules can easily pass the blood–brain barrier. The same is true of greasy molecules and amino acids. "Generally speaking," he explained, "those are the rules of the blood–brain barrier."

Al McGartland of EPA asked what it would take to get a dose–response curve for humans, rather than lab animals, that would apply to a subpopulation in the United States. This would be important information for setting policy.

Corkey responded that researchers have access to a large variety of human tissue and they could examine in detail the precise time course, dose–response, and targets within any given tissue. She believes that there is an increasing emphasis on the use of human or humanized tissues.

Blumberg said that he was part of a working group that was studying the cost of endocrine disrupters. The work was focused on the European Union because it had legislation on that topic coming up. "One of the surprising and sobering things for me," he said, "was despite the huge volume of data that we have, how little of it we could use for the exercise of trying to figure out what is the attributable fraction of disease burden due to chemicals. We need good longitudinal studies. We need a lot more biomonitoring than we have. Those two things would go a long way toward being able to link … laboratory animal studies with human outcomes."

An audience member offered some context to McGartland's question. EPA, the member noted, must try to quantify the health benefits that a regulation is expected to produce. This means getting numbers for such things as the number of cases of type 2 diabetes prevented or the number of strokes avoided. At this point in time, that sort of quantification is very challenging.

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Other Possible Contributors to Obesity

In addition to the wide array of environmental chemicals that people are exposed to in their daily lives, emerging evidence suggests that a number of other exposures may possibly increase an individual's chances of developing obesity or metabolic disease. These include sugar, artificial sweeteners, antibiotics, and viruses, and they were the subject of the workshop's fourth session.

OBESITY OF INFECTIOUS ORIGINS

Could obesity be triggered by certain types of infections? There is growing evidence that this may be the case. Nik Dhurandhar, professor and chair of the Department of Nutritional Sciences at Texas Tech University, discussed what is known about "infectobesity," a term he coined to describe obesity of infectious origin.

The Concept of Infectobesity

The question of whether obesity is an infectious disease, Dhurandhar said, has many similarities to a question that was asked many years ago: is gastric ulcer an infectious disease? At one time that seemed a ridiculous notion, and medical students were taught that the main causes of gastric ulcer were stress and spicy foods. Now, of course, it is widely recognized that ulcers are caused by the bacterium *Helicobacter pylori*. Obesity, too, may one day be recognized as—at least in some cases—the product of infection.

At present, Dhurandhar said, a number of pathogens have been shown to cause obesity in animal models. The first was reported in 1982 in *Science* magazine: canine distemper virus, which causes obesity in mice. A variety of other pathogens, including viruses, scrapie agents, bacteria, and even parasites, cause obesity in different animal models, he said.

Among these are several adenoviruses. Dhurandhar and colleagues were the first ones to describe the adipogenic—that is, fat-creating—properties of an adenovirus, in this case, the avian adenovirus SMAM-1, and they were also the first to describe the adipogenic properties of a human adenovirus, adenovirus type 36 (Ad36). Other research groups later discovered additional adipogenic adenoviruses, such as Ad37 and Ad5.

Dhurandhar said that for the purpose of his presentation he would be focusing on Ad36 to illustrate various attributes of infectobesity, mainly because that it is the microbe whose adipogenic properties have received the most study. Ad36 is one of more than 50 known human adenoviruses, and it is antigenically unique from the rest of them. It was first isolated in Germany in the late 1970s from a fecal sample from a girl who was suffering from diarrhea, a clue that the virus might be responsible for causing gastrointestinal disturbances.

Animal Models of Infectobesity

Dhurandhar and his colleagues have done a large number of experiments in which they infect animals with Ad36 and look for changes. Ad36 has been associated with a significantly greater prevalence of obesity in a large number of animal models, including chickens, mice, rats, and marmosets.

In one experiment with marmosets, for example, the infected animals gained fat about three times as much as the uninfected animals after 6 months. The infected animals also had significantly greater body fat at the end of those 6 months (Dhurandhar et al., 2002).

In an experiment done with mice on chow diets, Dhurandhar and his colleagues used three groups of mice: the uninfected controls; a group infected with Ad2, a nonadipogenic adenovirus used as a control for infection; and a group infected with Ad36. The group infected with Ad36 gained more body weight and more body fat, but what was perhaps even more interesting was the glucose levels of the three groups. The fasting glucose levels of the mice infected with Ad36 dropped steadily as they aged from birth to 12 weeks, whereas the levels stayed constant in the other two groups. The insulin levels also dropped in the mice infected with Ad36. The implication is that the Ad36-infected mice had much better glycemic control than the other two groups of mice.

Dhurandhar and colleagues did a similar experiment with the same three groups of mice but this time fed them high-fat diets. Such diets

usually increase the amount of fat stored in the liver and deteriorate glycemic control. The results showed that the animals infected with Ad36 seemed to be somewhat protected in terms of how much fat accumulated in the liver and had better glycemic control. A great deal of fat accumulated in the liver tissue of the control mice and the Ad2-infected mice, while the Ad36-infected mice had much less fat accumulation; indeed, the levels of fat in the livers of Ad36-infected mice fed the high-fat diet were much closer to the fat levels seen in control mice fed the standard chow diet, which have very little fat in their livers (Krishnapuram et al., 2011).

A group led by Jae-Hwan Nam of South Korea found that Ad36 requires the presence of the cytokine monocyte chemotactic protein 1 (MCP-1) to produce its adipogenic effect in mice. In particular, they showed that knockout mice missing the gene for the manufacture of MCP-1 do not get obese when infected with Ad36 (Na and Nam, 2012).

In another experiment, Nam and colleagues showed that an anti-inflammatory agent, mulberry extract, attenuates Ad36-induced obesity. And in yet another experiment they showed that vaccination of mice against Ad36 prevented the development of obesity after they were infected with Ad36.

In general, Dhurandhar said, animals infected with Ad36 have no overt symptoms other than the obesity; there is no additional mortality, for example. Ad36-induced obesity can also be passed from one animal to another through the normal infection routes—both through direct contact with an infected animal or by injection of the blood of an infected animal into the veins of an uninfected animal. The animals that are infected with Ad36 through direct contact or injection of blood get obese as they age. In short, obesity can be transmitted from one animal to another like an infection. This fulfills a Koch's postulate, which is used to determine the infectious nature of a disease.

Finally, Dhurandhar said, Ad36 infection does not have much effect on food intake, so it seems unlikely that the infection produces obesity through an increase in caloric intake. Dhurandhar said that he and his colleagues have not made objective measurements of the activity of Ad36-infected animals, but they are kept in cages, and no obvious differences in their activity levels have been observed.

Mechanism of Action

A large number of studies have examined the possible mechanisms of action for viral infections leading to obesity, Dhurandhar said, and he summarized their results in this way: it appears that the virus-induced expansion of adipose tissue is due to an increased proliferation, commitment, and differentiation of adipose tissue-derived stem cells as well as lipid accumulation in adipocytes.

This effect of Ad36 on lipid accumulation is dose dependent, Dhurandhar said. The greater that the viral load is, the more lipids accumulate—to a point. At a certain viral load, the effect levels off. Furthermore, an antiviral drug, cidofovir, that kills or blocks Ad36 reduces the level of lipid accumulation in the cells.

The lipid accumulation in adipocytes is very specific to the presence of the infection. Cells that have Ad36 accumulate lipids, while those that are free of the virus do not.

Dhurandhar then described a working model, in terms of cell signaling, of what the virus appears to be doing. On the one hand, it seems to increase the cyclic adenosine monophosphate (cAMP) pathway by acting on the cAMP-responsive element-binding protein (CREBP) pathway. On the other hand, the Akt pathway is activated. The combined effect is to increase the proliferation of adipocyte progenitors, leading to their differentiation and to lipid accumulation.

One interesting finding, Dhurandhar said, is that Ad36 increases glucose uptake by cells. This can be seen in experiments with adipose tissue biopsy specimens from humans. If the tissue is divided and cultured in two sections and if one of those two sections is infected with Ad36, the infected tissue shows significantly greater glucose uptake than the noninfected tissue. There is a similar effect in skeletal muscle tissue. Thus, Dhurandhar said, Ad36 appears to increase glucose uptake by both adipose tissue and human skeletal muscle cells.

Thus, the working model looks like this, Dhurandhar said: Ad36 infects adipocyte progenitors and increases their commitment and differentiation, resulting in increased adipogenesis; the virus also increases glucose uptake inside the cell (see Figure 5-1). In summary, he said, there are more adipocytes that have more lipids, and they are taking in more glucose. That may explain the increased adiposity that is also accompanied by enhanced glucose clearance from the systemic circulation.

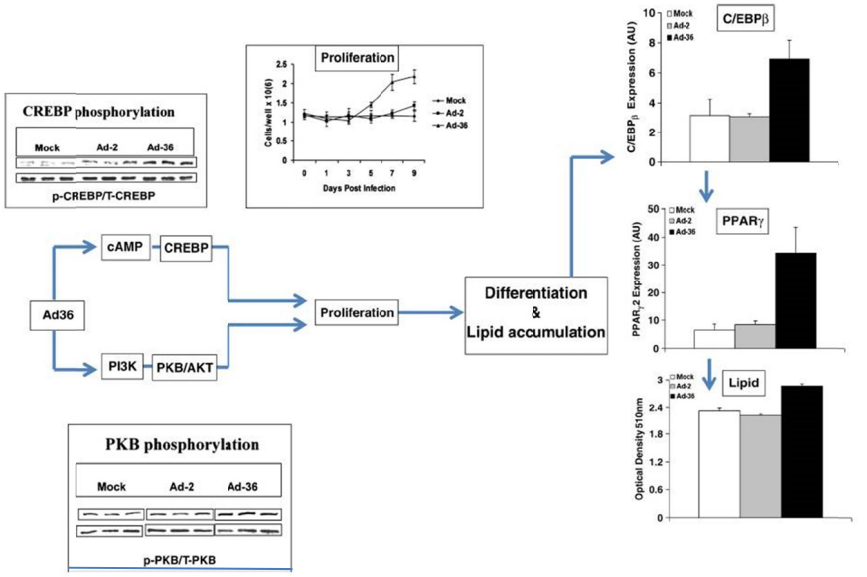

FIGURE 5-1 Working model of how Ad36 triggers lipid accumulation.
NOTE: AU = arbitrary units; C/EBPβ = CCAAT/enhancer-binding protein β; PI3K = phosphatidylinositol 3-kinase; PKB = protein kinase B; p-PKB = phosphorylated protein kinase B; PPARγ = peroxisome proliferator-activated receptor γ; T-PKB = tyrosine-phosphorylated protein kinase B.
SOURCES: Dhurandhar presentation to workshop, March 3, 2015. From Dhurandhar, 2012.

Human Studies

Dhurandhar then moved to a discussion of human studies. "The million dollar question," he said, "is, Do certain infections cause human obesity?"

There are a number of challenges to doing human studies, he said. One is that obesity has an insidious onset. An obese person may not remember what happened 3 or 5 years ago to trigger the obesity, for example, whether there was an infection around the time of the onset.

A second challenge is the presence of multiple etiological factors for obesity, such that it is very difficult to attribute obesity to any one factor. If you perform a study comparing people who have had a certain obesity-related infection with those who have not, the people in the control group with no history of the infection may still have become obese for some other reason.

A third challenge is that the infection by itself may not be enough to trigger obesity. Other factors may be needed. Thus, some people who get the infection become obese and others do not, and it may be difficult to determine what is going on.

Finally, ethical considerations preclude infecting people for an experiment, so, unlike in animal studies, it may be impossible to ever show a direct cause-and-effect relationship between an infection and obesity in humans. Dhurandhar compared the situation with that with smoking and lung cancer. There has been no direct experimental proof that smoking causes lung cancer in humans, so the proof relies upon strong indirect evidence of the connection.

So, Dhurandhar said, he and his colleagues have set out to uncover similar indirect evidence to answer the question of whether infections cause obesity. They began by looking for people whose blood held neutralizing antibodies against Ad36, which indicated that they had been exposed to the virus at some time in the past. In one study with more than 500 subjects, they found that 30 percent of the obese subjects had Ad36 antibodies, while only 11 percent of the nonobese subjects did. Furthermore, the subjects in the study who had antibodies to Ad36 had a significantly greater body mass index (BMI) than the subjects who did not have antibodies. Even within the nonobese group, those with the Ad36 antibodies were likely to be heavier than those who were negative for the antibodies. As a control, the researchers also looked at antibodies against two viruses that have not been associated with obesity, Ad2 and Ad31. For those viruses, there was no such relationship between the presence of antibodies and BMI or obesity.

Dhurandhar's group also carried out a human twin study looking at the relationship between the presence of antibodies and obesity (Atkinson et al., 2005). They recruited 90 twin pairs and tested their blood for the presence of the antibodies. They retained only those twin pairs in which one twin was positive for the antibodies and the other was negative. This left them with 26 pairs of twins, 20 of which were identical twins and the other 6 of which were fraternal twins. Twins usually have very similar BMIs, but among these 26 pairs of twins, the twins who were antibody positive had significantly higher BMIs than those who had not been exposed to Ad36. The implication is clear: infection with Ad36 may make it more likely that a person will gain weight and become obese. "I think this is the closest one can come to determining the role of Ad36 in human obesity, short of infecting them," Dhurandhar said.

Another study examined 1,500 Caucasian, Hispanic, and African-American men, women, and children who were screened for the presence of Ad36 (Krishnapuram et al., 2011). Analysis of the data showed that a previous Ad36 infection was associated with better glycemic control and lower hepatic lipid levels. This result is very similar, Dhurandhar noted, to the results of the experiments in mice that were infected with Ad36.

A 10-year prospective study of 1,400 men and women found similar results. The subjects were screened for exposure to Ad36 at the beginning of the study and followed for 10 years. Those who were positive for Ad36 antibodies at the beginning had a significantly greater increase in body fat over the next 10 years than those who did not have the Ad36 antibodies, and they exhibited significantly less decline in their glucose control than their antibody-negative counterparts. Compared with the findings of cross-sectional studies, this prospective study provides stronger evidence about the possible role of Ad36 in increasing fat.

There is evidence that, at least in some countries, the prevalence of Ad36 infections is rising. Dhurandhar showed some data from Sweden tracking the rates of Ad36 infections in lean Swedes over time, and the data showed that the prevalence increased from about 7 percent in the mid-1990s to nearly 20 percent in 2009. During that same period, Dhurandhar noted, the prevalence of obesity increased in Sweden in both men and women.

Summarizing the data, Dhurandhar said meta-analyses have found that most—although not all—of the studies report an association between exposure to Ad36 and an increased risk of obesity.

Significance and Implications

In closing, Dhurandhar spoke of the significance and implications of what is known about the connection between infections and obesity. Obesity is a complex disease with a multifactorial etiology, he said, and given that multifactorial etiology, it will be necessary to employ a multifactorial treatment and prevention approach. In particular, infection-related obesity will likely have its own unique prevention and treatment strategies, and it may well be the case that the development of a vaccine against Ad36 will be easier than dealing with obesity by inducing widespread behavioral change.

He cautioned that he did not mean to suggest that all obesity is due to infection. It is clear that infection with Ad36 causes obesity in animals

and is correlated with human obesity, and there may be many adipogenic pathogens that have yet to be discovered. The important question, he said, is how much have infections contributed to the increase in obesity since 1980? "I do not know the answer to that question," he said, "but that is a question to ask."

Finally, Dhurandhar showed a series of PowerPoint slides with data from the Centers for Disease Control and Prevention on the geographical prevalence of asthma, influenza, and obesity. Looking at the changes in the prevalence of asthma over time, it is clear that the pattern is random, which makes sense because asthma is a noninfectious disease. However, looking at the changes in the geographical prevalence of influenza over time, clear patterns seem to show focal points and the spread from state to state. Again, this makes sense because influenza is an infectious disease, and one would expect to see such changing geographical patterns over time. Finally, he showed a series of slides showing the prevalence of obesity over time and noted that the pattern was very similar to what was seen with influenza but not similar to that seen with asthma.

"I want to leave you all with this question," he said. "Why does the spread of obesity in the United States resemble an infectious disease?… I truly do not know the answer to that question. To me, it does look like an infectious disease spreading through the United States."

Discussion

Lynn Goldman of the Milken Institute of Public Health at George Washington University opened the discussion session with a question about the modes of transmission of Ad36. Is there, for example, maternal–fetal transmission? That is, are some people born with the virus? She also asked what the disease looks like in its acute stage. Are people acutely ill when they have this infection?

Dhurandhar answered that no specific information about what Ad36 does in people is available. In general, there are several categories of adenovirus infections. Some of them are linked with upper respiratory tract infections, some are linked with conjunctivitis, and some are linked with gastrointestinal disturbances. Because it was first isolated from a girl suffering from enteritis, it may be one of the classes of adenoviruses that cause gastrointestinal disturbances, but nothing is known for sure, and it does not produce diarrhea or any other major symptoms in animals.

As for the issue of mother-to-child transmission, the answer is not known. Dhurandhar said he would like to do that experiment and wrote a proposal requesting a grant, but it did not get funded.

Linda Birnbaum of the National Institutes of Environmental Health Sciences (NIEHS) asked if it would be worthwhile to do studies looking at what happens when people are exposed both to infectious agents and to environmental chemicals, because it is known that environmental exposures can alter susceptibility to infectious agents. Dhurandhar said that this would be a good research question. Indeed, some studies have shown that it is only when a virus acts in collaboration with various other factors that the virus expresses its phenotype.

ANTIBIOTICS AND OBESITY

Several studies have suggested the possibility that the use of antibiotics during infancy may increase a child's chances of later developing obesity. For example, experiments with mice have shown that if pregnant mice are given antibiotics at about the time of birth, the pups are more likely to grow into obese adults. Charles Bailey, an assistant professor of clinical pediatrics at the University of Pennsylvania and the lead investigator for the Data Coordinating Center of PEDSnet, a collaboration among several pediatric academic centers working to provide a standardized model for clinical care, provided an overview of what is known about the potential connection between antibiotic use and obesity in children. He spoke by phone.

The Role of the Microbiome

How might the use of antibiotics increase the chances of developing obesity? The most likely explanation, Bailey said, is that the antibiotics would differentially affect gut microbes. Several studies have suggested that a decreased diversity of gut microbes and, in particular, a shift to a small number of higher-risk species is associated with a higher prevalence of obesity. The mechanisms behind this are unclear, he said. One possibility is that the gut microflora has an effect on energy metabolism and how a person's body uses calories. Yet another possibility is that changes in the microflora result in changes in a person's digestive patterns, which in turn cause changes in food-seeking or calorie-consuming behavior. It is also possible, Bailey said, that antibiotics affect a person's immune responses and, in particular, lead to the creation of chronic low-level inflammatory stimuli, which could in turn

predispose a person to the development of obesity via such mechanisms as increased endogenous steroid release and insulin resistance.

In his presentation, Bailey focused on the possibility that infancy and early toddlerhood is a critical period for the establishment and stabilization of the gut microbiome, making this a particularly sensitive period for the use of antibiotics. Studies with older children and adults have shown a tendency for a sort of "microbiomic homeostasis," that is, a tendency for the gut microbiome to return to its original composition if some outside influences disturb it. The question, then, is, When exactly is this preferred composition of the gut microbiome set?

The establishment of the microbiome begins at birth or even before, Bailey said. Parents clearly have some effect on a child's microbiome, because studies looking at the composition of the gut flora have shown that components of the microbiome in children are "inherited," in the sense that they are concordant between a child and the child's parents and are very similar between twins. Components of a child's microbiome also seem to reflect the environment rather than the parents. So it is possible that the first few years of life are a critical period in establishing the composition of the gut microflora and that a disturbance during that time—such as a disturbance caused by antibiotic use—could change the composition of the microbiome and affect a child's likelihood of becoming obese.

The only way to know for sure whether this happens is to examine the data, so Bailey reviewed a number of observational studies that have looked for a connection between antibiotic use in childhood and the development of obesity.

Earlier Studies

Before describing these studies, Bailey pointed out that the sort of population-level research that he was talking about depends on a large-scale collaboration among not just investigators but also patients. "In the era of electronic health records and health information exchanges," he said, "we have a new kind of substrate to look at the way health is played out in the delivery of normal clinical care that incorporates the work of lots of unnamed collaborators seeing patients every day and lots of patients who have entrusted us with their data."

Bailey began by describing two studies that laid the groundwork for the study of antibiotics and obesity. The first was a cohort study in the United Kingdom that followed more than 10,000 children from birth

through 9 years of age. The children in the cohort were mainly upper middle class and 93 percent Caucasian. Surveys and parental recall were used to estimate environmental exposures for the children, while growth parameters were measured directly and obesity rates could be estimated directly at several time points up through 7 years of age (Trasande et al., 2013).

A retrospective review of the data from this study found an increase in obesity at 3 years of age if antibiotics had been taken in the first 6 months after birth but not if they had been taken subsequently. The BMI among 7-year-old children in the study who had taken antibiotics between 15 and 23 months after birth was also a slightly higher. Thus, there was some connection between antibiotic use and extra weight or obesity, but it was not definitive (Trasande et al., 2013).

The second study followed a cohort of nearly 30,000 children in Denmark. It used parental recall to determine antibiotic usage and gathered growth data with a questionnaire administered at 7 years of age. Interestingly, the data showed a major effect of whether the mother was overweight. There was a 54 percent increase in the risk of being overweight among children whose mothers were not overweight and who had been given antibiotics in the first 6 months after birth; conversely, there was a 46 percent decrease in risk of being overweight among children whose mothers were overweight and who had been given antibiotics in the first 6 months after birth (Ajslev et al., 2011). This finding of a decrease in the risk of being overweight after antibiotic use is unique to this cohort, Bailey said. Still, both studies suggest that perturbing the microbiome that one inherits in part from one's parents may affect the long-term risk of obesity.

A recent study in Canada found an association between antibiotic exposure in infancy and a later risk of obesity risk, but only in boys. "Whatever the mechanism behind the correlation is," Bailey commented, "it is not going to be simple and straightforward."

He also noted that the designs of these studies—the use of parental recall for information about exposure to antibiotics and a reliance on cross-sectional measurements of growth parameters at discrete time points—have both advantages and disadvantages. They can get very good information about covariates, including lifestyle and environmental factors, for example, but they are limited in their ability to get detailed longitudinal information on the degree of antibiotic exposure and the growth trajectories of the children.

The Current PEDSnet Study

Bailey then shifted to discussing the current study that he is involved with, beginning with a description of the PEDSnet Collaborative Network. This is a data-sharing partnership of eight large children's hospitals that are committed to the development of learning health systems in pediatric clinical care. The network is interested in obesity because it is one of the more pressing public health problems among children, he said.

In 2010–2011, six of the eight hospitals in the network took part in a pilot study on the subject of the role of antibiotics in the development of obesity. The purpose of the study, which examined a 2-year block of outpatient data on about half a million children, was to see if routine clinical data could be effectively used to answer questions regarding obesity and the treatment of obesity.

The study focused on clinical data regarding BMI. Because pediatricians routinely record the heights, weights, and BMIs of their patients, if for no other reason than they are necessary to determine the doses of medications, the records provided a relatively rich source of data, Bailey said. "We have a lot of opportunity to offset noise in the data by looking at the behavior of the data in aggregate."

Checking their data against results from the National Health and Nutrition Examination Survey (NHANES), the researchers found that the two agreed very well. In particular, their estimates of the prevalence of obesity among the children in the pilot study sample matched the obesity rates from NHANES quite closely, which gave them confidence that it was reasonable to use data from the clinical records to measure obesity in populations of children receiving both well-child care and acute care.

The pilot study also validated the use of prescription data as a measure of prescription drug use among children seen by doctors and other health care workers in the network. The prescription data do have some gaps, Bailey acknowledged—such as prescriptions from outside the network and drugs prescribed but not used—so the data have a particular set of systemic biases. However, he noted, other studies have taken different approaches as a way to balance out those biases.

Bailey also explained that the use of the height, weight, and BMI data provides a view of the prevalence of obesity among children very different from that obtained by the use of such administrative data as claims data or data from state-level data sets. The reason is that, according to their data, physicians recorded a diagnosis of obesity in only

about 18 percent of their juvenile patients who met the criteria for obesity according to their BMI. Indeed, he said, except for physicians in dedicated weight management clinics, no one is documenting obesity diagnoses for more than about one-third of their patients who meet the BMI criteria for obesity. Thus, any studies that rely on administrative data will miss about 80 percent of children who are obese according to BMI criteria.

Having validated the approach of using clinical data to study obesity, the group of researchers then took a close look at whether different exposures to antibiotics among children could explain at least some of the children's long-term chances of developing obesity. To do that they examined 15 years of data from the records of children in one large health care system in the mid-Atlantic region of the United States. The records held information on both prescription medications and growth measurements, and for a majority of the children in the study, there were at least 8 or 9 years of records. This made it possible to assemble a longitudinal cohort.

The researchers looked only at children who had received primary care in the network within the first year after birth and who had also had a longitudinal follow-up in the network at least past their third birthday. Ultimately, after eliminating subjects for which there was not enough information, the researchers were left with a cohort of 65,000 children who reflected the structure of the network, with one-third of them living in urban neighborhoods in and around Philadelphia, Pennsylvania, and the remaining two-thirds living in suburban neighborhoods. Nearly half of them were identified as belonging to some racial or ethnic minority, and approximately 40 percent were covered by a public insurance provider at some point during the study period.

To assess antibiotic exposure, the researchers collected data on prescriptions for antibiotics that were written for the subjects before 2 years of age. Antibiotics were classified as either narrow spectrum or broad spectrum. The data showed that the vast majority of the antibiotic prescriptions were for common childhood infections, such as ear infections, pneumonia, or sinus infections.

To determine obesity rates, the researchers collected data on BMI for 3 years beginning at 24 months of age. Obesity was defined as being at or above the 95th percentile for BMI according to norms from the 2000 NHANES data, while the cutoff for overweight was the 85th percentile for BMI calculated from the NHANES data.

Among the study's preliminary observations was that the use of antibiotics is very common in early childhood, Bailey said. Roughly two-thirds of the children in the study had some antibiotics prescribed to them before their second birthday. Furthermore, a substantial number of the children were given antibiotics multiple times before they were 2 years old. These repeat exposures were typically for the treatment of common childhood infections rather than for the treatment of chronic medical conditions that might result in growth trajectories that were different from those of the majority of the children.

A multivariate analysis of the data found a number of factors to be correlated with an increased risk of obesity at ages 2, 3, and 4 years. Boys were more likely than girls to be obese. Hispanic ethnicity but not membership in other racial and ethnic groups was associated with increased obesity. Public insurance coverage—a rough indicator of a lower socioeconomic status—was also associated with an increased risk of obesity, although there was no difference in obesity rates between children in urban versus suburban practices.

There was a strong association between obesity and a diagnosis of asthma. Some of that was probably due to steroid usage, Bailey noted, which is itself an independent predictor of increased obesity risk. The fact that children with asthma are less likely to be active may also play a role, he said, as may the inflammation associated with asthma.

Taking all of those factors into account, the researchers then calculated the increased risk of obesity—the "hazard ratio"—associated with taking antibiotics at various ages. Not only were children exposed to antibiotics more likely to be obese than children who were not, but there was also a trend toward a greater risk of obesity as the number of different antibiotics that a child had been exposed to increased.

Bailey broke the analysis down by broad-spectrum versus narrow-spectrum antibiotics. There is a dramatic difference there, he noted. For the broad-spectrum antibiotics, there was a trend toward a greater risk of obesity with exposure to a greater number of antibiotics, but that trend was not apparent for the narrow-spectrum antibiotics.

The data point to two conclusions, Bailey said. The first is that exposure to antibiotics early in childhood—in particular, in infancy—is associated with a small but persistent increase in obesity later in childhood. The second is that the majority of this effect appears to be associated with broader-spectrum antibiotics. "This is a significant clinical point," he said. "There is substantial evidence that narrower-spectrum drugs are adequate therapy for most of the kinds of infections

that are being treated in this population. The decision to use broader-spectrum antibiotics will often hinge on physician preference or convenience of dosing or other nonantimicrobial parameters. It is important to underscore that there may be unintended effects at work here."

It remains to be seen exactly what is mediating the association between antibiotics and obesity risk, if indeed the antibiotics are playing a causal role. Because the obesity occurs well after the antibiotic use, the association cannot explained by temporary deflections in a child's growth trajectory or by perturbations in the gut microbiome at the time of exposure, he said. His group is particularly interested in the possibility that the use of antibiotics causes lasting changes in the composition of the microbiome that persist beyond infancy, and it is currently conducting a study looking at the direct effects of antibiotic exposures on the composition of gastrointestinal bacteria that may shed some light on the precise mechanism.

Bailey then offered a number of caveats concerning his study that are related to its design. For example, certain biases are caused by the fact that it relies on health care system data. Specifically, although there is information on diagnoses and antibiotic prescription, Bailey pointed out that there is much less information about longitudinal factors that affect the family, lifestyle, and diet. This is particularly important, he added, because the effect sizes that they are observing are relatively small, on the order of a 10 to 20 percent increase in the risk of obesity. In the future, he said, it will be important to combine this type of study with the sorts of birth cohort studies that he described earlier, which provided detailed insight into lifestyle factors. Another caveat is that it was a regional study, so there is the possibility that it reflects geographic or local practice effects.

In summing up, he said that it is clear that the problem of obesity will not be solved with a silver bullet and that instead it will require a large number of incremental changes, some of which will be useful to only certain subsets of patients. In particular, he said, it seems possible that the careful use of antibiotics—tailoring them to the appropriate indications and to the appropriate antimicrobial spectrum—could have an effect on the risk of obesity at the population level. Thus, it makes sense to do further research on such things as the establishment and maintenance of the gut microbiome and the determination of "obesity-friendly" health care practices.

Discussion

In the brief discussion period that followed his presentation, Bailey first addressed a question from Linda Birnbaum of NIEHS about whether it might also be worth examining the effects of antiviral medication use in children. Bailey answered that while this is worth looking into, the rate of use of antiviral medications in children is much, much lower than the rate of use of antibacterial medications, so he did not believe that it would be possible to see any possible effects of antivirals in a study population of the size that he had used. It might also be worthwhile, he said, to look at the effects of antifungal use in children. To his knowledge, he said, no one has tested the effects of antifungals on the gut microflora.

Sheela Sathyanarayana from the University of Washington mentioned the experiment that Bailey had described, in which antibiotics were given to mice during the perinatal period and led to obesity later on, as the pups grew. Has anyone looked at whether antibiotics given to human mothers in the human perinatal period have an effect on their children's risk of obesity? Bailey said he would love to see such a study and that it should be feasible in the right setting, but it has not been done.

SUGAR AND OBESITY

It is no surprise to anyone that sugar plays a role in obesity—after all, sugar supplies calories in an appealing and easy-to-eat form, and the consumption of too many calories steadily over an extended period of time leads to obesity. But does sugar—and, in particular, a certain type of sugar, namely, fructose—play a different and more direct role in this disease? That was the subject addressed by Ayca Erkin-Cakmak, a clinical research associate at the University of California, San Francisco, who has been investigating the effects of a reduced amount of sugar in the diet on the metabolic health of obese children.

She began by showing a couple of videos, one made in 1970 and one made much more recently, of doctors saying that it is calories, not sugar per se, that people have to be careful of—that as long as you keep the total calories low enough you do not gain weight and that sugar itself poses no risks. Erkin-Cakmak said that this view is too simplistic and that science has something else to say.

According to science, she said, some calories are more likely to cause disease than others. Different types of calories are metabolized differently, she explained. For example, a person who consumes a cup of

almonds with 160 calories absorbs only 130 calories because of the fiber in the almonds. In the case of proteins, the body is required to supply a certain amount of energy to use their amino acids for energy, so the net calorie gain is less than the number of calories in the protein. She also noted that although some fats are healthier than others, 1 gram of fat counts as 9 kilocalories no matter what type of fat. "So a calorie is not a calorie," she said.

Erkin-Cakmak then reminded the audience of the definition of toxicity. Toxicity refers to the degree that a substance can damage an organism. In that definition, there is no distinction between acute and chronic toxicity. If fructose is to be considered a toxic substance for the human body, it must pose a risk factor independent of the effects of its calories and independent of the obesity that those calories can cause. Furthermore, one must establish a causal relationship between the fructose and whatever negative consequences it is associated with.

One of the criticisms of the claim that fructose is toxic to humans is that the toxicity has been shown in animal models and not in humans and that it has been demonstrated at doses that are in excess of what humans normally ingest. But, Erkin-Cakmak said, she would be talking about human data and the doses routinely ingested by people.

The main problem with fructose is not obesity, she said. People do not die from obesity. Instead, people die from various components of the metabolic syndrome, particularly diabetes. Diabetes is a disease that has cost a fortune to treat and prevent, so that is the problem resulting from the ingestion of fructose that she has been investigating.

Thirty percent of the adult population in the United States is obese, Erkin-Cakmak noted, and 80 percent of the obese population is sick with various syndromes: type 2 diabetes, cardiovascular disease, hypertension, and hyperlipidemia. "They have all these components of metabolic syndrome because they exceeded their daily intake and they became fat," she said. Or is that really it? Forty percent of people in the United States who are of normal weight also suffer metabolic dysfunction, she said—again, type 2 diabetes, hyperlipidemia, and hypertension. The prevalence is less among those who are of normal weight than among those who are obese, but the metabolic dysfunction is still a problem. Almost half of the population of the United States is metabolically unhealthy, she said. "It looks like there is an exposure which affects the [entire] population."

What could that exposure be? Erkin-Cakmak showed a graph of U.S. sugar consumption from 1822 to 2005. That consumption grew steadily until the 1930s, when it was approximately stable until the 1970s, when

people became concerned about fats in their diets and replaced some of them with sugar, at which point sugar consumption began growing sharply again. The rise in sugar consumption is correlated with the appearance of various health issues, such as cardiovascular problems and type 2 diabetes. That does not prove causation, she noted, but there is clearly a relationship between the increasing amount of sugar consumed and the development of metabolic problems.

Whatever relationship there is between sugar consumption and diabetes is confounded by obesity, Erkin-Cakmak said, because obesity is strongly correlated both with sugar consumption and with diabetes. However, she continued, although there is a strong relationship between diabetes and obesity, the two conditions are not concordant. Some countries have a relatively high prevalence of obesity but a low prevalence of diabetes, while other countries have a high prevalence of diabetes but a relatively low prevalence of obesity. Thus, diabetes is not a subset of obesity, she concluded. Indeed, while the prevalence of obesity is increasing worldwide by 1 percent per year, the prevalence of diabetes is increasing at about 4 percent per year. If diabetes were a subset of obesity, they should be increasing at the same rate.

Plausibility of the Sugar–Diabetes Connection

How might fructose be leading to diabetes? One possible connection is through liver disease. Fatty liver disease can be caused by either alcohol or sugar, and histologically, the damage to the liver looks the same for the two types of fatty liver disease.

Nonalcoholic fatty liver disease has become an epidemic in the United States, Erkin-Cakmak said. Today, one-fourth of African Americans, one-third of Caucasian Americans, and almost half of Latinos have steatosis, or the abnormal retention of lipids within the cells of the liver, and 5.5 percent of the U.S. adult population is suffering from nonalcoholic fatty liver disease (Browning et al., 2004). Autopsies of children from the ages of 5 to 19 years who died from causes other than metabolic problems showed fatty liver disease in 13 percent of them, and the number jumps to 38 percent if the children were obese (Schwimmer et al., 2006).

There are three kinds of fat, Erkin-Cakmak noted: visceral fat, subcutaneous fat, and liver fat. While visceral fat is not good, liver fat is even worse and is associated with poor health. For example, a study done in South Korean adults found that nonalcoholic fatty liver disease was a

primary predictor of type 2 diabetes, after such factors as age, sex, BMI, and alcohol consumption were controlled for.

Another study tried to model changes in insulin dynamics as a function of either visceral fat or liver fat. In the models, when the researchers held the liver fat constant, they found that they could see no difference in insulin dynamics between those with low levels of visceral fat and those with high levels. However, when visceral fat was held constant, larger amounts of liver fat were correlated with greater insulin resistance. Thus, the liver fat seems to be more closely related to changes in insulin dynamics than visceral fat.

Mechanism of the Sugar–Diabetes Connection

An explanation of the mechanism behind the sugar–diabetes connection begins with the observation that fructose is not glucose, Erkin-Cakmak said. While glucose is essential for every single cell in the body, fructose is utilized by cells only if it is necessary. "The common wisdom is a calorie is a calorie and sugar is just empty calories," she said, "but chronic fructose exposure promotes liver fat accumulation." This in turn promotes the metabolic syndrome and also increases protein glycation, which promotes cellular and structural aging.

When a person consumes glucose, only 20 percent of that glucose enters the liver, while the rest is consumed by the body, particularly the muscles. Most of the glucose that enters the liver is stored as glycogen, while a very small amount of it enters the mitochondrial tricarboxylic acid (TCA) cycle and generates adenosine triphosphate (ATP). A tiny amount leaves the mitochondria and contributes to de novo lipogenesis; the newly synthesized lipids are transported as triglycerides out of the liver. Thus, glucose consumption does not add lipids to the liver cells.

Ethanol is quite different, with 80 percent of the ethanol consumed entering liver cells. The ethanol that enters the liver cells goes into their mitochondria and overruns the mitochondria, exceeds the TCA cycle's capacity, and then leaves the mitochondria via the citrate shuttle and contributes to de novo lipogenesis. The newly synthesized lipids leave the liver cells as triglycerides. This is why alcoholics suffer from hypertriglyceridemia, high blood levels of triglycerides. Some of the newly synthesized lipids, very low density lipoproteins, participate in the generation of liquid droplets that are stored in the liver, which results in alcoholic fatty liver disease.

In the case of fructose, 100 percent enters the liver cells. It is not used in glycogen synthesis. Instead, it overruns the mitochondria in the same way that ethanol does, with lipids stored in the liver and triglycerides being exported into the bloodstream. This is how alcohol and fructose are metabolized in the same way.

The results of this can be seen in a study by Jean-Marc Schwartz at San Francisco General Hospital. Normal-weight adults were given either a fructose-based diet or a complex carbohydrate diet, where the two diets had exactly the same number of calories. Those who had the fructose-based diet stored 27 percent more lipids in their livers than those who were on the complex carbohydrate diet. Erkin-Cakmak said that she and her colleagues had recently finished a very similar study in African-American and Latino obese adolescents and saw very similar results.

A second problem with fructose is the browning reaction, also known as the Maillard reaction or nonenzymatic glycation. This browning reaction generates reactive oxygen species from the fructose. In the human body, this glycation happens seven times faster for fructose than for glucose, producing reactive oxygen species seven times as fast.

Turning to human studies, Erkin-Cakmak showed results from the EPIC-Interact study in Europe that looked at the relationship between the consumption of sugar-sweetened beverages and diabetes. The researchers found that those who consumed one or more cans of sugar-sweetened beverage per day had a 29 percent greater risk of developing diabetes, after adjusting for energy intake and BMI.

In an international study looking at diet and diabetes, researchers used data from the Food and Agriculture Organization on the total number of calories consumed and the consumption of fruits, oil, sugar, meat, cereals, roots, nuts, and vegetables. They combined those data with data on diabetes prevalence worldwide from the International Diabetes Federation as well as economic data from the World Bank World Development Indicators Database. An analysis of the data showed that of all the food types, only sugar was correlated with diabetes prevalence. Furthermore, each additional 150 calories consumed was associated with a 0.1 percent increase in diabetes prevalence; however, if those additional 150 calories were from a can of soda, the increase in diabetes prevalence was 1.1 percent; that is, the prevalence of diabetes was 11 times greater if the additional calories were from a can of soda than from other sources. The researchers estimated that 25 percent of the cases of diabetes worldwide is explained by sugar consumption (Basu et al., 2013).

This study addresses some of the Bradford Hill criteria for causal medical inference, Erkin-Cakmak said: dose, duration, directionality, and precedence. Higher doses of fructose consumption lead to greater increases in diabetes prevalence. The duration of exposure is related to the development of diabetes, and the direction of causality is clear. Precedence is also clear, because whenever the availability of sugar increased in a country, 3 years later the diabetes prevalence increased in the same country.

Given all of these different forms of evidence pointing to the role of fructose in metabolic syndromes and particularly in diabetes, Erkin-Cakmak concluded by saying that understanding of the role of diet in disease must change.

Discussion

In the discussion session following the presentation, Linda Birnbaum of NIEHS asked if there is any information about what fructose consumption might do to the microbiome. Erkin-Cakmak responded that she believes that fructose can be toxic to the microbiome and that, conversely, alteration of the microbiome might lead to a more excessive amount of fructose absorption.

Barbara Corkey of Boston University asked about what role factors other than sugar consumption might play in the development of diabetes, given that a number of other factors have been changing in parallel with the increased consumption of sugar, such as exposure to the plasticizers in the soda containers. It is troubling to attribute causation to one of these factors and eliminate all of the others from consideration, she said. Erkin-Cakmak replied that this was a good point and that she did not believe that the authors of the paper had considered changes in environmental exposures over time.

Nik Dhurandhar of Texas Tech University asked Erkin-Cakmak about the role of sugar in obesity. She replied that obesity is not the main problem. “Being metabolically healthy or unhealthy is the problem.”

NONCALORIC SWEETENERS AND OBESITY

For several decades people have used sugar substitutes—noncaloric sweeteners—as a way of sweetening food without adding the calories that come with sugar, but could those noncaloric sweeteners themselves cause obesity? That was the question addressed by Kristina Rother, a clinical investigator in the Diabetes, Endocrinology, and Obesity Branch at the National Institute of Diabetes and Digestive and Kidney Diseases.

Rother broke her presentation into three sections: an overview of artificial sweeteners and how they convey sweetness, a look at studies that have reported an association between artificial sweetener use and obesity, and a review of the data and concepts that either support or rebut a causal role for artificial sweeteners in the development of obesity.

Artificial Sweeteners and How They Convey Sweetness

Six artificial sweeteners are currently regulated by the U.S. Food and Drug Administration (FDA). The first one to be put on the market was saccharin, Rother said, and it is about 300 times sweeter than sucrose. Then there are aspartame (200 times sweeter), acesulfame potassium (200 times sweeter), and sucralose (600 times sweeter). The ones that were developed the most recently are neotame and advantame. The last two are also the two sweetest, with each one being 10,000 and 20,000 times sweeter than sucrose. They are so sweet, Rother said, that they are difficult to work with because an incredibly small amount makes a product taste extremely sweet. No products have yet been made with advantame, although there will be some in the future, she predicted.

FDA also sets the accepted daily intake (ADI) for each of the sweeteners. This is the amount that a person can ingest each and every day throughout his or her lifetime without experiencing any adverse health consequences. For saccharin, this is 5 milligrams per kilogram of body weight, or about the amount in three sodas sweetened with saccharin. For sucralose, the ADI is equivalent to five sodas per day, and for acesulfame potassium the ADI is equivalent to about 30 sodas per day. Most adults will not reach the ADI, Rother observed, but a publication by South Korean scientists showed that children can easily reach the ADI because of their small body size. If they drink two sodas a day, children may already be beyond the ADI.

To explain how artificial sweeteners work, Rother began with a description of the sense of taste. The tongue's taste buds are concentrated mainly on the sides and the back of the tongue. In each taste bud there is one cell that responds to only one taste—either salt, sour, sweet, bitter, or umami. When one of these cells is activated, it sends a signal via the cranial nerves to the insula in the brain, reporting what has been tasted.

Interestingly, these taste receptors are located not only on the tongue or the oral pharynx, Rother said. There are taste receptors all over the mouth as well as in the intestine, in the beta cells of the pancreas, and

even in the lungs. "What they do in the lungs, we really have no clue," she said. "There are things that we really don't understand yet."

In the intestine, endocrine cells have sweet taste receptors that respond to sugars by increasing the level of incretin hormones. These hormones lead to an increase in insulin levels and thus a decrease in blood glucose levels. It makes no difference to these sweet taste receptors in the intestine whether you have ingested carbohydrates, sugars, or an artificial sweetener—the receptors will respond in the same way.

One of the important incretins produced by the cells in the intestines is glucagon-like peptide 1 (GLP1). This hormone acts not only to increase insulin but to slow gastric emptying, decrease appetite, and decrease the levels of glucagon.

Studies Reporting an Association Between Artificial Sweetener Use and Obesity

A number of studies have suggested an association between the use of artificial sweeteners and obesity. As an example, Rother described a study by Sharon Fowler and colleagues whose results were published in 2008. They examined data from the San Antonio Heart Study, which enrolled more than 2,000 people, mostly white Americans and Hispanic Americans. When the researchers running the study enrolled the participants, they assessed their food intake and asked them how much regular soda and diet soda they drank. These people were then followed for 7 to 8 years (Fowler et al., 2008).

Fowler and her colleagues used the data from the study to see how the BMI of the participants changed over the course of the study and to compare that change with the consumption of diet sodas. What they found was a clear relationship between increases in BMI and soda consumption, with those people who drank more diet sodas experiencing a greater increase in their BMI over the 7 to 8 years of the study than those who did not drink diet sodas or who drank fewer diet sodas (see Figure 5-2). For example, people who did not drink sodas at all had an average increase in BMI of 1.0—equivalent to a person who was 5 feet 6 inches tall and weighing 155 pounds (BMI = 25) gaining about 6 pounds. Because most people gain weight as they age, that 6-pound gain over 7 to 8 years is a reasonable weight gain. However, the BMI of people who drank an average of 3 to 10 sodas per week increased by about 1.5 over that same time period. For a given individual, Rother noted, the difference

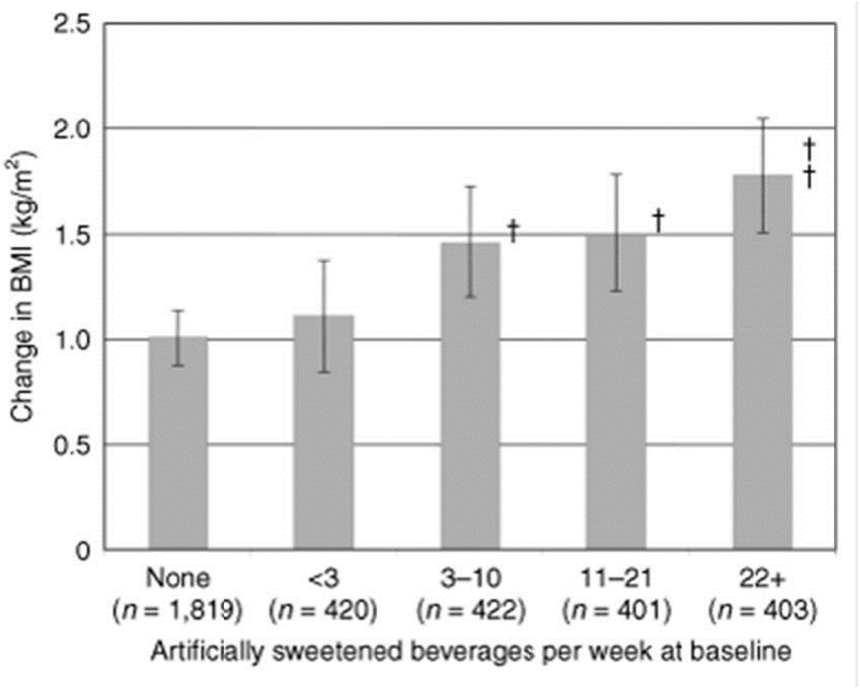

FIGURE 5-2 Change in body mass index (BMI) as a function of diet soda consumption after 7- to 8-year follow-up: versus none, $p<0.01$(†) versus none, $p<0.001$ (‡).
SOURCES: Rother presentation to workshop, March 3, 2015. From Fowler et al., 2008.

in weight gain is not particularly noticeable—only an additional 3 pounds for that 5-foot-6-inch, 155-pound person. But, she said, at the population level, "this is very important."

In short, there is evidence of a relationship between the use of artificial sweeteners and obesity, but studies such as the study of Fowler and colleagues identify only a correlation between the two. They say nothing about causality, that is, whether the use of the artificial sweeteners actually did something to cause the weight gain. Rother examined that question next.

Data and Concepts Supporting or Rebutting a Causal Role for Artificial Sweeteners in Obesity

To examine whether there is a causal relationship between artificial sweeteners and obesity, Rother began by discussing in vitro studies and then moved to animal studies and, finally, studies with human populations.

Researchers have used in vitro tests to study the effects of artificial sweeteners on a number of different types of cells, including preadipocytes, 3T3-L1 cells, mature adipocytes, and human mesenchymal stem cells. A variety of different effects have been observed, Rother said. For example, saccharin, sucralose, and acesulfame potassium have all been shown to cause preadipocytes to turn into adipocytes more quickly. In mature adipocytes, artificial sweeteners cause a decrease in lipolysis, or the breakdown of lipids, so that more lipids accumulate in the cells. There is also decreased lipolysis in human mesenchymal stem cells. That last result, Rother said, was unpublished data obtained in collaboration with her colleagues Sabyasachi Sen and Allison Sylvetsky at George Washington University that they were planning to present at a meeting of the Endocrine Society later during the same week that the workshop was held.

Referring back to Barbara Corkey's workshop presentation on the previous day, Rother noted that Corkey had reported that various artificial sweeteners increase the amount of insulin secreted from rodent pancreatic beta cells in vitro. The same increase in insulin secretion has also been shown in MIN6 cells in response to sucralose and saccharin, she said.

Artificial sweeteners are also known to be bacteriostatic, that is, they inhibit the growth of bacteria. This is generally a useful function because artificial sweeteners are used in a variety of products, including lip balm and toothpaste. Dentists are happy about artificial sweeteners in toothpaste, Rother said, because they help prevent the growth of bacteria in the mouth and around the teeth and gums. Other research has shown that artificial sweeteners—sucralose in particular—suppress intestinal microflora, the complex ecosystem of bacteria that live in the intestines and help with the digestion of food.

An important paper published in *Nature* in October 2014 showed that this effect on the intestinal microflora could be linked to obesity, Rother said. A group of researchers led by Eran Elinav from Israel examined the effects of feeding saccharin to mice (Suez et al., 2014).

The saccharin-fed mice got significantly fatter than glucose-fed mice, which led to the question of why. The researchers found that there was no difference between the two groups of mice in either energy intake or energy expenditure, which led them to deduce that it was the changed microbiome in the saccharin-fed mice that made them fat. In particular, the mice became more efficient at digesting their food because certain pathways in the microbiome that help the mice use the calories that they digest are upregulated; that is, the response of those pathways to calories is increased.

To verify that this was really what was going on, the researchers transplanted the microbiomes from the saccharin-fed mice and the glucose-fed mice into other, germ-free mice and examined those mice. What they found was that the microbiomes of the mice into which the microbiomes had been transplanted ended up resembling those of the particular type of mice from which they had received the transplants: The mice with transplants from saccharin-fed mice had higher levels of glucose in their bloodstreams and got fatter than the mice with transplants from the glucose-fed mice.

The Israeli researchers also looked for differences between people who regularly used artificial sweeteners and those who did not consume artificial sweeteners. There were a variety of differences: the artificial sweetener users had higher BMIs and higher levels of hemoglobin A1C, for example. Everything was a little worse for that group, Rother said, but she added that she did not find the human data in that paper to be particularly convincing, other than showing that the microbiome in people who consume artificial sweeteners looks different from the microbiome in those who do not, which is not particularly surprising. It will be important to go beyond these sorts of associations in people and start examining causation, she said.

Some interesting data could appear from some clinical trials now under way in Sweden, in which researchers are testing human microbiome transplantation as a treatment for obesity. "We will see what the human studies show," she said.

A different line of research has examined the effects of the consumption of artificial sweeteners by lactating mothers. Studies have shown, for example, that lactating rats concentrate acesulfame potassium sixfold in breast milk. Rother indicated that preliminary studies done in her lab have found higher concentrations of acesulfame potassium in human milk than in the mother's blood serum. "I think that when we do

cleaner studies," she said, "we will be able to show that acesulfame potassium also accumulates in human breast milk."

Could that affect the baby? Studies in rats have shown that offspring that were exposed to artificial sweeteners during pregnancy and lactation had a higher sweet taste preference later in life. Thus, it is possible, Rother said, that children whose mothers consumed large amounts of artificial sweeteners during pregnancy or nursing might develop a preference for sweeter foods.

Rother's research group has also examined the short-term effects that the consumption of artificial sweeteners has on the blood levels of glucose and different hormones. They carried out oral glucose tolerance tests with 22 healthy adolescents and young adults ranging in age from 12 to 25 years in which changes in the blood levels of glucose and certain hormones were tested at various points in time after the subject was given a large dose of glucose. Before giving them the glucose and starting the test, they gave the subjects either mineral water or a diet soda with sucralose and acesulfame potassium. Those who had been pretreated with the diet soda had significantly higher levels of GLP1 during the test. When they repeated the test with 11 subjects with type 1 diabetes, they saw the same effect.

This would seem to be a good result, because GLP1 decreases the rate of gastric emptying, so that drinking a diet soda before eating, say, a pizza would cause a person to get full sooner and not each as much. "In fact," Rother said, "some nutraceuticals have been started to be developed that mix all kinds of artificial sweeteners based on the principal that maybe you can get your GLP1 up."

But there is more to the story, she said. A study of 17 obese women (average BMI of 42 kilograms per square meter) found that giving them sucralose before an oral glucose tolerance test made their test results worse (Pepino et al., 2013). In particular, during the test the levels of both glucose and insulin were much higher in the women who had consumed sucralose than in those who had just had water.

Rother's group did the same experiment with 31 middle-aged adults of normal weight (average BMI of 26 kilograms per square meter). They found the same thing: giving them artificial sweeteners before an oral glucose tolerance test led to higher insulin levels during the test. One problem, she said, is that there is a great deal of variability in the outcomes of oral glucose tolerance tests, so such tests will require a large group of subjects to produce results that are statistically significant. However, she said she believes that it is worth following up on this

experiment because the size of the difference that they observed—a 20 percent increase in the amount of insulin in the bloodstream over time—is clinically relevant. "That is as much increase as you get with metformin or with weight loss or with all kinds of things," she said.

Those studies were all looking at acute effects; that is, they focused on changes that occurred in the minutes and hours after consumption of artificial sweeteners. Rother then described experiments that looked for long-term effects. There are actually very few of these, she said. Two of them were published in 2012 in the same issue of the *New England Journal of Medicine*. One was a randomized trial of the effects of diet beverages on the weight of adolescents, while the other was a similar trial but with children instead of adolescents.

In the first one, the researchers randomly split a group of 224 overweight and obese adolescents into two groups (Ebbeling et al., 2013). One got diet drinks delivered to their homes to replace their usual sugar-sweetened drinks. The others were given supermarket gift cards with no instructions so that they could buy what they wanted. Although the group receiving the diet drink gained less weight, on average, after 1 year, at the end of the 2-year study there was no difference in weight gain between the two groups. The replacement of sugary drinks with diet drinks had failed to help the adolescents gain less weight.

In the other study, performed in the Netherlands, 641 children who already drank sugar-sweetened sodas were divided into two groups, with one of them continuing to drink soda (one can per day) and the other given one can of an artificially sweetened drink per day. After 18 months the researchers looked for differences between the groups. All of them gained weight, of course, because they were growing children, but the sugar-sweetened group gained more weight than the children who received the drink with the artificial sweetener. The main problem with the study, Rother said, is that there was no group of children who drank just water, so it is impossible to know how children who drink diet sodas would fare versus those who drink just water, but the experiment did clarify one thing: "We know now that if you drink soda, you gain weight beyond what you should gain," Rother said. "If you replace it with artificial sweeteners, you gain less weight."

Rother ended her talk by commenting that the human brain is perfectly capable of differentiating between caloric sweetness and noncaloric sweetness. Research has shown that while artificial sweeteners activate the insula in much the same way that sucrose does, sucrose activates certain parts of the brain that artificial sweeteners do not, in particular, dopamine-

dependent areas. That raises the question of whether sweetness with calories provides a different sort of reward to the brain than sweetness without calories and whether that makes a difference, particularly with chronic use. It is an issue that needs to be investigated further, she said.

In summing up, Rother said that there is no convincing evidence that artificial sweeteners prevent or alleviate obesity in humans. On the other hand, there are clear data showing that, in vitro, artificial sweeteners lead to more adipogenesis, less lipolysis, and more insulin secretion than sucrose. It is also clear that artificial sweeteners can affect the intestinal microflora and, in lab animals at least, that they can lead to higher glucose levels and greater weight gain. In humans, however, it has been very difficult to establish causality. We know that artificial sweeteners change the microbiome and that it is possible, by looking at the microbiome, to tell the difference between someone who consumes artificial sweeteners and someone who does not. But do artificial sweeteners cause people to gain weight because of these changes? Rother believes that there are plausible reasons for the connection between artificial sweeteners and obesity but that we are far away from answering that question on the basis of rigorous clinical studies.

Discussion

In the discussion session following Rother's presentation, Barbara Corkey began by asking about the various doses of artificial sweeteners used in the various experiments. Rother answered that the in vitro experiments generally used levels higher than those that would be seen in humans consuming a reasonable amount of artificial sweeteners, but her research group is hoping to be able to do some in vitro experiments that reduce the doses to levels that are realistic for human users of artificial sweeteners. On the other hand, at least some of the experiments in lab animals—in particular, the mice experiment described in *Nature*—used doses that are comparable to the ADI in humans.

Linda Birnbaum commented that she believes that there are probably many people who drink large amounts of diet sodas every day and that there is probably a wide range of exposures in the population. In particular, there may be a subset of the population that routinely consumes levels of artificial sweeteners that are much higher than most health researchers would expect.

Corkey added that even educated people may not understand how prevalent artificial sweeteners are in food. Someone who picks up a

container of yogurt and sees that it contains acesulfame potassium, for example, is not likely to realize that this is an artificial sweetener. A related issue is that many people look for low-calorie and low-sugar foods in the belief that they are healthier and forget that this means that they contain artificial sweeteners. Rother mentioned one of her projects in which parents of children were asked if they would give their children artificial sweeteners and most of them said no, but when they were asked to choose products for their children, they chose a lot of products that said "No sugar added"—exactly the products that would have artificial sweeteners (Sylvetsky et al., 2014).

PANEL DISCUSSION

Lynn Goldman of George Washington University started off the panel discussion following the final presentation of the session by asking the four presenters to talk about what she saw as an emerging theme at the workshop: that it is not obesity so much as related metabolic changes that are the main health consequence to be concerned about.

Erkin-Cakmak agreed that the main issue is whether a person is metabolically healthy or unhealthy. She added that in a recent study conducted with African-American and Latino populations—both of which are generally metabolically unhealthy—she and colleagues found that they could improve the subjects' metabolic health by giving them a healthier diet, even one that gives them the same number of calories that they had been consuming, for just 10 days.

Rother disagreed somewhat, commenting that, in general, adiposity is associated with increased inflammation and is clinically associated with more cardiovascular risk. Although not everybody needs to lose 50 pounds to be healthy, it is important to accept that, in general, obesity is associated with certain health conditions.

Dhurandhar offered two quick points. First, he said, while it is important to focus on the metabolic consequences of obesity, those are not the only consequences of obesity. "There are numerous other [nonmetabolic] comorbidities or adverse conditions that are linked with obesity that we may overlook if we only focus on diabetes or cardiovascular disease," he said.

Second, he said, while research usually involves isolating one factor out of many and examining how it affects other things, in nature various factors work together and thus need to be considered in conjunction with one another. He mentioned as an example a study of animal models of celiac disease that found four different factors had to exist at the same time for the celiac disease to be expressed.

Dhurandhar asked whether, because antibiotic exposure is a marker for infections, there is a higher rate of obesity among children who have more infections. A related question came from a webcast audience member who asked if the association between antibiotic use and obesity can be confounded by a relationship between infections and obesity, assuming that antibiotics were being prescribed for obesity-causing pathogens.

Bailey replied that in the health services study that he and his colleagues did, they found no association between obesity and such infections as colds or upper respiratory infections. Furthermore, once the use of antibiotics was taken into account in the multivariate analysis, such infections as inner ear infections were not associated separately with obesity. Given that close to 90 percent of ear infections are viral in origin, he said, he thinks that it is unlikely that children who are more susceptible to clinical viral infections are also more susceptible to obesity.

Rother offered an anecdote to illustrate how various confounding factors leading to obesity may be at play. A case study trying to understand obesity in Kuwaiti children found that the strongest factor for childhood obesity was maternal employment. This could be due to several reasons, he said. He knows from experience that working mothers sometimes ask for antibiotics for their sick children because they do not want to miss work. On the other hand, a working mother is less able to supervise what her children are eating at various times of the day or how much television they are watching. It can be difficult to tease apart these various factors.

Janet Young, a webcast audience member, asked whether probiotic treatment might either complement an antibiotic treatment or take the place of an antibiotic treatment and how this might affect obesity. Rother said that it is extremely hard to get evidence-based answers to such questions and therefore little is known on the topic.

Corkey commented that despite the general consensus that obesity is not healthy, there is no healthy treatment for obesity, which raises the question of what clinicians should be doing about it. “You talk about people going to the grocery store,” she said. “What kind of advice should we give to people about how to conduct their eating lives?”

Erkin-Cakmak said she is raising her toddler with as little salt, sugar, or artificial sweeteners as possible and is feeding him mainly unprocessed food that is cooked from scratch. A recent birthday party indicated that the approach may be working, because her son, when he tried the birthday

cupcake with frosting pronounced it "yucky" and threw it in the trash can. She explained that cooking from scratch, avoiding processed food, and consuming real food would be the key to become metabolically healthy. That's what she and her colleagues are encouraging in their patients who are seen in the clinic.

Dhurandhar said that the problem is that scientists do not yet know enough about the disease of obesity or its complexity and multifactorial etiology, similar to cancer. However, he added, the cause and the treatment of a disease are two separate issues. Celiac disease, for instance, can be treated by restricting gluten, but gluten does not cause it. Not knowing the best treatment for cancer does not mean that clinicians do not try to treat it. "I think that is where we are in terms of obesity treatment right now," he said. "We have a blanket treatment to offer for obesity today," which is to eat less and move more, regardless of the cause. "That is what underscores the need for research so that we can consider all of these causes, contributory factors, and come back with a better and really effective biologically meaningful treatment and prevention strategy that can take the weight off meaningfully and keep it off. We are not there yet."

Bailey added that the best advice that he can offer people at this point in time is "moderation in all things.… I end up recommending a balance, a balance in diet, a balance in exercise, a balance in activity, and, to steal the line from Mark Twain, a balance in balance, too."

Rother added that there is not enough evidence to answer certain questions with certainty, such as whether people should stop drinking artificially sweetened sodas. Thus, she said, she agrees with Dhurandhar that moderation is a good strategy.

Corkey elaborated that in the past people have been given advice for which there was no scientific evidence and that has occasionally been proven wrong. "For example," she said, "our focus on lipids has been all wrong. Poor eggs suffered for many years." She continued by commenting that scientists really do not know what causes obesity and that there is very little useful, outside of surgery and a few moderately effective medications, to treat it. Thus, she said, it is important to make the situation clear to the public and emphasize that instead of looking to researchers for answers right now, the public should recognize that what is needed is much more funding to do the research to find those answers.

Linda Birnbaum of NIEHS responded that it is not necessary to wait for 100 percent understanding and certainty before taking action. A lot of information is available from mechanistic studies, animal studies,

observational studies, and the few clinical intervention trials that have been done, she said. "You put it all together and there are certainly some warning flags that we need to look at."

Responding to Corkey's question about advice to individuals on what they should do differently, Birnbaum said that she believes that individual behavioral change is much less effective in the long term than public policy changes. A major issue that needs to be addressed, she said, it that while fresh foods are the healthiest option, they are not available to the entire population, and this situation particularly affects people from disadvantaged backgrounds, who are most at risk of obesity.

Young asked a question about the presence of small amounts of glyphosate—the pesticide sold as Round Up—in foods, particularly corn and high-fructose corn syrup. A paper published by Shehata and colleagues (Shehata et al., 2013) reported that many pathogenic gut bacteria are resistant to glyphosate, whereas many beneficial bacteria are susceptible to it. Could the presence of glyphosate in corn syrup be a confounding variable in the results showing a link between fructose and metabolic dysfunction? Goldman answered that she has not seen any studies addressing the issue, but that it is a reasonable question to ask.

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6

Research Needs

The fifth session of the workshop was devoted to a panel session on research needs. Roundtable member Hal Zenick of the U.S. Environmental Protection Agency (EPA) introduced the four discussants, who discussed the field's research needs from various perspectives, and then presided over a wide-ranging discussion that involved the discussants, Roundtable members, and workshop participants.

A PERSPECTIVE FROM NIEHS

The first discussant was Linda Birnbaum, director of the National Institute of Environmental Health Sciences (NIEHS) and a Roundtable member. She began with a brief review of some of NIEHS's efforts in the area of the environment and obesity.

NIEHS held its first symposium on the fetal origins of obesity in 2004. A number of years later it announced a program to explore the connections among environmental chemicals, obesity, and diabetes. It began funding grants for that program in 2011 and currently has funding for 57 grants related to obesity, 32 of which involve human cohorts, 20 of which are studies with animal models, and 5 of which are mechanistic studies.

The institute's work with the National Toxicology Program has shown, she said, that it will be important to think more broadly about which chemicals to consider. Given that it is practically impossible to test all the 80,000 chemicals that are currently in commerce, it will be important to get a better sense of how to prioritize which chemicals to screen.

An important need is to identify environmental chemicals that cause weight gain or changes in body composition, she said. Another important need is to understand the windows of susceptibility. It is likely that children are much more susceptible to the effects of environmental chemicals than adults, but exactly when are they the most susceptible?

In examining the effects of environmental chemicals, it will be important to look past their effects on adipocytes and think also about their effects on other tissues, including the gastrointestinal tract, the pancreas, the liver, muscle tissue, and the brain. "We need to remember that the brain is controlling much of our behavior, both conscious and unconscious, and we need to focus on that," she said. There are satiety centers in the brain, for instance, but the brain also controls circadian rhythms, and they can play a role in overeating. Toxicologists know that they may get very different dose–response curves if they dose their animals at different points in the circadian cycles.

In doing toxicological studies, it would be valuable to maintain the lab animals long enough to see effects, particularly when the research is looking at developmental effects. Researchers often hold animals just to weaning, Birnbaum noted, or perhaps until puberty, but to see the sorts of effects on weight and metabolism that are of interest, it will be important to keep the animals at least until they are 1 year of age.

Another question that researchers should ask themselves is if they are using the right animal model. Effects can vary from one species to another, from one strain to another, and from one sex to another. For example, some strains of mice gain only a small amount of weight on a high-fat diet, while others grow to gargantuan proportions.

In humans, researchers studying obesity should remember to take into account such factors as race/ethnicity, socioeconomic status, and even birth order. One interesting difference between Europe and the United States is that new mothers in Europe are more likely to nurse their children for a significant amount of time. That can affect obesity. Gestational diabetes is another factor that may play a role, Birnbaum said, but not enough is known about it to say for sure.

She commented that obesogens are likely to affect more than the risk of obesity; they have various other health effects as well. A chemical that is very bioactive is probably going to do different things in different tissues, she said, particularly in context-dependent endocrine-mediated processes.

In studying obesity in both animals and humans, body weight gain may not be the best metric to use, Birnbaum said. In particular, body composition is extremely important. So researchers should probably consider more than just body mass index.

The microbiome is likely to play an important role in obesity, she said, and learning more about the microbiome in general and about its role in obesity in particular should be a focus of future research.

Concerning epidemiological studies of the effects of various environmental factors on obesity, Birnbaum said that not only are prospective longitudinal studies needed, but also researchers should think about using randomized controlled intervention trials. Such trials can be ethically done in an environmental context, and there is certainly potential for them in the future, she said.

Researchers should be shifting their focus from solely on obesity to paying more attention to the metabolic syndrome and diabetes, Birnbaum suggested, because it is not obesity per se that is the major health risk but, rather, diabetes and metabolic issues.

It would be valuable to coordinate the epidemiological studies with the animal studies, she said. "The toxicologists want to think that they are the ones finding a thing and then the epidemiologists go and look," she said. "I think it may be more the other way around, that frequently it's the epidemiologist who sees an association that nobody has ever considered before and then the toxicologists need to see if there is biological plausibility there."

Econometrics studies will also be important, Birnbaum said. By putting a dollar value on the costs of obesity, diabetes, and the metabolic syndrome, it should be possible to drive policy changes.

The current paradigm for obesity in the United States is one of intervention and treatment, Birnbaum noted. Certainly it will be important to continue to improve on ways to intervene and treat, but it would be much better to learn how to prevent obesity in the first place. "That is going to have to start not only in children but actually prenatally and maybe even preconception," she said. "I am not giving anyone a bye on exercise and diet, but are we making it harder for people to control their weight? I think that is the question."

A PERSPECTIVE FROM USGS

The next discussant was Suzette Kimball, the acting director of the U.S. Geological Survey (USGS) and a member of the Roundtable. The issue of the environment and obesity intersects with work that is occurring at USGS, she said, and her presentation was from that perspective. In particular, she said, the body of research on environmental exposures and the role that they play in increasing the risk of obesity and other health challenges that is emerging is related to the research done at USGS, which focuses on environmental conditions and factors.

Recognition that chemical exposures may play a role in the development of obesity can lead to the development of new avenues for

risk reduction and prevention, she said, but this requires determination of which particular toxicants are increasing the risks of obesity and other health issues.

The research gaps that need to be addressed in this area are not unique to obesity research, Kimball noted. Research on the mechanisms by which environmental chemicals cause adverse health effects and the way that these chemicals may interact in the environment to provide cumulative effects is needed. New and better methods to measure the presence of these chemicals in the environment are also needed.

Kimball then spoke about some of the challenges she sees in the area. They include understanding the potential health effects of chronic exposures to extremely low concentrations—that is, less than 1 part in 1 billion—and gaining greater knowledge about the potential increased vulnerability of various populations, including the elderly, the very young, and those who have other complicating health factors. Another challenge is figuring out how exposures in early life stages have effects much later in life or even in subsequent generations.

From the perspective of USGS, she said, a major research challenge is identifying and characterizing the environmental drivers of exposure. The fate and transport of environmental chemicals—as well as human exposure to such chemicals—are affected by both natural and anthropogenic changes in the environment, she said. The human-driven changes in the environment vary across the world, depending on societal demands for land and natural resources and the changes in resource consumption that are driven by economic prosperity. Those changes are compounded by natural Earth processes, climate trends, and related climatic events.

Scientists at USGS specialize in understanding the factors at the interface of the environment and health, Kimball said. That is, they work to characterize the interactions among the physical environment, the living environment, and people to understand how various processes affect human, wildlife, and ecological exposures to environmental disease agents.

A recent focus at the agency has been endocrine-disrupting chemicals (EDCs). USGS spends approximately $5 million per year on research related to these chemicals, Kimball said. However, it also collaborates on such research with many other agencies, such as EPA, the U.S. Department of Agriculture, the U.S. Army Corps of Engineers, and various state public health and agricultural agencies, nongovernmental organizations, and universities. “So the actual figure that is dedicated to this research is much larger than that $5 million,” she said.

In 2014, USGS developed a national strategy for research on EDCs, Kimball said, and that strategy focuses on the science related to the occurrence, exposure pathways, and effects of these chemicals on natural resources, including both terrestrial and aquatic wildlife. Some of the research challenges and research directions identified in that strategy have a great deal in common with the work on these chemicals that the public health sector is undertaking.

The research needs identified in the USGS strategy include the systematic evaluation of sources; the distribution and state of EDCs in the environment; identification of the routes of exposure to EDCs of fish, wildlife, and humans; studies of how EDCs accumulate in animal and human tissues; research into the effects of simultaneous exposures to mixtures of chemicals; investigations into the mechanisms of action of EDCs and the mechanisms by which EDCs cause adverse effects; improvements in analytical techniques, laboratory methods, and biological assays for identifying EDCs in the environment; investigations into less studied endocrine pathways involving metabolism, behavior, fat storage, bone development, and immunity; and the identification of new potential EDCs. "Those challenges that we have identified as a research strategy specifically for EDCs can, as I said, connect very well to the kinds of challenges that I heard articulated over the last day and a half," she said.

A PERSPECTIVE FROM EPA

The third discussant was John Rogers, the director of the Toxicity Assessment Division of the National Health and Environmental Effects Research Laboratory in the Office of Research and Development at EPA.

He began by saying that one thing that struck him about the topics discussed at the workshop was that the standard regulatory developmental toxicity tests at EPA would miss most of what was being talked about. For one thing, when EPA scientists do teratology studies, they take the fetuses from the mother just before they would be born, so they do not see effects that appear later in life. And even in multigenerational studies, he said, they do not follow the offspring for very long. Furthermore, things like insulin resistance, elevated blood pressure, or changes in body composition that did not affect body weight would not be detected at all in the experiments. He also noted that even when there was elevated body weight in some of the studies, the extra weight was not seen as an adverse effect. In short, he said, we could be missing some of the biggest problems we are seeing now in children, like obesity and diabetes.

In research on developmental exposures, Rogers said, a key research need is to learn about critical periods. Do the critical periods all come before birth? And even if the critical periods are prenatal in humans, a lot of the development—especially neurobehavioral development—that occurs in utero in humans happens after birth in rodents. This raises the question of what sorts of animal models are needed to model the human situation as best as possible.

Many different questions arise concerning the appropriate animal models, he said. A variety of inconsistencies appear among studies, and some of those may be due to differences in the animal models used in the different studies. Given that there are important differences among species—between the mouse and rat, for example—and even among strains of the same species, how do you choose which is the most appropriate? Not enough is known about those differences yet. Other issues are the following: What kind of diet should be used for animal models? Do the animals need to be put on a high-fat diet to stress their systems and demonstrate that they respond differently if they have a high caloric intake? What should be the exposure period and the dose of test agents?

Dose can be a particularly tricky issue because of nonmonotonic dose–responses. For example, a low dose of a substance might stimulate some pathway that leads to obesity, but increasing the dose might trigger toxic mechanisms that start to drive the weight down, so at some point the response changes from weight gain to weight loss. In particular, if the dose at the start is too high, it might seem that there is no effect or even that the substance causes weight loss when at a lower dose it can lead to obesity.

One of the most important things for researchers to do with animal models, Rogers said, is to understand the mechanisms that explain how certain environmental exposures lead to obesity. One particular focus, he said, should be how insulin affects other endocrine systems.

Researchers should be looking for animal models for some of the things that affect the body composition of offspring in humans, such as maternal obesity, preexisting obesity prior to pregnancy, high maternal weight gain during pregnancy, and gestational diabetes. With such models, researchers could start to ask how these various factors make the offspring more likely to be affected by the mother's exposure to certain environmental chemicals.

Many researchers working with animals models look only at selected endpoints and do not observe other outcomes, Rogers said. It would be

useful for researchers to start doing more complete evaluations and to measure the various aspects of the metabolic syndrome—not just obesity, but insulin resistance, blood pressure, and blood lipids—as well as changes in the stress response.

Because animal tests take longer to perform if the animals have to be kept for up to 1 year and tested at the end of that time, it would be useful to have biomarkers that would provide early indications of later effects. That could considerably shorten the time that it takes to run such tests.

Researchers should recognize that body weight is not a particularly good endpoint for work with either animal models or humans. Body composition, in particular, measures of fat content and location, is a much better indication of what is going on.

AN OBESITY PERSPECTIVE

The fourth discussant was Nik Dhurandhar from Texas Tech University, who said that his comments would be grounded mainly in the obesity perspective and what he sees as research in that area.

If one thinks of obesity as the result of an energy surplus, he said, the obvious solution is to reduce that energy surplus by decreasing energy intake or increasing energy expenditure, which in turn is done either by increasing activity or by increasing metabolic need. Clinicians have tried to attack obesity in this way by asking people to eat less or to move more or by using drugs or surgery to reduce intakes or increase expenditures.

However, Dhurandhar said, one should also ask what is upstream of increased energy intake or decreased activity. The answer, he said, is a dysregulation of energy balance. People who are not obese are able to maintain their body weight, but it is not done consciously. People cannot say at the end of the day exactly how many calories that they have consumed or expended, yet they have balanced the two. That means that they are regulating their body weight at some subconscious level, which in turn implies that in those people who are not able to maintain their body weight, there is a dysregulation of energy balance, which is what leads to the energy surplus. What is interesting to him, Dhurandhar said, is the question of what causes this dysregulation of energy balance and does so only in some individuals.

He then showed a slide that he had borrowed from Claude Bouchard at the Pennington Biomedical Research Center at Louisiana State University and that he had modified slightly. It was a partial list of dozens of putative contributors to weight gain in four categories: the social environment, the physical environment, behavior, and biology.

"So this is a reality," Dhurandhar said. "In one individual, several of these factors may be operational, and in another individual, it could be a completely different set of factors that may be determining that person's body weight or obesity." The point is, he said, that if people focus on just one or a few factors in trying to determine why people are obese, it will likely be inadequate.

To explain the problem, he offered a metaphor: suppose there is a buffet and you are concerned about the calorie consumption of people enjoying this buffet. You decide that you are going to focus on one of those dishes for its caloric contribution to that person's caloric intake, and perhaps you reduce the amount of that dish that a person is allowed to eat or you completely remove that dish from the buffet. But if you remove a particular product that is contributing to caloric intake, there are still many others on the table that can make up the difference. "That is what I think of as digging a hole in water," he said. "It is really going to get filled by something else." The moral is this: a focused attempt on just one aspect that contributes to obesity may not yield the desired results.

Therefore, Dhurandhar said, it makes sense to refer to "obesities" rather than just "obesity" to make it clear that multiple conditions that may have similar symptoms but that are really different types of conditions are involved. He compared the situation to jaundice, which describes a symptom but not what has caused it, such as viral hepatitis, cirrhosis, cancer, or some other condition.

Therefore, he said, it is important that researchers identify the true causes of obesity—the truly upstream causes, not the midstream causes. "As a physician," he said, "I always like to say that when somebody presents with a cold, I don't treat the nose; I treat what caused that cold. Maybe there's an allergy; maybe there is an infection. I think that is a strategy that requires a multifactorial approach to a multifactorial disease."

DISCUSSION

Linda Birnbaum began the discussion by noting that the collection of environmental chemicals that had been talked about at the workshop was much smaller than the complete list of chemicals of concern. There was no mention of pesticides, for instance, and little talk of nicotine and tobacco smoke, even though there are some striking data connecting maternal smoking with both obesity and type 2 diabetes in offspring. Many of the environmental chemicals of concern work through endocrine-mediated mechanisms, she noted, and much of the work looking at

endocrine disruptions has focused on estrogen, androgen, and thyroid hormone, but there are many other endocrine systems that can be disturbed.

Jerry Heindel of NIEHS asked the speakers to comment on next steps to advance the field of environmental exposures and obesity.

Nik Dhurandhar answered that one of the first things that needs to be done is to identify what is not known, and he said that the workshop had been a good start to that end. One thing to keep in mind, he said, is the difference in clinical treatment and research. While clinicians today need to offer the best tools in the toolbox for the treatment of obesity, it is the job of researchers to move things forward and to develop strategies and treatments that are truly effective in producing meaningful and durable weight loss for the majority of people suffering from obesity. For instance, research is needed to understand better the various factors that contribute to obesity and to determine which of those are preventable and which are not.

Finally, he commented that while it may seem simple for people to simply eat less, it is not. Although eating is under volitional control in the sense that you can decide whether to put food in your mouth at any given moment, that does not mean that it is easy for someone to control their eating patterns over the months and years that it takes to lose weight and keep it off. He emphasized the importance of treatments that work not only in clinical trials, at the hands of talented researchers, but also in the real world, at the population level.

Paul Sandifer from the College of Charleston and Hollings Marine Lab said that he had been struck during the workshop by the paucity of animal models used in studies of obesity. In particular, he noted, only one study mentioned an aquatic vertebrate, which was the zebrafish. He suggested that many organisms may provide useful experimental models for this work. Because so many of the EDCs are waterborne or water mediated, he said, it might offer some additional insights to deal more with fish, which live in a water environment all the time.

Suzette Kimball responded to that comment by saying that there is a fairly robust body of ongoing research concerning bioaccumulation, looking at how these chemicals of interest reach higher and higher levels as you move up the food chain, and fish are the species of choice in that work. Because fish are also a human food source, it is important to understand the role that bioaccumulation might play in exposing people to high levels of obesogens.

Birnbaum added that it would also be important to study bioaccumulation in terrestrial animals as well because some of the patterns of bioaccumulation appear to be different between aquatic and terrestrial species.

Kristina Rother of the National Institutes of Health (NIH) suggested that it would be useful if different researchers could combine resources in a way that allowed each of them to focus on what they do best. As an example, she offered a hypothetical scenario: "I'm a clinician; I don't have easy access to adipocytes. Barbara wants to know the concentrations of artificial sweeteners that occur under [a] normal daily intake situation (information I have), and somebody else needs a liver enzyme experimental setup to test something else. Even within just this group, we could potentially help each other so that I don't have to do adipocyte experiments but can focus on my breast milk analyses."

A member of the webcast audience suggested that research on obesity and diabetes could be helped with the establishment of a major research initiative, something that would be similar to the human genome project but that would also be multinational.

Birnbaum responded that NIH is in the process of launching a major research initiative on precision medicine that will eventually involve more than 1 million subjects compiled mainly from existing studies. "The idea," she explained, "is to have access to the electronic health records and expand that with additional questionnaires, so you would have both biomedical exams and extensive questionnaire data and potentially take biological specimens and so on." The project might offer the opportunity to include some environmental factors as well, she said. She also said that while a large international trial would be very useful, it is extremely difficult to start studies with these very large cohorts. Finally, she said that what researchers really need to examine in these trials is early life exposures. There are a number of large birth cohorts in numerous countries around the world in which such early life exposures are now being studied, and there are similar, albeit much smaller, studies being carried out in the United States.

Barbara Corkey of Boston University said that it will be important to find better and more effective ways to share data and information among researchers. By sharing data and information, scientists and other workers can focus on what they do best. "For example," she said, "handling the data is a very special skill set that, for example, basic scientists rarely possess, but it's a very important aspect of interpreting

data. So we should work together to achieve these things rather than each trying to learn them."

John Rogers suggested that a similar thing is true in terms of the levels at which different scientists work. By coordinating in vitro work with animal studies and with human studies, it would be possible to get a much stronger, more cohesive picture. That would not be difficult to do if the different research approaches were designed together.

One audience member asked what sort of funding proposals researchers should be submitting, given that everyone agrees that understanding obesity will require understanding multiple factors and how they interact but that the funding agencies still seem to respond best to very focused proposals. Are the funding agencies ready for some interdisciplinary research with experts from various fields coming together and trying to answer questions about obesity?

Birnbaum responded that NIH tries to find the proper balance between individual research grants and program funding that brings together a variety of different types of research. The more that is spent on the one, the less there is to spend on the other. Furthermore, NIEHS has developed some approaches to increasing interdisciplinary work. One example is the Victor Program, in which one investigator receives an individual RO1 grant and as many as two other investigators can be added on to do additional studies that were not in the original grant but that are related to it. Still, Birnbaum said, it often is the case that to get a grant funded, an investigator must write the proposal in a very narrow way, which, given the nature of obesity, risks missing the forest for the trees. It is an issue that funding agencies and researchers must work further on.

Lynn Goldman of George Washington University turned the discussion to the broader public health perspective on obesity. She noted that chronic diseases associated with obesity cost the economy billions of dollars every year and that the prevalence of obesity is twice what it was in the 1960s. Even though obesity rates have been leveling off in the youngest children, they are not going back down to where they used to be. So the nation faces an enormous future cost associated with obesity as well.

It is important, she noted, to look at the interventions that are happening to identify and disseminate those that are actually working, because some interventions are clearly more effective than others. Right now there is a very scattershot approach to obesity prevention, with government agencies, states, foundations, and companies all making

efforts. From a public health perspective, it would be very useful, Goldman said, to be able to select from all of these effects those that are actually working and then to promote them, while at the same time doing the necessary research to understand the problem fundamentally. "We can't really afford 10 or 20 or 30 years of understanding the entire biology of a system before we start taking action to protect people," she said.

The same thing is true for treatment, she said, because so many people are already obese and are struggling with such diseases and diabetes and the metabolic syndrome.

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Obesity Policy Solutions Discussed at the Workshop

Faiyaz Bhojani of Royal Dutch Shell presided over the last session, which was dedicated to the discussion of policy solutions to reduce exposure to chemicals associated with the development of obesity. A policy, he said, can be thought of as a vision for the future that outlines priorities and the expected roles of the stakeholders and groups. A policy, broadly defined, also includes building consensus and informing people or the public, he said, emphasizing that informing the public is a topic that deserves greater attention than it sometimes gets. A policy needs the commitment of a state, an organization, or other entity to implement it, and it requires not only the laws or regulations that define the policy but also a variety of other mechanisms involved in its implementation.

Concerning policies regarding environmental exposures and obesity, Bhojani said, a key question will be whether sufficient and clear evidence should be required before the next step is taken, whether that would be too late and the next steps should be taken in the absence of such evidence, and, if that is the choice, exactly how that would be done.

The session consisted of presentations by four speakers[1] and then a wide-ranging discussion period involving not just the speakers but the Roundtable members and members of the workshop audience.

[1] This summary reflects the order of the presentations as they were given at the workshop, which differed slightly from the workshop's agenda.

PRESENTATIONS

Jeanne Conry

The first discussant was Jeanne Conry, the assistant physician chief at Kaiser Permanente, who was representing the American Congress of Obstetricians and Gynecologists (ACOG), where she introduced an agenda on reproductive health and environment. She spoke by phone.

As background, she said that ACOG is the nation's leading group of physicians who provide health care for women. It has about 57,000 members, and it works both to educate physicians and to advocate for policy changes.

Obstetricians-gynecologists (OB-GYNs) see themselves as primary care providers, caring for women across their life spans, Conry said. In particular, they see women before, during, and after pregnancy, so they have the opportunity to discuss care with women during these times, which is important in terms of environmental exposures, where many of the critical periods occur during pregnancy or in the first few years afterward. Pregnancy care should be viewed as an investment in the next generation, she said.

Research has shown that children are born, in some respects, "prepolluted," she said. That is, there are harmful environmental chemicals that are ubiquitous, and one study found that 43 chemicals were seen in virtually every pregnant woman in the United States. Furthermore, research has shown that environmental chemicals can cross the placenta, and a number of the environmental chemicals that women are exposed to are known to affect fetal development. Exposure to mercury during pregnancy, for instance, is known to affect cognitive development, and diethylstilbestrol (DES) can even have a transgenerational impact. So it is clear that exposures during pregnancy can have serious effects on a child's health.

In 2013, ACOG and the Society for Reproductive Medicine came out with a committee opinion on reproductive health and the environment designed to educate OB-GYNs. It was based on current research, Conry said, and one of its main messages is that not all exposures are created equal. For instance, underserved and minority populations are disproportionately affected by environmental chemicals. And women of reproductive age with occupational exposures to toxic chemicals are particularly vulnerable to adverse reproductive health outcomes.

The most important point for OB-GYNs to understand about environmental chemicals, Conry said, is that, unlike pharmaceuticals, they enter the marketplace without detailed research on their reproductive toxicity, so not much is known about them in that respect.

OB-GYNs do not have to be experts in environmental health, she said, but they should be able to do an intake exam and inform their patients about what their potential exposures are.

ACOG's main policy-centered message in this area is that controlling toxic exposures relies on working toward the greater good, Conry said. Many toxic exposures cannot be controlled by individual actions, and thus, changes in the levels of environmental chemicals generally occur only in response to changes in national policy. So, once ACOG had a committee opinion to work from, it began working at the national and state levels to regulate environmental chemicals.

ACOG has identified various policy gaps. An important one is that while pharmaceuticals must be shown to be safe and effective before being used in humans, manufactured chemicals must be shown to be harmful to people before they can be removed from manufacture. This is particularly important to emphasize to OB-GYNs, she said, because while they are familiar with the rules regulating pharmaceuticals, many are not familiar with the world of environmental chemicals, where chemicals are released into the environment without careful study of their effects on humans.

That difference is critical in educating physicians on the topic, she said, because they quite often say that they cannot offer advice about environmental exposures because there is no good research on the topic. "They simply don't understand how different it is between chemical and environmental exposures and that the burden of proof needs to shift," Conry said.

ACOG has several specific recommendations on the topic. First, at the individual level, physicians should educate women to do such things as eat healthy, wash fresh fruits and vegetables, and look at what their exposures are. More importantly, though, it recommends that the U.S. Congress should enact meaningful preventive and protective chemical safety legislation so that the burden of proof is transferred from individuals and physicians to the chemical industry. Specifically, the legislation should identify and reduce exposures to toxic environmental chemicals, should require the U.S. Environmental Protection Agency (EPA) and other federal agencies to take all necessary actions when reviewing substances to guarantee health and safety, and should fund

rigorous scientific research into the causes and prevention of birth defects.

Nearly two-thirds of voters worry about chemicals in consumer products, Conry said, and almost 90 percent support legislation that ensures that products are safe for human use. The support spans demographic and partisan lines, she said. So it is incumbent on everyone in health care each to make their voices heard in support of such action.

Sonya Lunder

The next presenter was Sonya Lunder, a senior analyst at the Environmental Working Group, a nonprofit advocacy organization. Lunder described the group's mission to be to evaluate the available scientific information and look for opportunities for policy intervention and also for public education.

Concerning environmental chemicals that promote obesity, she said, the good news is that there are ample data that may be used to take action and reduce public exposures to the endocrine disrupters like bisphenol A (BPA), arsenic, and persistent organic pollutants that had been presented as case studies at the workshop. Much is known about these chemicals from research on hormone disruption, she said, and these chemicals are ripe for intervention.

The scientific and regulatory communities have generated a great deal of biomonitoring data, and scientists can, in many cases, identify the populations that are at risk. Collectively, scientists can identify the sources of exposure via pathways like consumer products and industrial chemical releases and can state with certainty that people are being harmed by these exposures, particularly because the chemicals are among the better-studied endocrine disrupters.

Lunder said that her role as an advocate is not only to learn from the data but also to identify those sources of human exposure to chemicals that are known to be harmful. A major challenge that the public health community faces is that the United States is behind much of the rest of the developed world in its screening of chemicals on the market and in its ability to prioritize out the hazards posed by chemicals. She traced much of the problem to the Toxic Substances Control Act (TSCA) of 1976, which grandfathered in 62,000 chemicals that were already on the market and were assumed to pose no unreasonable risk to human health or the environment. "We obviously see that that is not a safe assumption

to make about the chemicals that were in widespread use at that point," she said.

EPA faces an incredible burden in trying to take action on those chemicals that were grandfathered in, and so far, under its authority under TSCA, it has been able to ban only polychlorinated biphenyls. The agency also faces a challenge in its screening of new chemicals, she said. Its new chemical program has only 90 days to evaluate chemical applications, and about half of the new chemicals submitted have very little or no data about physical or chemical properties and health endpoints. And, in reality, the companies have little incentive to develop those data because then they would have to be disclosed for the new chemical submission process. "EPA has approved 40,000 new chemicals since TSCA was enacted," Lunder said, "and the names and basic identities of these chemicals are, in many cases, trade secrets and not disclosed—even within EPA, let alone to the greater research world or to the public."

Furthermore, because there is a rapid turnover in the chemical market, 20 percent of the high-volume chemicals in one reporting cycle actually are not even produced in significant volumes in the next. So one very important policy need is finding a way to rapidly screen and address chemicals during the period of time that they are on the market, given that there will always be an influx of new chemicals.

Still, despite these problems, Lunder said, she believes that environmental interventions hold great promise in averting health problems such as obesity and other metabolic disorders. The reason is that, unlike attempts to change individual behavior practices, which often revert to the original practices even after changes have been made, environmental interventions have the potential to create permanent changes that affect an entire population. In many cases, she said, environmental interventions have been cost-effective ways of reducing exposure to hormone disrupters.

Even in the absence of governmental actions, there can be effective responses to the presence of environmental chemicals, Lunder said. The general public is incredibly concerned about the role of environmental chemicals in health, and this has resulted in some rapid changes in the marketplace, such as the removal of BPA from the liners of baby formula containers. Liquid baby formula was commonly sold in cans whose linings contained BPA, resulting in low levels of BPA in baby formula, a product that could be 100 percent of a child's diet for the first 6 to 12 months of life. The removal of BPA from the liner of baby formula cans

is a great example of a quick market change that could drastically reduce exposure during a critical period, she said.

In addition to such positive moves as the ban on phthalates in some children's products, some hard lessons about the complexities of making chemical substitutions have also been learned, Lunder said. For example, she said, a number of savvy consumers began to avoid products with high levels of high-fructose corn syrup, which led to an increased use of organic brown rice syrup as a sweetener in many processed foods. "And now we are realizing that rice, and especially brown rice, is loaded with arsenic," she said, "so people who are buying supposedly more healthy processed food are actually buying food with arsenic in it."

The Environmental Working Group, Lunder said, has been working to identify the most obvious problems concerning consumer exposures, problems for children, and the safety of the food supply and trying to fix them. It has, for example, been performing product testing to identify the presence of various chemicals in foods as a way of raising awareness among consumers and researchers. It has developed several apps for consumers that are intended to highlight the widespread use of endocrine-disrupting chemicals, including an online database of about 80,000 food products, and a similar app that rates cosmetics, soaps, and lotions. It has written numerous public comment letters and lobbied on behalf of TSCA reform for the support of research and biomonitoring for toxic chemicals. "I would like to encourage you guys to join us in this effort," she said.

Finally, Lunder commented that Kristina Rother's statistic about the role of soda in promoting obesity—that drinking only a soda or two per day is associated with a significant increase in body mass index over a long period of time—is very helpful for the public to hear, and it could be very motivating for some people. That sort of clear information that pinpoints problems and helps people prioritize in a nonjudgmental way is really helpful, she said. Researchers should strive to provide more such information.

Judy LaKind

The next discussant was Judy LaKind, the president of LaKind Associates and an adjunct associate professor in the Department of Epidemiology and Public Health at the University of Maryland School of Medicine and the Department of Pediatrics at the Milton S. Hershey Medical Center. She spoke by phone.

LaKind described a systematic review of the epidemiological literature on the relationship between chemical exposure and obesity and obesity-related illnesses that she and her colleagues carried out. In particular they examined BPA, phthalates, and their associations with obesity, diabetes, and heart disease. The papers appeared in *Critical Reviews in Toxicology* in 2014 (Goodman et al., 2014; LaKind et al., 2014a).

Showing a slide that included all the studies that had been published at the time of the review of BPA and obesity, LaKind illustrated the range of results that had been observed. From the slide, she noted, it was obvious that there had been a large number of studies on the subject but also that the results had been inconsistent.

In individual studies there were many times when the authors found inconsistent results, depending on how obesity was assessed, LaKind said, and there were also inconsistencies in results across studies when authors used the same approach for assessing obesity. "So, when you look at the totality of the data, I think what you will see is that it neither supports nor refutes the hypothesis of an association between BPA and obesity," she said.

In the case of the relationship between BPA and diabetes, there were fewer studies but still a substantial number of them, and again, there was a great deal of inconsistency in results across studies. LaKind and her colleagues observed similar inconsistencies for reports on associations between urinary BPA levels and various types of assessments for heart disease. Similar inconsistencies were found for studies examining associations between the levels of phthalate metabolites and obesity, type 2 diabetes, and cardiovascular disease. Overall, the literature does not shed light on whether BPA or phthalates are associated with these three health outcomes.

There are various reasons for this problem, LaKind said. One issue is that these studies are focused on short-lived chemicals. Researchers are quite good at measuring and interpreting data on persistent chemicals, such as dioxins, but best practices for measuring and interpreting biomonitoring data on short-lived chemicals are in the early stages (LaKind et al., 2014b), and studies often include measures that are unlikely to properly capture exposures that are relevant for the time frame of interest (LaKind et al., 2012). In the studies that she and her colleagues looked at, too few samples were generally taken to capture the variability that occurs from hour to hour and day to day or over longer

periods of time. Many of those papers acknowledged that this was a problem, she said.

A second problem arises from matrix adjustment issues. Many of the studies look at urinary measures of chemicals, and it is known that urine dilution varies from person to person and day to day. LaKind pointed out that the best approaches for accounting for these variations are still debated. The method used can have a significant effect on the analyses of associations between exposure and outcome, and, indeed, depending on the method used for matrix adjustment, it is possible to get completely different associations from the same data (Goodman et al., 2014; LaKind and Naiman, in press).

A third issue is the lack of consistency in study design (LaKind et al., in press). This makes it very difficult to compare one study with the next. Yet another issue is that most researchers performing these studies have not taken diet into account as a confounding variable, yet diet is one of the main exposure pathways for BPA, and diet is also critical to understanding obesity and obesity-related disease.

The take-home message, LaKind said, is that research that promotes and provides higher-quality exposure estimates should be supported if this research is to be useful in public health decision making.

Sheela Sathyanarayana

The last presenter was Sheela Sathyanarayana, an associate professor of pediatrics in the Department of Environmental and Occupational Health Sciences at the University of Washington and chair of EPA Children's Health Protection Advisory Committee. She was representing the American Academy of Pediatrics at the workshop.

She began by describing some of the characteristics of good environmental policy. To start, it should take all stakeholder opinions into account. It should be well written, concise, and easy to understand. In particular, because many members of the public have a difficult time understanding environmental exposures, policy needs to be written in a way that they can easily understand and that allows them to implement it in their daily lives.

It can be very difficult to write a policy that is effective in achieving its goal, Sathyanarayana said. Many times policies are put into place without evidence about whether they will be effective, and it is only years later, after studies have been done to examine the results, that it becomes clear that they were not effective.

Good environmental policy should consider the weight and strength of the evidence. Without concrete evidence concerning the harms of different chemicals, one can use screens to detect and prioritize chemicals and then do a risk assessment to estimate the various risks. Risk assessment is at the core of a lot of environmental policy that gets put into effect, Sathyanarayana said.

Good environmental policy should also take into account a cost–benefit analysis. This should include the cost of imposing the regulation but also the cost of doing nothing.

Who should be putting forth the policy? There are many possibilities: medical schools can carry out changes in medical school curricula. The American Academy of Pediatrics could revise its guidelines to clinicians. And even within an organization it is not always clear who should put forth the policy. Within the American Academy of Pediatrics, for example, it could be the Council on Environmental Health, the Council on Endocrinology, or perhaps representatives from the entire organization.

The individual states can put policies in place. Sathyanarayana explained that Washington State has placed limits on the amounts of certain chemicals that can be in children's products to protect their health. "But I can say, having sat on the governor's committee to implement these regulations, it is really difficult to implement on the ground level," she added.

The federal government can also put policies into place. For instance, the Chemical Safety Improvement Act was introduced in the Senate in 2013 to reform TSCA, but it has not been moving forward.

To conclude, Sathyanarayana spoke about the difficulties of making a difference through changing individual behavior. She works with the Pediatric Environmental Health Specialty Unit, a national organization of networks across the country that does environmental health consults for families. It provides a document that offers various recommendations on how to reduce exposures to phthalates and BPA: buy low-fat dairy products such as skim milk and low-fat cheeses, buy fresh or frozen fruits and vegetables when possible, avoid canned and processed foods, and so on. The document also recommends to people that they avoid certain plastics and, ideally, to use stainless steel or glass when possible.

To determine how effective it is to provide families with written guidelines on reducing exposures to phthalates and BPA, Sathyanarayana and colleagues performed a study. They recruited 10 families with two children between the ages of 4 and 8 years. Half got a catered dietary

intervention, and the other half got educational handouts. They expected that the half with the catered intervention would have lower levels of phthalates and BPA at the end of the trial because they were eating food that was carefully prepared to avoid contact with materials containing these chemicals. On the other hand, they expected that the half given the written guidelines would show little or no change because they were unlikely to change their food preparation and eating patterns significantly.

What they found at the end of the trial, however, surprised them. The half given the educational handouts showed no change, as expected, but the half given the catered meals showed a large increase in phthalate concentrations in their blood. It turns out that the ground coriander used in preparing the catered meals had a huge amount of phthalate in it. The lesson is this: it can be very difficult to advise people on how to avoid these exposures because even the most careful, doctor-designed programs may not do what they are expected to do.

DISCUSSION

The discussion period began with a question from a Web audience member, Janet Young, who referred to Judy LaKind's comments about inconsistencies in studies on the effects of environmental chemicals and commented that in 2009 *Chemical and Engineering News* reported that gut bacteria influence the toxicity and effectiveness of pharmaceuticals. Her question was, Wouldn't the individual gut microbiome signature affect the toxicity of exogenous chemicals and, therefore, research outcomes?

LaKind responded that it is not an area she works in, so she could not speak to the issue directly, but there are dozens or even hundreds of different factors that affect the outcomes that are observed. However, she added that it would not surprise her if the microbiome was found to play a role and add to the complexities in examining exposure–outcome associations.

Lynn Goldman of George Washington University agreed with LaKind, saying that she has reviewed many of these studies herself, and the exposures that are likely of most importance—those that have occurred over years or decades or even prenatally—are generally not the exposures that can be measured. The studies end up measuring more immediate, short-term exposures, which do not provide any sense of the cumulative burden.

Linda Birnbaum of the National Institute of Environmental Health Sciences also agreed, commenting that many human epidemiological

studies of the short-lived chemicals, even if they are longitudinal, are often based on a single urine measurement, and it is well known that for some of the short-lived chemicals, this can be completely misleading. There are some studies with BPA levels that indicate that it requires at least four to seven urine measurements to have any idea of what the average urine levels may be, and even then there may be no good idea of what the peak concentrations are, which may also be important.

Sheela Sathyanarayana commented that it is known that the highest exposure concentrations for many of these chemicals, because they are derived from the diet, come at night after an entire day of eating. Thus, when they do their studies they ask their study participants to get samples at night. Most of the epidemiological studies do not do this, however, so Sathyanarayana estimated that the exposure estimates are underestimating the actual body burdens by a significant amount.

Dennis Devlin from ExxonMobil said that one of the concerns that has been expressed in a small industry group that has been working on revisions to the TSCA is whether EPA will find it too difficult to declare that chemicals are safe for human use, which is one of the approaches that many are calling for in the revision of TSCA. Because EPA's culture is designed more for saying no to hazardous chemicals, he suggested, the agency might have a difficult time saying, "Yes, this chemical is completely safe." One particular concern is that EPA might be swayed by the history of chemicals that were once thought to be safe and that later turned out to threaten health or the environment in one way or another. So, he asked, will this proposed new role for EPA demand a tremendous cultural change at the agency, and how difficult will it be to create that change?

Goldman answered that, having served as an assistant administrator at EPA, she does indeed believe that EPA is capable of making such judgments; indeed, it already makes such decisions in a variety of situations, as in determining significant new use rules. The real challenge for EPA, she said, is identifying which chemicals are likely to be bioactive and to pay much more attention to those and their intended uses. Nor did she think that EPA will be particularly bothered by the possibility that a chemical judged to be safe at one point turns out years later to have unexpected consequences. "Is it possible the judgment will change? Well, I hope so. Science moves onward, and things do change, so I think that is important."

She added that she believes that the U.S. Food and Drug Administration (FDA) will have to be part of the big picture of regulating

various chemicals that may play a role in obesity. If a chemical is used as a food additive, for example, EPA is not allowed to regulate that use; instead, FDA must decide on its safety. "So the capacity of FDA to be able to scrutinize those [chemicals] and, if you may, the willingness of FDA to use its authority to look at those [are] extremely important," she said. Reforming TSCA may be necessary for improvement in this area, but it will not be sufficient. FDA will also have to pay attention to such chemicals.

Furthermore, she added, the private sector has a major role to play in all of this as well. It has already been demonstrated how market demand can influence such things as the presence of BPA in food containers, but it is also the case that a number of corporations are moving away from some of these substances on their own. Johnson & Johnson, for example, took the formaldehyde out of its baby shampoos without any order from EPA. Thus, many changes may be made without the creation of new regulations, but those changes will not come quickly enough, she said, as long as there are such large gaps in knowledge about these chemicals.

Frank Loy commented that TSCA reform is the biggest single legislative effort that the health community and the environmental community are going to see but that it will inevitably be a bipartisan effort, which means that, from the health community's point of view, it will not be the absolutely ideal bill. It is going to have serious flaws, he said. Thus, the health community and the nongovernmental organization community will have to decide whether it is good enough. It is impossible to predict exactly what the bill will look like, but it will not satisfy everybody.

One of the things that the bill will have, he predicted, is preemption; that is, it will prohibit certain state-level rules or certain future state-level rules from making some tests tougher than the level set forth in the bill itself. "We are going to have to make decisions like that," he said, "and I would just say, having been in the game of politics for a while … if we do not get a bill this time, we are not going to get one for another *x* years. So taking an imperfect bill and dumping it is probably going to leave us where we are for a very long time."

Nsedu Witherspoon of the Children's Environmental Health Network asked for some key messages that public health and child health policy advocate communities may be able to prioritize and rally around to reduce some of these exposures and related health outcomes.

Sonya Lunder suggested targeting some "egregious" uses of endocrine-disrupting chemicals in consumer products. As examples, she

pointed to the polyvinyl chloride (PVC) used in medical tubing and also in food processing. Much of the contamination in food happens during the processing stage, she said, so getting the PVC out is of critical importance. "I think we have reached the point where that should be a no-brainer," she said, "and I would love everybody's help on that."

Birnbaum cautioned, however, that one must think carefully about alternatives. Newborns in the neonatal intensive care unit may be particularly vulnerable to the diethylhexyl phthalate softener used to make PVC medical tubing, but they still require some sort of tubing in their treatment, and whatever is chosen as a substitute may have its own, lesser-known problems.

Concerning the issue of key messages, Goldman suggested that one bit of advice for parents is that they should themselves take control over different aspects of what their children are exposed to. For instance, they should pay attention to the commercial advertising that their children see—during Saturday morning children's programming, for instance—because many of those commercials are for junk food, and studies have shown that young children are not critical about advertising messages. Parents should also take control over what their children are served at school, she suggested. They should work to get the sodas and the junk food out and the fresh fruits and vegetables in.

Henry Anderson of the Wisconsin Division of Public Health said that it would be very useful for consumers to have an idea of exactly where different chemical exposures are coming from. It would be very helpful, for instance, if the public were told whether they should worry about eating canned food or whether they need to worry about food that has been kept in plastic containers for several days.

LaKind responded that she thought that this was a very good point. With the advent of biomonitoring, it has become possible to get very good measurements of what a person has been exposed to, but it is often difficult to interpret all those data because the idea of source apportionment—knowing exactly how much exposure is coming from each type of source—has been lost. While the traditional methods of exposure science, which inherently included source apportionment, might feel outdated to some people, she said, they are extremely important and capture information not available from biomonitoring.

Sathyanarayana commented that such source apportionment can be very complicated for a number of reasons, one of which is that manufacturers change their formulations constantly. In personal care products, for example, the concentration of chemicals tested one year may

be different in the exact same product when tested the next year, she explained. It is the same with foods, she added. There are often changes in processing over time, which changes the sorts of chemicals that the foods are exposed to—and contaminated with—during processing, so it can be difficult to say exactly where certain chemical exposures arise.

Linda McCauley of Emory University asked if any of the clinicians at the workshop might comment on the best ways for health practitioners to talk with members of the public about the various issues related to environmental exposures and obesity.

Jeanne Conry replied that ACOG is working with its board certification unit to provide information for physicians to read as part of their board certification so that they are exposed to and understand some of what is going on in this area. It is an incremental process, she said, working with the board certification and then with the education of medical students and with the education of residents.

Sathyanarayana described the sort of scenario that she might see in her clinic and how she deals with it. An obese 5-year-old is visiting the clinic, and Sathyanarayana has about half an hour with him or her. She first talks about the growth curve, making sure that the parents understand that the child is gaining weight at a rate that is more rapid than normal and making sure that they understand what obesity is, why it is important, and what it can lead to. Then she talks about the child's diet. If there are obvious problems—such as one case where the family was consuming five 2-liter bottles of soda every week—she explains what they should be doing instead. And she also talks about other things to do around the home to keep the child safe and reduce exposures.

LaKind offered two closing thoughts. First, she suggested that in doing studies of the effects of environmental exposures and obesity, it will be important for the research teams to include exposure scientists, who could offer valuable expertise and insights. Second, she reiterated an earlier point that it will be important to be careful with alternative products. Otherwise, there may be a move to alternatives that end up posing a greater risk (LaKind and Birnbaum, 2010).

Sathyanarayana referred back to the observation that evaluation of the interplay between environmental exposures and obesity is a broad, multidisciplinary field, and she said that just as attacking its issues will require researchers from different areas to work together, coming up with effective policies will require policy makers to work together to come up with comprehensive policies that address all the different aspects of obesity management—not just the environmental exposures but also all

the other components that were not specifically addressed in the workshop.

Goldman said that she had been struck by the differences in the federal response to, on the one hand, issues of physical activity and diet and, on the other hand, issues related to environmental exposures and obesity. For the first, she noted, there has been remarkable White House leadership, with the First Lady being very engaged, bringing together people from federal agencies, foundations, and industry to work on the issue. But the second issue has not been a part of that. The question, then, is how to get issues related to environmental exposures and obesity to be considered part of that broader effort to fight obesity. Dealing effectively with environmental exposures and obesity will require an effort broader than that which has been waged to date, she said. "It needs to bring in more of the science, and it needs to bring in FDA, EPA, and others to be able to assume their responsibilities in this…. So we have a way to go before we have a policy framework that actually is addressing these issues."

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Workshop Agenda

March 2–3, 2015

National Institute of Environmental Health Sciences
Building 101, Rodbell ABC
111 T.W. Alexander Drive
Research Triangle Park, NC

Workshop Objectives:
The workshop will explore the role of chemical exposures in the development of obesity through sessions focused on a life span view, possible biologic pathways and environmental influences, and effects of food additives and antibiotics. Speakers will make links between exposure to environmental chemicals and increased incidence of weight gain, glucose tolerance and insulin sensitivity, inflammation, and aspects of metabolic syndrome in animal models and human studies. Two panels at the end of the workshop will provide a chance to discuss opportunities for new research and possible policy actions to address exposure to chemicals associated with the development of obesity.

March 2, 2015

8:30 am	**Welcome and Objectives** Frank Loy, LL.B. Roundtable Chair
	Linda S. Birnbaum, Ph.D., DABT, ATS Roundtable Member Director, National Institute of Environmental Health Sciences

Session 1: Framing the Problem	
Objectives:	Explore the multiple pathways involved in the risk of obesity from two perspectives: public health and environmental health. Discuss how these disciplines intersect and diverge in their focus on the causes of obesity. Set the stage for the discussions to follow.
8:45 am	**Introduction to Session and Speakers** Frank Loy, LL.B. Roundtable Chair
8:50 am	**Public Health Overview** William H. Dietz, Ph.D. (*by phone*) Director, Sumner M. Redstone Global Center for Prevention and Wellness Milken Institute School of Public Health George Washington University
9:10 am	**Environmental Health Overview** Jerry Heindel, Ph.D. Health Scientist Administrator, Population Health Branch National Institute of Environmental Health Sciences
9:30 am	**Discussion** (20 minutes)

Session 2: Life Span View of the Role of Chemical Exposures and Obesity	
Objective:	Provide a life span view of obesity focusing on chemical exposures from pregnancy to childhood to adulthood.
9:50 am	**Introduction to Session and Speakers** Gwen Collman, Ph.D. Director, Division of Extramural Research and Training National Institute of Environmental Health Sciences

10:00 am	**The Role of Prenatal Exposure to Persistent Organic Pollutants on Childhood Obesity: Evidence from Epidemiological Studies** Damaskini (Dania) Valvi, M.D., M.P.H., Ph.D. Research Fellow, Department of Environmental Health Harvard T.H. Chan School of Public Health
10:30 am	**Endocrine-Disrupting Chemicals, Onset of Puberty, and Obesity** Frank M. Biro, M.D. Director of Research, Adolescent and Transition Medicine Cincinnati Children's Hospital Medical Center Professor, Department of Pediatrics University of Cincinnati College of Medicine
11:00 am	**Break** (20 minutes)
11:20 am	**OBELIX (OBesogenic Endocrine Disrupting Chemicals: LInking Prenatal EXposure to the Development of Obesity Later in Life): An Integrated Approach to Studying the In Vitro, Clinical, and Epidemiological Effects of Endocrine-Disruptor Exposure Prenatally and in Early Infancy** Juliette Legler, Ph.D. (*by phone*) Professor and Deputy Head, Department of Chemistry and Biology Institute for Environmental Studies Vrije University Amsterdam
11:50 am	**Discussion** (50 minutes)
12:40 pm	**Lunch Break** (60 minutes)

Session 3: Biologic Pathways and Environmental Influences

Objectives:	Discuss biologic pathways involved in obesity and how these could be disturbed by environmental chemical exposures.
1:40 pm	**Introduction to Session and Speakers** Henry Anderson, M.D. Roundtable Member State Health Officer Wisconsin Division of Public Health
1:50 pm	**Identifying Environmental Chemicals to Test for Obesity and Diabetes Outcomes: Clues from Toxcast High-Throughput Screening Data** Scott S. Auerbach, Ph.D. Molecular Toxicologist, Biomolecular Screening Branch, National Toxicology Program (NTP) National Institute of Environmental Health Sciences
2:20 pm	**The Effects of Persistent Organic Pollutants (POPs) on Adipose Tissue Function and Inflammation: In Vitro and In Vivo Models and Studies in Humans** Robert Barouki, M.D. Professor University Paris Descartes
2:50 pm	**Transgenerational Effects of Obesogens: Tributyltin** Bruce Blumberg, Ph.D. Professor, Developmental and Cell Biology School of Biological Sciences Professor, Biomedical Engineering The Henry Samueli School of Engineering University of California, Irvine
3:20 pm	**Break** (20 minutes)

3:40 pm	**Effects of Perinatal Exposure to BPA on Obesity and Metabolic Disease Later in Life** Beverly Rubin, Ph.D. Associate Professor of Integrative Physiology and Pathobiology Sackler School of Graduate Biomedical Sciences Tufts University
4:10 pm	**Effects of Environmental Chemicals on Energy Metabolism and Insulin Secretion** Barbara Corkey, Ph.D. Zoltan Kohn Professor Boston University School of Medicine
4:40 pm	**Panel Discussion** (50 minutes)
5:30 pm	**Adjourn for the Day**

March 3, 2015

8:30 am	**Welcome Back and Introduction** Lynn Goldman, M.D., M.S., M.P.H. Roundtable Vice-Chair Dean, Milken Institute School of Public Health George Washington University

Session 4: Nutrients, Food Additives, Antibiotics

Objective:	Present research on how antibiotics and agents in our food, such as high-fructose corn syrup and artificial sweeteners, may play a role in the development of obesity.
8:40 am	**Introduction to Session and Speakers** Lynn Goldman, M.D., M.S., M.P.H. Roundtable Vice-Chair

8:50 am	**Infectobesity: Obesity of Infectious Origins** Nikhil V. Dhurandhar, Ph.D. (*by phone*) Chair, Department of Nutritional Sciences Texas Tech University
9:20 am	**Antibiotics and Obesity** Charles Bailey, M.D., Ph.D. (*by phone*) Assistant Professor of Clinical Pediatrics, Divisions of Hematology & Oncology Children's Hospital of Philadelphia
9:50 am	**Sugar and Obesity** Ayca Erkin-Cakmak, M.D., M.P.H. Clinical Research Associate University of California, San Francisco
10:20 am	**Noncaloric Sweeteners and Obesity** Kristina Rother, M.D., M.H.Sc. Chief, Diabetes, Endocrinology, and Obesity Branch National Institute of Diabetes and Digestive and Kidney Diseases
10:50 am	**Panel Discussion** (50 minutes)
11:40 am	**Lunch Break** (60 minutes)

Session 5: Research Needs

Objective:	Identify opportunities for new research directions based on the discussions at the workshop.
12:40 pm	**Introduction to Session and Discussants** Harold Zenick, Ph.D. Roundtable Member Former Director of the Office of Research and Development National Health and Environmental Effects Research Laboratory U.S. Environmental Protection Agency

12:50 pm	**Discussants:** Linda S. Birnbaum, , Ph.D., DABT, ATS Roundtable Member Suzette M. Kimball, Ph.D. Roundtable Member (*by phone*) Acting Director, U.S. Geological Survey John M. Rogers, Ph.D. Director, Toxicity Assessment Division National Health and Environmental Effects Research Laboratory U.S. Environmental Protection Agency Nikhil V. Dhurandhar, Ph.D. (*by phone*) President, Obesity Society
1:20 pm	**Panel Discussion** (60 minutes)

Session 6: Policy Solutions

Objective:	Discuss possible actions to reduce exposure to chemicals associated with the development of obesity.
2:20 pm	**Introduction to Session and Discussants** Faiyez Bhojani, M.D. Roundtable Member Chief Medical Officer, Global Manufacturing and Chemicals Royal Dutch Shell
2:30 pm	**Discussants:** Jeanne Conry, M.D. (*by phone*) Assistant Physician-in-Chief, Kaiser Permanente Past President, American Congress on Obstetrics and Gynecology

Judy LaKind, Ph.D. (*by phone*)
President, LaKind Associates, LLC
Adjunct Associate Professor, Department of Epidemiology and Public Health
University of Maryland School of Medicine

Sheela Sathyanarayana, M.D., M.P.H.
Associate Professor, Departments of Pediatrics and Environmental and Occupational Health Sciences
University of Washington
Member, Council on Environmental Health
American Academy of Pediatrics

Sonya Lunder, M.P.H.
Senior Analyst
Environmental Working Group

3:00 pm **Panel Discussion** (50 minutes)

3:50 pm **Closing Remarks**
Frank Loy, LL.B
Roundtable Chair

4:00 pm **Adjourn**

B

Speaker Biographical Sketches

Scott S. Auerbach, Ph.D., is a molecular toxicologist in the Molecular Toxicology and Informatics Group within the Biomolecular Screening Branch of the National Toxicology Program (NTP) Division. His primary role is the analysis and interpretation of multivariate data sets (i.e., data sets containing data from microarray analysis, RNA sequencing, and high-throughput screening). His specific responsibilities include oversight of the NTP DrugMatrix Database and ToxFX, multivariate data analysis and modeling with the purpose of prioritizing chemicals for targeted toxicological assessment, use of machine learning approaches to develop multivariate data models that diagnose and predict toxicological pathology and disease, application of short-term in vivo transcriptomic studies to allow the identification of genomic benchmark dose values and mechanistic characterization, and pathway- and network-level characterization of toxicity-related omics results with the goal of understanding mechanisms of toxicity. He received a B.S. in biochemistry and molecular biology from The Pennsylvania State University and a Ph.D. in pharmacology from the University of Washington.

Charles Bailey, M.D., Ph.D., is a member of the Divisions of Oncology and Hematology at Children's Hospital of Philadelphia and an assistant professor of clinical pediatrics at the University of Pennsylvania. As part of the general oncology group, he cares for patients with a variety of tumors. His focus is on leukemia and lymphoma and particularly on acute lymphoblastic leukemia, the most common type of childhood cancer. In addition to caring for patients on the inpatient service and in the clinic, Dr. Bailey serves as part of the steering committee for the division's Leukemia/Lymphoma Group. Dr. Bailey also serves as the lead investigator for the Data Coordinating Center of PEDSnet, a collaboration across pediatric academic centers to provide a standardized model for clinical data that are accessible for observational research and clinical trials as part of the PCORnet national research network (an initiative of the Patient-Centered Outcomes Research Institute [PCORI]).

Other work includes the development of pediatric quality measures through the use of electronic records and clinical data to improve outcomes for children with complex diseases.

Robert Barouki, M.D., is a biochemist and molecular biologist whose main focus during the past 15 years has been understanding the mechanisms of toxicity of environmental pollutants, such as dioxin. In particular, he has studied the biological consequences following the activation of the dioxin receptor aryl hydrocarbon receptor (AhR). Dr. Barouki initially focused on consequences related to cellular stresses, such as oxidative stress and endoplasmic reticulum stress. He subsequently studied the different effects triggered by different ligands of the AhR using, in particular, omics technologies, suggesting that part of the toxicity may be related to the disruption of endogenous functions. Recently, his main focus has been on developmentally relevant cellular effects that are disrupted by the AhR, notably, the epithelial–mesenchymal transition. In addition to cancer development, he is now focusing on the effects of pollutants on adipose tissue functions and on the nervous system in rodents and in Caenorhabditis elegans. His additional projects include clinical studies in obese individuals, as well as studies on the toxicity of drugs and ethanol and the development of relevant biomarkers in humans.

Linda S. Birnbaum, Ph.D., DABT, ATS, is the director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP), located in Research Triangle Park, North Carolina. She oversees a $740 million budget that funds multi-disciplinary biomedical research programs, prevention, and intervention efforts that encompass training, education, technology transfer, and community outreach. In most years, NIEHS supports more than 1,000 research grants. A board-certified toxicologist, Dr. Birnbaum has served as a federal scientist for nearly 35 years. She received a Ph.D. in microbiology from the University of Illinois at Urbana-Champaign, and has held several different positions with both NIEHS and the U.S. Environmental Protection Agency. She is a diplomate of the American Board of Toxicology, a fellow of the Academy of Toxicological Sciences, a Collegium Ramazzini Fellow, and a member of the National Academy of Medicine.

Dr. Birnbaum is an adjunct professor at the University of North Carolina at Chapel Hill and at Duke University. She is a former vice president of the American Aging Association, a former chair of the Division of Toxicology of the American Society of Pharmacology and

Experimental Therapeutics, and a former president of the Society of Toxicology.

Frank Biro, M.D., is the director of research, adolescent, and transition medicine at Cincinnati Children's Hospital Medical Center and a professor in the Department of Pediatrics at the University of Cincinnati. His work focuses on pubertal maturation; that is, how the timing of puberty is impacted by the physical, chemical, and social environment and how changes associated with puberty impact adolescent and adult morbidity and mortality. Dr. Biro is currently working on several grants funded through the National Institute of Environmental Health Sciences, the National Cancer Institute, and the National Institute of Child Health and Human Development. Dr. Biro is the principal investigator of the Cincinnati Breast Cancer and the Environment Research Program (BCERP), which is a joint effort of researchers from Cincinnati Children's Hospital Medical Center and the University of Cincinnati, local breast cancer support and service organizations, and local breast cancer survivors and advocates. The goals of BCERP include researching the impact of environmental factors on puberty through epidemiological studies, researching the relationship between biochemical processes associated with obesity and cancer susceptibility, and researching the relationship between the age of breast development and the onset of menarche, the first menstrual cycle. The Growing Up Female project is a collaborative program in which BCERP research examines the role that genetic-level markers and social, environmental, and lifestyle factors play in the timing of puberty. The researchers are specifically interested in defining how diet and environmental exposures affect when a girl starts puberty, how a girl's genes and her social environment affect when she starts puberty, and what personal and environmental factors are associated with how a girl matures through puberty.

Bruce Blumberg, Ph.D., is currently a professor of developmental and cell biology, pharmaceutical sciences, and biomedical engineering at the University of California, Irvine. Dr. Blumberg received a Ph.D. in biology from the University of California, Los Angeles (UCLA). His postdoctoral training was in the molecular embryology of vertebrate development at the School of Medicine at UCLA. He was appointed as a staff scientist at The Salk Institute for Biological Studies in La Jolla, California, and joined the faculty at University of California, Irvine, in 1998.

Jeanne Conry, M.D., Ph.D., is assistant physician-in-chief at Kaiser Permanente Roseville Medical Center and associate clinical professor of obstetrics-gynecology (OB-GYN) at the University of California, Davis. She has been practicing OB-GYN at Kaiser Permanente for more than 20 years. Dr. Conry's professional interests include menopausal health and preconception care. She has brought the value of those interests to her role as assistant physician-in-chief, in which she shaped the Roseville group's chronic conditions management program to include women's health needs and preconception care. She was also instrumental in overseeing the development of the Roseville Kaiser Permanente Women and Children's Center. Dr. Conry served as chair of the California Preconception Care Council from 2006 to 2010 and currently serves on the Centers for Disease Control and Prevention Select Panel on Preconception, a coalition of government and health care providers that seeks to improve pregnancy outcomes by emphasizing the need for healthy choices across the reproductive life span of women. Dr. Conry earned a medical degree from the University of California, Davis. She also holds a doctor of philosophy in biology from the University of Colorado Boulder.

Barbara E. Corkey, Ph.D., is the Zoltan Kohn Professor of Medicine and Biochemistry at the Boston University School of Medicine. She serves as director of American Diabetes Association Inc. and AdipoGenix Inc. and as director of the city-wide National Institutes of Health (NIH)-funded Boston Obesity/Nutrition Research Center. She serves as director of the Obesity Research Center at the Boston University School of Medicine. She has a long-standing interest in metabolism as it relates to obesity. Her main areas of research are obesity and the biochemistry of insulin in the adipose tissue. She has published more than 100 peer-reviewed articles and is frequently an invited speaker at national and international institutions and symposia. She was awarded the BEST award for her groundbreaking work in diabetes, and she is the recipient of an NIH MERIT Award (provides long-term, stable support to investigators whose research competence and productivity are distinctly superior), and the Charles H. Best Lectureship and Award from the University of Toronto. Dr. Corkey received a Ph.D. in biochemistry and biophysics from the University of Pennsylvania.

Nikhil V. Dhurandhar, Ph.D., is professor and chair of the Department of Nutritional Sciences at Texas Tech University, Lubbock, Texas. He is president of The Obesity Society for 2014–2015 and an editor of the *International Journal of Obesity*. As a physician and nutritional biochemist, he has been involved with obesity treatment and research for more than 20 years. Dr. Dhurandhar coined the term "infectobesity," that is, obesity of infectious origin. Dr. Dhurandhar and colleagues were the first to identify adipogenic effects of an avian adenovirus (SMAM-1) and a human adenovirus (Ad36) and the first to report on the beneficial effects of Ad36, particularly on glucose metabolism. He believes that simple explanations for causes of obesity are inadequate and novel approaches are required for its effective management. Dr. Dhurandhar has received research funding from the National Institutes of Health, the American Diabetes Association, the Federal Emergency Management Agency, and other nonprofit or commercial funding sources; has published more than 100 scientific articles and book chapters; and has served as a mentor or adviser for several students and postdoctoral fellows.

William Dietz, M.D., Ph.D., is the director of the Sumner Redstone Global Center for Prevention and Wellness at the Milken Institute School of Public Health at George Washington University. Previously, he held positions at the Centers for Disease Control and Prevention, Tufts University, and the Floating Hospital of New England Medical Center Hospitals. He is a past president of the American Society for Clinical Nutrition and the North American Association for the Study of Obesity and was a member of the advisory board of the Institute of Nutrition, Metabolism, and Diabetes of the Canadian Institutes for Health Research and of the 1995 Dietary Guidelines Advisory Committee. In 1998, Dr. Dietz was elected to the National Academy of Medicine. An author and editor, his books include *Clinical Obesity in Adults and Children and* Nutrition: What Every Parent Needs to Know. Dr. Dietz earned a B.A. from Wesleyan University, an M.D. from the University of Pennsylvania, and a Ph.D. from the Massachusetts Institute of Technology.

Ayca Erkin-Cakmak, M.D., M.P.H., received a medical degree from the Istanbul University Cerrahpasa School of Medicine in Istanbul, Turkey. After graduating from the medical school, she completed a pediatrics residency program at the Istanbul University Medical School Hospital. As a first-year resident, she did a 4-week rotation in the Department of Pediatrics at the Yale-New Haven Hospital, where she

had a chance to observe the differences between the health care systems in Turkey and the United States. That 1-month period convinced her to pursue a career in the United States. After residency, Dr. Erkin-Cakmak worked as a visiting physician and clinical researcher in the Pediatric Endocrinology Division at the Yale-New Haven Hospital. She participated in research projects investigating the effectiveness of different insulin regimens in the management of newly diagnosed type 1 diabetes and contributed to clinical studies evaluating the effect of metformin on menstrual irregularity in diabetic adolescents. Dr. Erkin-Cakmak completed training in public health at the University of California, Berkeley, School of Public Health, and specifically studied maternal and child health. She currently works at the University of California, San Francisco, as a clinical research associate with Dr. Robert Lustig in a study investigating the effects of reductions in the amount of sugar in the diet on metabolic health among obese children.

Jerrold (Jerry) Heindel, Ph.D., received a doctorate in biochemistry from the University of Michigan and worked in the area of reproductive biology and toxicology while on the faculty at the University of Texas Medical School at Houston and the University of Mississippi before coming to the National Institute of Environmental Health Sciences (NIEHS) to head its reproductive and developmental toxicology group. Twenty years ago he moved to the Division of Extramural Research and Training at NIEHS, where as a scientific program administrator he is responsible for designing, developing, administering, and assessing the impact of the NIEHS grants programs in endocrine disrupters, the developmental basis of diseases, reproductive toxicology, and obesity and diabetes.

Suzette M. Kimball, Ph.D., is acting director of the U.S. Geological Survey (USGS), the scientific agency of the U.S. Department of the Interior. Dr. Kimball was previously director of the Survey's Eastern Region. Dr. Kimball provides executive leadership of USGS geologic investigations on the past, present, and future conditions of Earth's environment, hazards, and resources. Specifically, she is responsible for basic earth science programs, including monitoring of worldwide earthquake hazards, geologic mapping of land and seafloor resources, the study of volcano and landslide hazards, and research and assessments of mineral and energy resources. As director of the Eastern Region of USGS, Dr. Kimball led multidisciplinary science programs in geology,

hydrology, biology, and geography covering the 26 U.S. states east of the Mississippi River, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands. The USGS Eastern Region includes more than 2,600 employees in about 120 locations. Dr. Kimball received a B.A. in English from the College of William & Mary, an M.S. in geology/geophysics from Ball State University, and a Ph.D. in environmental sciences/coastal and oceanographic processes from the University of Virginia.

Judy S. LaKind, Ph.D., is president of LaKind Associates, LLC, an adjunct associate professor in the Department of Epidemiology and Public Health, University of Maryland School of Medicine, and an adjunct associate professor in the Department of Pediatrics, Pennsylvania State University College of Medicine Milton S. Hershey Medical Center. Dr. LaKind is a health and environmental scientist with expertise in exposure science, assessment of human health risks, biomonitoring, scientific and technical analysis for regulatory support, and state-of-the-science reviews.

Dr. LaKind has spoken and published extensively on exposure- and risk-related issues, including children's exposures to environmental chemicals, the implications of uncertainty in the risk assessment process, weighing potential risks and benefits related to chemical use, the presence of environmental chemicals in human milk, and the time dependence and distributional analysis of exposure. Dr. LaKind has evaluated the use of human health risk assessment in the development of water quality criteria and has critically analyzed the environmental fate, behavior, and bioavailability of pollutants in the context of setting regulatory criteria. She has developed risk assessments for a variety of urban industrial sites, military bases, and firing ranges and has utilized state-of-the-science models for estimating blood lead levels in adults and children.

Dr. LaKind has taught graduate-level courses in risk assessment and aquatic chemistry at Johns Hopkins University and the University of Maryland. She serves on the editorial boards of the Journal of Toxicology and Environmental Health and Environment International and is past associate editor for the Journal of Exposure Science and Environmental Epidemiology. Dr. LaKind is a member of the World Health Organization (WHO) Survey Coordinating Committee for the WHO Global Survey of Human Milk for Persistent Organic Pollutants (POPs), a counselor with the International Society of Exposure Science, and a board member of the National Swimming Pool Foundation. She is a former

member of Maryland's Children's Environmental Health and Protection Advisory Council, the Lead Poisoning Prevention Commission, and the Maryland Pesticide Reporting and Information Workgroup. Dr. LaKind also served on the Institute of Medicine Committee on Blue Water Navy Vietnam Veterans and Agent Orange Exposure and the U.S. Environmental Protection Agency Science Advisory Board Panel on Perchlorate: Approaches for Deriving Maximum Contaminant Level Goals for Drinking Water.

Juliette Legler, Ph.D., is a toxicologist with training in environmental sciences, aquatic ecotoxicology, and molecular biology. She is a professor of toxicology and environmental health and deputy head of the Institute for Environmental Studies' Department of Chemistry and Biology at the Vrije University (VU) Amsterdam. She teaches toxicology at the B.Sc. and M.Sc. levels at VU and is coordinator of the M.Sc. ecology–environmental chemistry and toxicology specialization. Her research focuses on determination of the molecular mechanisms of toxicity of chemicals and their effects on humans and the environment. She is coordinator of the European Union OBELIX (OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life) and NWO-VIDI projects (NWO is a national research organization in the Netherlands; VIDI offers individual grants to researchers), which study possible links between early-life-stage exposure to endocrine-disrupting chemicals and the development of obesity later in life. In addition, she participates on various advisory committees, such as committees of the Dutch Health Council and the Organisation for Economic Co-operation and Development.

Sonya Lunder, M.P.H., is a senior analyst at the Environmental Working Group. Prior to joining the Environmental Working Group in 2002, Ms. Lunder managed a community health intervention at a Superfund site and worked on epidemiology studies at California's Environmental Health Investigations Branch. Her research at the Environmental Working Group focuses on toxic chemicals in food, water, air, and consumer products. Ms. Lunder holds a master's of public health in environmental health sciences from the University of California, Berkeley.

John M. Rogers, Ph.D., is the director of the Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency (EPA), Research Triangle Park,

North Carolina. Prior to that he served as chief of the Developmental Biology Branch, and he has been with EPA for 30 years. Dr. Rogers is also a graduate faculty affiliate in the curriculum in toxicology, University of North Carolina at Chapel Hill, and an adjunct professor at the North Carolina State University College of Veterinary Medicine. He received a Ph.D. in biology from the University of Miami and was a National Eye Institute postdoctoral fellow at the University of California, Davis. Dr. Rogers's research addresses mechanisms of abnormal development, including maternally mediated developmental toxicity, maternal nutrition, and the developmental origins of health and disease. Dr. Rogers is a past president of the Teratology Society, a member of the Society of Toxicology (SOT), a past president of the Reproductive and Developmental Toxicity Specialty Section of SOT, and a member of the International Society for Developmental Origins of Health and Disease.

Kristina Rother, M.D., M.H.S., is a clinical investigator in the Diabetes, Endocrinology, and Obesity Branch at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Dr. Rother received an M.D. degree at the University of Freiburg in Germany. She completed a residency in pediatrics at the Mayo Clinic and fellowships in pediatric endocrinology at the Mayo Clinic, Massachusetts General Hospital, and the Children's Hospital in Zurich, Switzerland. In 2008, Dr. Rother earned a master of health sciences degree in clinical research through a collaboration between the National Institutes of Health Clinical Center and Duke University Medical Center. She is the principal investigator on a bench-to-bedside study and has been in her current position since 2000. Dr. Rother's research has focused on islet cell transplantation, islet cell regeneration, and beta cell preservation in pediatric and adult patients with type 1 diabetes. Most recently, her research interests have included pediatric type 2 diabetes, how bariatric surgery works to resolve type 2 diabetes in adults, and the metabolic effects of artificial sweeteners.

Beverly Rubin, Ph.D., is associate professor of integrative physiology and pathobiology at the Tufts Sackler School of Graduate Biomedical Sciences. Her research focuses on gonadotropin-releasing hormone (GnRH), the primary hypothalamic signal regulating pituitary gonadotropin secretion, and its essential function for reproductive fertility. Patterns of GnRH release are sexually dimorphic, established perinatally, and altered with age. She is interested in further deciphering the exquisitely orchestrated

events involved in the regulation of GnRH secretion required to support reproductive cyclicity and ovulation in female mammals and in delineating age-related changes that contribute to reproductive decline. She is also examining how the development, function, and aging of the reproductive axis are influenced by early exposure to endocrine disrupters and studying how perinatal exposure to endocrine disrupters can exert long-term effects on body weight regulation.

Sheela Sathyanarayana, M.D., M.P.H., is an assistant professor of pediatrics and adjunct assistant professor within the Department of Environmental and Occupational Health Sciences at the University of Washington and an investigator within the Center for Child Health, Behavior, and Development at the Seattle, Washington, Children's Research Institute. She is a pediatric environmental health specialist. Her research interests focus on exposures to endocrine-disrupting chemicals, including phthalates and bisphenol A, and their impact on reproductive development. Currently, Dr. Sathyanarayana is the center director and clinical director for The Infant Development and Environment Study (TIDES), which is a multicenter cohort study of phthalate exposures in pregnancy and health outcomes in children. She is currently a co-chair for the U.S. Environmental Protection Agency's Children's Health Protection Advisory Committee. Dr. Sathyanarayana performs environmental health consults for health care professionals, governmental entities, and individual families related to environmental exposures and children's health. She also practices general pediatrics at Harborview Medical Center in Seattle, Washington.

Kristina Thayer, Ph.D., is director of the National Toxicology Program's (NTP's) Office of Health Assessment and Translation (OHAT), located on the campus of the National Institute of Environmental Health Sciences (NIEHS). OHAT conducts evaluations to assess the evidence that environmental chemicals, physical substances, or mixtures (collectively referred to as "substances") cause adverse health effects and provides opinions on whether these substances may be of concern, given what is known about current human exposure levels. OHAT also organizes workshops, state-of-the-science evaluations, and other analysis activities to address issues of importance in environmental health sciences. Before becoming director of OHAT, she held positions in the NTP Office of Liaison, Policy, and Review, the NIEHS Office of Risk Assessment Research, and the NTP Center for the Evaluation of Risks to Human

Reproduction. Prior to joining NTP/NIEHS, she was a senior scientist at the World Wildlife Fund and then at the Environmental Working Group.

Damaskini (Dania) Valvi, M.D., Ph.D., M.P.H., is a research fellow at the Harvard T.H. Chan School of Public Health (Boston, Massachusetts) and the Centre for Research in Environmental Epidemiology (Barcelona, Spain). She received a graduate degree in medicine from the University of Crete, Greece, and an M.P.H. and a Ph.D. in epidemiology from the Pompeu Fabra University in Barcelona. Her primary research focus is to study whether exposure to environmentally persistent and nonpersistent pollutants early in life may influence children's health later in life, with a special interest in studying obesity and diabetes. She is also interested in identifying (1) the sources of environmental pollutant exposures in pregnant women and children; (2) factors, including dietary, genetic, and epigenetic factors, that are modifying the effects of exposures to environmental pollutants on metabolic outcomes; and (3) the mechanisms that underlie the effects of environmental pollutant exposures on weight gain and metabolism in humans.