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Do Interactions Between Gut Ecology and Environmental Chemicals Contribute to Obesity and Diabetes?

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Abbreviation List:

ADME: absorption, distribution, metabolism, and excretion

BPA: bisphenol A

BMI: body mass index

DDE: dichlorodiphenyldichloroethylene

DES: diethylstilbestrol

ER: estrogen receptor

FIAF: Fasting-induced adipocyte factor

GR: glucocorticoid receptor

GST: glutathione S transferase

HCB: hexachlorobenzene

LPL: lipoprotein lipase inhibitor

PFOA: perflurooctanoic acid

PPAR-gamma: peroxisome proliferator-activated receptor-gamma

PAHs: polyaromatic hydrocarbons

PBBs: polybrominated biphenyls

PBDE: polybrominated diphenylether

PCBs: polychlorinated biphenyl

RXR: retinoid X receptor

S. aureus: *Staphylococcus aureus*

TLR5: Toll-Like Receptor 5

US: United States

UDP: uridine diphosphate

ABSTRACT

BACKGROUND: Gut microbiota are important factors in obesity and diabetes, yet little is known about their role in the toxicodynamics of environmental chemicals including those recently found to be obesogenic and diabetogenic.

OBJECTIVES: We integrate evidence that independently links gut ecology and environmental chemicals to obesity/diabetes, providing a framework for suggesting how these environmental factors may interact with these diseases, and identify future research needs.

METHODS: We examined studies with germ-free or antibiotic treated laboratory animals, and human studies that evaluated how dietary influences and microbial changes affected obesity/diabetes. Strengths and weaknesses of studies evaluating how environmental chemical exposures may affect obesity and diabetes were summarized, and research gaps on how gut ecology may affect the disposition of environmental chemicals were identified.

RESULTS: There is mounting evidence that gut microbiota composition affects obesity and diabetes, as does exposure to environmental chemicals. The toxicology and pharmacology literature also suggests that interindividual variations in gut microbiota may affect chemical metabolism via direct activation of chemicals, depletion of metabolites needed for biotransformation, alteration of host biotransformation enzyme activities, changes in enterohepatic circulation, altered bioavailability of environmental chemicals and/or antioxidants from food, or alterations in gut motility and barrier function.

CONCLUSIONS: Variations in gut microbiota are likely to affect human toxicodynamics and increase individual exposure to obesogenic and diabetogenic chemicals. Combating the global obesity/diabetes epidemics requires a multifaceted approach that should include greater emphasis

on understanding and controlling the impact of interindividual gut microbe variability on the disposition of environmental chemicals in humans.

Introduction

The prevalence of adult obesity in the United States (US) has risen dramatically over the last three decades from 14.5% (Flegal et al. 1998) to over 33% (Flegal et al. 2010). Medical costs of obesity are estimated to be between $147 and $168 billion per year in the US, and account for up to 16.5% of medical care costs (Cai et al. 2010; Cawley and Meyerhoefer 2010; Finkelstein et al. 2009). Childhood rates of obesity are rising in the US (Wang et al. 2011) and in many other countries (Wang and Lobstein 2006). Analysis of trends from 1980 to 2008 also have shown an increase in body mass index (BMI) (Finucane et al. 2011) and diabetes (Danaei et al. 2011) in the majority of geographic areas surveyed world-wide. The origins of the global obesity and diabetes epidemics are multifaceted, with growing evidence that multiple environmental factors contribute to their development. This is exemplified by emerging evidence of the role of gut microbial ecology in obesity and Type I and Type II diabetes (Musso et al. 2011; Qin et al. 2010), as well as evidence from human studies and animal models that environmental chemicals may contribute to the development of these diseases (Baillie-Hamilton 2002; Carpenter 2008; Casals-Casas and Desvergne 2011; Grün 2010; Heindel and vom Saal 2009; La Merrill and Birnbaum 2011; Newbold et al. 2008). While interindividual variability in the gut microbiome affects the metabolism of pharmaceuticals (Clayton et al. 2009) and some environmental toxins (Dean and Ma 2007), the impact of gut ecology on the absorption, distribution, metabolism, and excretion (widely abbreviated in the pharmacology literature as ADME) of xenobiotics, including obesogenic and diabetogenic chemicals, has received little to no attention. We review the scientific evidence that independently links gut ecology and environmental chemicals to obesity and diabetes, providing a framework for suggesting how these environmental factors

may interact with these diseases, and identify future research needs to further our understanding of these relationships.

The Gut Microbiome and Obesity

Gut microbes outnumber human cells by a factor of 10, yet we know surprising little about many of these organisms. The metagenomic sequencing of the human microbiome reveals that there are 3.3 million non-redundant genes, with over 99% of the genes being of bacterial origin (Qin et al. 2010). This gene set is 150-times larger than the human genome. While certain microbial species appear to be shared by groups of individuals (Arumugam et al. 2011), with over 50 species shared by 90% of the individuals studied, there is considerable variation in both the types of microbes and in the diversity of microbial functional genes found between individuals (Qin et al. 2010). The notion of a conserved core of functional genes in the microbiome has been supported by studies in monozygote and dizygote twin pairs, though major differences in the abundance of microbes at the phylum level were observed in the microbiome of obese compared to lean twins (Turnbaugh and Gordon 2009; Turnbaugh et al. 2009a). No relationship between specific phyla and obesity has been found in a more recent study, although significant associations between obesity and inferred microbial metabolic activities such as energy harvesting and osmolyte production (based on the presence of genes predicted to encode specific enzymatic activities) were found (Arumugam et al. 2011). This is consistent with the observations of Calvani et al. who detected differences in the levels of microbial metabolites in the urine of obese compared to lean individuals (Calvani et al. 2010).

The first suggestion that changes in adiposity may influence gut microbiota was made in a study of intestinal by-pass patients (Bjorneklett et al. 1981). More recently, bariatric surgery has been

shown to alter gut ecology (Furet et al. 2010; Zhang et al. 2009), and improve glycemic control in Type II diabetics (Ahima and Sabri 2011; Meijer et al. 2011). The mechanisms by which bariatric surgery corrects hyperinsulinemia are unknown (Reed et al. 2011; Tam et al. 2011) nor is information available on the duration of this effect (Schauer and Rubino 2011). While it is not known whether the changes in microbial populations observed in by-pass surgery patients are directly related to changes in diabetes status, some studies suggest changes in bacteria populations may be related to obesity. Changes in specific bacterial populations post-bariatric surgery include reduction in methanogenic Archaea (Furet et al. 2010; Zhang et al. 2009). Zhang et al. hypothesized that in obese patients, methanogens could accelerate the fermentation of plant polysaccharides by lowering hydrogen gas produced during fermentation, leading to higher acetate production and increased energy harvesting, although that work was based on an extremely small sample size (Zhang et al. 2009). Others have suggested that genetic factors may play an important role in determining the levels of gut methanogens (Hansen et al. 2011).

Experiments with germ-free animals or animals treated with antibiotics, have resulted in conflicting evidence regarding a role for gut microbes in the development of obesity. Some studies have demonstrated that germ-free mice are resistant to diet-induced obesity when fed a Western-type high sugar and fat diet (Backhed et al. 2004; Backhed et al. 2007), whereas a more recent study using a different mouse line (C3H) found the opposite effect (Fleissner et al. 2010). In one mouse model, major differences were observed in the proportion of the different bacterial phyla in genetically obese mice and lean mice. Genetically obese ob/ob mice had a 50% reduction in the abundance of Bacteroidetes, and a proportional increase in Firmicutes (Ley et al. 2005). A metagenomic analysis revealed that the microbiome of obese mice had a higher

percentage of genes associated with energy extraction than that of lean mice (Turnbaugh et al. 2006). Further work in the ob/ob mouse model demonstrated that this trait of increased energy extraction was transferable: weight gain and total body fat was higher in germ-free mice that received gut microbiota from obese mice than microbiota from lean mice. These differences were observed even though food consumption was the same (Turnbaugh et al. 2006).

In addition to their role in energy harvesting in the gut, microbiota may also affect obesity and diabetes risk via several other mechanisms, including regulation of fat storage (Backhed et al. 2004), control of metabolic endotoxemia-induced inflammation (Cani et al. 2008; Cani and Delzenne 2007), and affecting the level of satiety factors such as glucagon-like peptides and leptin (Cani et al. 2009; Ravussin et al. 2011; Sanz et al. 2010). For instance, C57BL/6J wild type mice raised from birth with conventional gut microbiota had a 42% higher body weight and suppressed levels of Fasting-induced adipocyte factor (FIAF) compared to germ-free mice. The broader relevance of this observation is unclear, since no differences were found in circulating FIAF levels of conventional C3H mice fed a Western or high fat diet as compared to germ-free mice (Fleissner et al. 2010). Gut epithelial FIAF is a lipoprotein lipase inhibitor (LPL). Repression of its expression increased LPL activity and increased storage of triglycerides in adipocytes (Backhed et al. 2004).

The metabolic inflammation hypothesis is based on the observation that mice fed a high fat diet show changes in microbiota associated with increased intestinal permeability and go on to develop metabolic endotoxemia and inflammation. In genetically obese mice (ob/ob), gut microbiota composition affects plasma levels of endotoxin, presumably through altered gut permeability, which then leads to endotoxemia, inflammation, and metabolic changes that may influence the risk of diabetes and obesity (Cani et al. 2008). It has also been observed that

prebiotics in humans can influence gut microflora which in turn affect the levels of gut satiety factors, including glucagon-like peptide 1 and peptide YY (Cani et al. 2009). Diet and weight loss studies in obese mice and human studies characterizing how microbial communities are affected by diet, suggest complex interactions between diet, adiposity, gut microbiota, satiety hormones levels, and inflammation (Jumpertz et al. 2011; Muegge et al. 2011; Ravussin et al. 2011; Turnbaugh et al. 2010; Turnbaugh et al. 2009b).

There also may be host genetic-gut ecology links that affect immune function and the development of the suite of changes linked with metabolic syndrome (Vijay-Kumar et al. 2010). Mice lacking Toll-Like Receptor 5 (TLR5), which is important in immune system recognition of bacterial antigens in the colon, are hyperphagic with increased food consumption resulting in hyperlipidemia, hypertension, insulin resistance, and increased adipocity. Transferring gut microbiota from these TLR5 knock out mice to germ-free wild type mice also resulted in hyperphagia and many of the same symptoms of metabolic syndrome. Unlike ob/ob mice that demonstrated phylum level differences in microbiota, the TLR5 knock out and wild type mice had similar proportions of Firmicutes and Bacteroidetes. However, there were marked differences in certain bacterial species in the TLR5 knock out mice compared to wild type mice. Surprisingly, Letran et al. did not observe basal inflammation or other metabolic changes in TLR5 knock out mice, although they did note a reduction in flagellin-specific CD4 T cells following *Salmonella* infection (Letran et al. 2011). The discrepancy between these reports, which used genetically identical mice, was suggested to lie in the different microbiota colonizing the mice at the different facilities. Thus, despite the apparently different outcome, these reports help to illustrate the importance of the gut microbiota and its complex interaction with the immune system.

Other studies suggest that microbiota may influence weight gain or loss and adiposity in humans. Ley et al. (2006) showed that obese humans had a lower Bacteroidetes to Firmicutes ratio than lean humans, but that this ratio increased with weight loss (Ley et al. 2006). Armougom also found lower levels of Bacteriodetes in obese individuals (Armougom et al. 2009), although no difference in this ratio was found by Arumugam et al. (Arumugam et al. 2011).

Weight gain during pregnancy can affect gut microbial populations and incidence of obesity in offspring (Collado et al. 2008, 2010). Breast versus formula feeding practices, and vaginal versus Cesarean section delivery also appear to affect the gut ecology of infants and may have relevance for obesity (Hallstrom et al. 2004; Musso et al. 2010; Penders et al. 2006; Penders et al. 2005). Another analysis of human microbiomes suggested that fecal microbiota composition in infants may predict later weight gain in children (Kalliomaki et al. 2008).

While there is ongoing debate about the relevance of phylum level differences in obese and lean individuals, research in these areas is still in its early stages. In order to thoroughly test the human adiposity-gut microbe hypothesis there is a need for additional carefully controlled experiments as well as larger epidemiological studies.

Gut Microbiome and Diabetes

In addition to the growing number of studies that suggest gut microbiota may affect the development of obesity, several studies suggest that the nature of the gut microbiota is linked to Type II diabetes. This includes a study that found men with Type II diabetes had significantly reduced ratios of fecal Firmicutes including Clostridia compared to non-diabetic controls. Plasma glucose was positively correlated with both the ratios of Bacteroidetes to Firmicutes, and

of the *Bacteroides-Prevotella* group to *Clostridium coccoides-Eubacteria rectale* group. In addition, the diabetic group also had more *Beta-proteobacteria* than non-diabetic controls. The authors suggested that the Bacteroidetes and Proteobacteria groups may affect diabetes risk via an endotoxin-induced inflammatory response, since both are gram-negative bacteria with lipid polysaccharide outer membranes (Larsen et al. 2010). While this is the first study to show changes in microbial populations between Type II diabetics compared to non-diabetics, the study is based on a small number of subjects (n=36), and these results need to be replicated in larger studies. It should also be noted that individuals in both the control and diabetic groups had a wide range of BMIs.

Other researchers have investigated if gut microbiota affects glycemic control and glucose tolerance using animal models of Type II diabetes. In ob/ob mice, treatment with antibiotics (norfloxacin and ampicillin) for two weeks decreased levels of both aerobic and anaerobic bacteria in the gut. Antibiotic-treated ob/ob mice had significantly improved glucose tolerance. This improved glucose tolerance in antibiotic-treated mice was attributed to multiple factors, including reduced liver triglycerides, increased liver glycogen, increased adiponectin and reduced lipopolysaccarides in the plasma (Membrez et al. 2008). The authors suggested that changes in the microbiota improved glucose tolerance via changes in metabolic and inflammatory pathways. Other animal studies have examined if there are differences in insulin resistance and glycemic control between germ-free mice and mice with conventional gut microbes. Germ-free mice fed a high fat diet consumed fewer calories, excreted more fecal lipids, and weighed less than conventional high fat diet fed mice. The germ-free mice also had reduced fasting and non-fasting insulinemia and improved glucose tolerance. The authors suggested these results support a role for gut microbiota in insulin sensitivity (Rabot et al. 2010).

Relatively few studies have evaluated the role of microbes on Type I diabetes. It has been suggested that increased gut permeability (commonly called "leaky gut") may affect the absorption of antigens that can attack and damage pancreatic beta cells (Vehik and Dabelea 2011). Increased gut permeability has been observed in subjects with Type I diabetes (Bosi et al. 2006). Since gut microbes can affect intestinal permeability (Garcia-Lafuente et al. 2001), gut ecology may play a role in the development of Type 1 diabetes (Neu et al. 2010). Another hypothesis by which microbes may cause Type I diabetes is via the production of bacterial toxins that can directly damage or affect the function of pancreatic beta cells. The *Streptomyces* toxin, bafilomycin A1, when injected into mice, resulted in smaller pancreatic beta cells and impaired glucose tolerance (Myers et al. 2003). This toxin can be produced by soil microbes and infect root vegetables such as potatoes. Other microbial toxins, such as streptozotocin, have been used to induce diabetes in an experimental animal model (Like and Rossini 1976). Little is known about other microbial toxins that may directly attack pancreatic beta cells and affect Type I diabetes. For Type II diabetes, there is some speculation as to whether manipulating gut microbiota may have therapeutic benefits, including whether prebiotics, postbiotics, antibiotics or even microbial transplantation may have clinical significance (Kootte et al. 2011).

Obesogenic and Diabetogenic Environmental Chemicals

The possible role of chemical toxins in the development of rising rates of obesity world-wide was first proposed by Baillie-Hamilton (Baillie-Hamilton 2002). Grün and Blumberg (Grün and Blumberg 2006, 2007) suggested that certain environmental pollutants, called "obesogens" can disrupt or interfere with the body's homeostatic controls of adipogenesis, lipid metabolism, or

energy balance. Adipose pathways involving nuclear receptors, such as the estrogen receptor (ER), retinoid X receptor (RXR), peroxisome proliferator-activated receptor-gamma (PPAR-gamma), and glucocorticoid receptors (GR) were some of the first proposed molecular targets of environmental obesogens (Grün and Blumberg 2007). Others have suggested that endocrine disrupting chemicals that affect adipose and glucose-related pathways should be categorized into a subgroup called "metabolic disrupting chemicals" (Casals-Casas and Desvergne 2011). Expanding the obesogen hypothesis, several researchers have proposed that environmental chemicals may act during critical windows of prepubertal and pubertal development to alter pathways involved in food intake, insulin sensitivity, lipid metabolism, and adipocyte development (Heindel and vom Saal 2009; Wolff et al. 2008).

The level and strength of evidence (human versus experimental animal or cell culture studies), the mechanism of action, and whether a dose-response effect or a low-dose effect (U-shaped response curves) is observed, vary by chemical. While there are relatively few studies that have examined whether environmental factors play a role in Type I diabetes (Howard and Lee 2011), associations between the incidence of Type II diabetes and exposure or use of a number of environmental chemicals is well supported in the human epidemiological literature for dichlorodiphenyldichloroethylene (DDE) (Codru et al. 2007; Cox et al. 2007; Lee et al. 2006; Rignell-Hydbom et al. 2009; Son et al. 2010; Turyk et al. 2009a; Turyk et al. 2009b; Ukropec et al. 2010), hexachlorobenzene (HCB) (Codru et al. 2007; Ukropec et al. 2010), highly chlorinated polychlorinated biphenyls (PCBs) (Codru et al. 2007; Lee et al. 2006; Lee et al. 2010; Ukropec et al. 2010; Wang et al. 2008), dioxin (Henriksen et al. 1997; Kang et al. 2006; Michalek and Pavuk 2008), chlordane (Cox et al. 2007; Everett and Matheson 2010; Lee et al. 2007a; Lee et al. 2006; Lee et al. 2010, 2011; Son et al. 2010), and occupational exposure to agricultural

insecticides and herbicides (chlordane, heptachlor, chlorpyrifos, diazinon, alachlor, cyanazine, and trichlorofon) (Montgomery et al. 2008). Without mechanisms of action, however, it cannot yet be determined if all of the chemicals identified play a potential causal role, or if co-exposures result in detecting some chemicals that do not have a biological effect on diabetes risk.

For other chemicals, there is a clearer picture of effects in human populations and mode of action. Globally, high levels of arsenic in water supplies have been associated with increased incidence of Type II diabetes (Chen et al. 2007; Navas-Acien et al. 2006; Rahman et al. 2009; Tseng 2007). Mechanistic studies suggest arsenic may impair insulin secretion from pancreatic beta cells, and induce changes in gene expression that affect pancreatic insulin secretion and insulin resistance in peripheral tissues (Diaz-Villasenor et al. 2007; Diaz-Villasenor et al. 2006). While a diabetogenic effect of another metal, cadmium, was noted in rats exposed neonatally (Merali and Singhal 1980), and suggestive evidence on fasting glucose levels in humans was reported (Schwartz et al. 2003), no mechanism of action has been identified.

Although there is strong evidence of a mechanism of action for other chemicals, few, if any studies have documented whether past or current exposure levels in humans poses a risk. For example, there is strong mechanistic data supporting tributyltin as a developmental obesogen, especially through nuclear receptor signaling. PPAR-gamma is one of the key regulators of cell growth and differentiation of adipocytes. Tributyltin is an agonist for both PPAR-gamma, and the retinoid X receptor (RXR-alpha, -beta and -gamma) (Grün and Blumberg 2006; Grün et al. 2006), and can sensitize human and mouse stromal stem cells to differentiate into adipocytes (Inadera and Shimomura 2005; Kirchner et al. 2010). Pubertal exposures in male mice cause an increased body weight gain, hepatic steatosis, hyperinsulinemia and hyperlipidemia (Zuo et al. 2011). However, the effect of environmental tributyltin exposure on related obesity disorders in

human populations has not yet been investigated. More studies are needed to define the extent of human exposure from tributyltin's use in anti-fouling marine paints, as a stabilizer in polyvinyl chloride plastics, and its use in wallpaper, textiles and floor coverings (Antizar-Ladislao 2008; Appel 2004; Kannan et al. 2010).

For several estrogenic environmental chemicals including bisphenol A (BPA), the alkylphenols nonyl- and octylphenol, diethylstilbestrol (DES), and genistein, there is evidence from animal or tissue culture models that these environmental estrogens affect a variety of other receptor-mediated, cellular, and molecular targets linked to adipose and or glucose metabolism. Glucocorticoid receptor (GR) signaling is central to adipocyte differentiation. Using the 3T3-L1 preadipocyte cell line, Sargis and colleagues demonstrated that BPA stimulated GR and increased lipid accumulation in the differentiating adipocytes (Sargis et al. 2010). BPA can also influence glucose transport in mouse 3Y3-F442A adipocytes, enhancing the level of a key glucose transport protein GLUT4 (Sakurai et al. 2004). Other studies have shown that BPA can suppress the release of adiponectin (which can affect insulin sensitivity and resistance) from adipocytes or adipose explants from human patients (Hugo et al. 2008).

The evidence of early life exposure to BPA and effects on obesity from animal studies is not consistent, and appears to be affected by route of exposure, gender and species (Miyawaki et al. 2007; Rubin et al. 2001; Ryan et al. 2010). There is not strong evidence in human studies of an effect on obesity at current levels of BPA exposure for humans (Lang et al. 2008; Melzer et al. 2010).

Using 3T3-L1 adipocytes, researchers have found that the alkylphenols octyphenol and nonylphenol upregulate the expression of the resisten gene, which affects insulin resistance and

decreases adipocyte differentiation. Male rats treated with octylphenol show increased serum levels of glucose (Lee et al. 2008). While nonylphenol has been widely detected in adipose tissue of humans, a positive relationship between measures of obesity such as BMI and adipose levels of this environmental estrogen has not been shown (Lopez-Espinosa et al. 2009).

Mice exposed neonatally to the non-steroidal estrogen DES show an initial weight loss followed by an increase in body fat by 2 months of age (Newbold 2010). DES exposure increases serum leptin and triglycerides levels, and changes the expression of several genes involved in fat distribution (Newbold et al. 2007). Cohort studies have not yet determined whether there is a higher incidence of obesity in DES mothers, or their children exposed to DES *in utero* even though this compound has been found to have multigenerational affects on other endpoints such as female cervical cancer and male urogenital malformations (Palmer et al. 2009; Troisi et al. 2007).

For the phytoestrogen genistein, effects on gene expression of adipose-related factors, including induction of phospholipase A2 group 7 and phospholipid transfer protein genes, were seen at low, but not high doses in a rodent study (Penza et al. 2006). This suggests that for some environmental chemicals, especially those with hormonal action, low-dose effects need to be examined rather than relying on traditional high-dose response effects. U-shaped response curves have been reported for other environmental chemicals, including some congeners of polybrominated diphenylether (PBDEs) flame retardants including PBDE-153, and certain PCBs (Lee et al. 2007b; Lee et al. 2011; Lim et al. 2008), suggesting that for metabolic syndrome, obesity, and diabetes future research needs to determine if other chemicals may have low-dose effects.

Generalizations of effects on obesity or diabetes risk cannot be extrapolated to an entire class of chemicals. For instance, Type II diabetes-related effects of PCBs, and brominated flame retardants (polybrominated biphenyls (PBBs), and PBDEs), appear to be more closely associated with highly halogenated forms of these chemicals (Everett et al. 2007; Lee et al. 2010; Lim et al. 2008). Only certain types or metabolites of phthalates appear to be associated with obesity or diabetes in humans (Hatch et al. 2008). In rodent studies, while diisobutylphthalate shows some evidence of affecting obesity via PPAR pathways (Boberg et al. 2008), evidence for other phthalates, including diethylhexyl phthalate, is less consistent (Casals-Casas et al. 2008; Feige et al. 2010).

For other chemicals, such as the PPAR agonist perfluoroctanoic acid (PFOA), there is emerging but inconsistent evidence of an association with obesity and diabetes. While there is some evidence of a higher incidence of diabetes in those occupationally exposed to PFOA(Lundin et al. 2009), a large-scale cross sectional epidemiology study did not observe a relationship between PFOA levels and Type II diabetes or fasting glucose levels (MacNeil et al. 2009). Rodent studies indicate PFOA can be transmitted from the dam to pup during lactation (Fenton et al. 2009) and there is some evidence of PFOA being a developmental obesogen in mice (Hines et al. 2009), but studies in rats have not observed an effect of early-life PFOA exposure on plasma insulin or leptin levels (Boberg et al. 2008).

Disposition of Environmental Chemicals

In addition to the host-microbe interactions and the direct effects of the chemicals discussed above, we suggest that microbes may affect obesity and diabetes by altering the ADME of

environmental chemicals. Microbially mediated effects on ADME could include the direct activation of chemicals (Van de Wiele et al. 2010; Van de Wiele et al. 2005; Wallace et al. 2010), production of microbial metabolites that compete for limited host biotransformation capacity (Clayton et al. 2009; Wallace et al. 2010), alteration of host biotransformation enzyme activities (Claus et al. 2011; Meinl et al. 2009), changes in enterohepatic circulation (Meijer et al. 2006), or altered bioavailability of environmental chemicals and/or antioxidants from food (Kemperman et al. 2010; Lhoste et al. 2003; Oishi et al. 2008; van Duynhoven et al. 2010). Increased bioavailability may also be brought on by changes in gut motility and barrier function. While there is evidence that the ADME of environmental chemicals may be affected by many of these microbial-mediated pathways, no studies have evaluated how the ADME of obesogenic or diabetogenic chemicals are affected by variations in the human microbiome. There is a need to determine the effect of microbes on the bioavailability of environmental chemicals, and the direct biotransformation of persistent organic pollutants (Dean and Ma 2007; Possemiers et al. 2009).

Using an *in vitro* model which simulates the human intestinal microbial system (biota cultured from human feces), it has been demonstrated that colonic microbiota were capable of transforming polyaromatic hydrocarbons (PAHs) to the bioactive estrogenic metabolites 1-hydroxypyrene and 7-hydroxybenzo(*a*)pyrene, while stomach and small intestine digests of the PAH did not produce estrogenic metabolites (Van de Wiele et al. 2005). This suggests that colonic microbes are capable of direct biotransformation of parent compounds into active metabolites. Gut microbes were found to thiolate and methylate arsenic in both human and animal models (Pinyayev et al. 2011; Van de Wiele et al. 2010). Exposure to high levels of arsenic have been associated with both increased risk of bladder cancer as well as higher

incidence of diabetes in areas with contaminated water supplies and/or seafood (Chen et al. 2007; Coronado-Gonzalez et al. 2007; Kim and Lee 2011; Navas-Acien et al. 2006; Rahman et al. 2009; Yen et al. 2007). While these microbial model systems suggest the capacity for biotransformation of environmental chemicals in the gut, especially the colon, their impact on systemic pollutant levels is not known. The extent of biotransformation variation by the gut microbes, so called "presystemic metabolism" (Grundmann 2010) of different individuals is also not known. Despite phylogenetic diversity, the implied functional metabolic redundancy observed in the gut metagenomes of individuals (Turnbaugh et al. 2009a) raises the question as to whether or not important differences exist between the enzymatic capacities of individuals. While no large scale functional studies have been to done to characterize the interindividual variation in gut microbe enzymatic capacity, the available data suggests that variations in gut microbiota affect environmental chemical disposition (Rowland et al. 1985; McBain and MacFarlane 1998). This has been indirectly established in the area of colon cancer where variation in fecal enzyme activities has been found to correlate with cancer risk (Rowland 2009). Although not directly related to obesogens, the pharmacology literature provides valuable evidence demonstrating how chemical fate can be affected by variability in the host microbiome (Clayton et al. 2009; Sousa et al. 2008; Wallace et al. 2010; Wilson 2009). For example, a 2008 review identified 30 drugs that can be metabolized by gut microbiota, including chloramphenicol, whose metabolism shows considerable interindiviual variability that is dependent on the presence or absence of specific bacteria genera (Sousa et al. 2008). In addition, studies have shown that variations in gut microbiota can affect the metabolism of commonly used over the counter drugs, like acetaminophen (Clayton et al. 2009), and that limiting gut microbial metabolism of chemotherapy drugs can reduce drug toxicity (Wallace et al. 2010).

Recent evidence suggests that metabolism and toxicity of acetaminophen is affected by individual variations in the gut microbiome. Some gut bacteria, including *Clostridium difficle* metabolize tyrosine to *p*-cresol that can compete with acetaminophen for sulfonation in the gut. In individuals whose gut bacteria produce high levels of *p*-cresol, less acetaminophen undergoes sulfonation because of competition with *p*-cresol and more is glucuronidated. Researchers found that the ratio of sulfonated to glucuronidated *p*-cresol in the urine was predictive of acetaminophen toxicity. The same markers are likely relevant to metabolism of other compounds that rely on these pathways for detoxification (Clayton et al. 2009) including many that have been suggested to be environmental obesogens / diabetogens (see supplementary Table 1).

Another example of gut ecology-pharmaceutical interaction is the metabolism of the chemotherapeutic prodrug, CPT-11. Upon administration this prodrug is first activated by carboxyesterases in the liver to yield toxic SN38, which in turn is glucuronidated by uridine diphosphate (UDP)-glucuronosyltransferase to nontoxic SN38G. SN38G is excreted into the bile and returned to the gut where beta-glucuronidases from commensal gut bacteria remove the glucuronide. This reactivates the drug in the gut, which can in turn cause bloody diarrhea, limiting the dose that can be used in chemotherapy. To circumvent the unintended intestinal toxicity of SN38, researchers developed an inhibitor of microbial glucuronidase that was not toxic to gut microbes, but which prevented metabolism of SN38G, and thereby increased mouse tolerance to CPT-11 (Wallace et al. 2010). Microbial glucuronidase activity also has been shown to be important in activating food borne procarcinogens in the gut (Humblot et al. 2007), further illustrating the role of this enzyme in chemical metabolism.

The above examples underscore the reasons why recent reviews in the pharmacology literature have articulated the need for future drug development to include an integrated assessment of host

and environmental factors, including gut microbes that affect drug disposition (Grundmann 2010; Sousa et al. 2008; Wilson 2009; Wilson and Nicholson 2009). This topic has received little attention in the toxicology literature (Possemiers et al. 2009). In this regard pharmacometabolomics appears to be an important emerging tool for investigating how gut ecology may affect the fate of chemical toxicants and their contribution to diabetes and obesity risk. Given the established functional differences in the microbiomes of obese and lean humans (Arumugam et al. 2011), microbes may be an important source of variation in the ADME profile of obesogenic / diabetogenic chemicals that deserve increased attention.

This is especially important in light of evidence in animal models that suggest changes in gut microbiota not only effect levels of gut metabolic enzymes, but levels of hepatic enzymes as well (Claus et al. 2011; Meinl et al. 2009; Reddy et al. 1973). The ability of gut microbiota to affect levels of hepatic enzymes was initially demonstrated for carbohydrate metabolizing enzymes. Compared to germ-free animals, conventional animals displayed a significantly higher activity of hepatic glucose-6-phosphate dehydrogenase (Reddy et al. 1973). There is limited recent evidence that certain Phase I enzymes also can be influenced by gut microbiota. The expression and level of P450 enzymes Cyp3a11 and Cyp2c29 were significantly higher in the livers of mice with conventional gut microbiota compared to germ-free controls (Claus et al. 2011).

Several hepatic and gut Phase II enzymes also have been compared in germ-free and germ-free animals “reassociated” with conventional microbiota. These include glutathione transferases, glutathione peroxidase, epoxide hydrolases, acetyltransferases, and sulfotransferases. Levels of isoenzymes were compared in the liver, small intestine, cecum, and colon of germ-free and reassociated control rats. In most cases, germ-free animals had higher levels of colonic phase I and II enzymes than control rats with conventional microbiota although there was no effect of

germ-free status on the levels of enzymes in the small intestine. Differences were, however, observed in the level of liver enzymes in germ-free rats compared to reassociated rats of both sexes, with elevations in sulfotransferases. Hepatic epoxide hydrolase was elevated in germ-free animals as well, but only in females (Meinl et al. 2009).

Levels of hepatic biotransformation enzymes can also be affected by diet-induced changes in gut microbes. Levels of glutathione S transferase-Pi (the predominant GST isoenzyme) were lower in colon cells of germ-free animals compared to rats with conventional microbiota. The levels of this enzyme increased three-fold when the diet of rats with conventional microbiota was changed from a digestible maize starch to a high amylose starch. The authors suggested that amylose was fermented in the colon, and may have yielded short-chain fatty acids such as n-butyrate, which may have induced GST (Treptow-van Lishaut et al. 1999). Changes in gut microbiota composition were not measured with the dietary change, so it is not known to what extent gut microbiota may have affected induction of GST directly, however, this report does suggest that changes in gut microbial activity (fermentation) correlate with changes in this Phase II enzyme that plays an important role in cellular detoxification (Di Pietro et al. 2010). The potential for diet/microbe enhanced induction of detoxification capacity demonstrated in the colon of animal models contributes to interest in the potential detoxification/anticancer affects of both pre- and probiotics, however, further human studies are required (Genuis 2011).

There is some evidence from laboratory animal studies that the polyphenols quercetin and catechin may influence levels of Phase II enzymes in the liver or gut (Lhoste et al. 2003; Wiegand et al. 2009), and that gut microbes play a role in polyphenol-mediated enzyme induction (Lhoste et al. 2003). Few studies have looked at the levels or activity of Phase II biotransformation enzymes in the human gut (Peters et al. 1991; Teubner et al. 2007). Little is

known to what extent activities of these biotransformation enzymes are affected by the wide variations in the human microbiome. Hence, we have virtually no information on how variations in gut ecology affect human ADME capacity with respect to environmental chemicals. Understanding how other dietary components, including polyphenols, may modify gut microbial populations and levels of Phase I and II enzymes, may yield important information relevant to interindividual variation in chemical metabolism (Kemperman et al. 2010; van Duynhoven et al. 2010).

Another area where little information exists is whether the enterohepatic circulation of environmental chemicals is affected by variation in gut microbial populations. Since many environmental toxicants undergo Phase II metabolism (see supplementary Table 1), those that are excreted in the bile may be further metabolized by the enzymes of gut microbiota including glucuronidases, leading to enterohepatic circulation and increased residence time in the body (Humblot et al. 2007). The importance of this process has been demonstrated by administration of non-absorbable fat (sucrose polyester) that decreases enterohepatic circulation and increases fecal fat excretion of the polybrominated diphenylether flame retardant, PBDE-47 (Meijer et al. 2006). To what extent enterohepatic circulation of lipophillic persistent pollutants, including diabetogens or obesogenic chemicals can be influenced by variations in gut microbial populations is not known, but the limited information available suggests that the variability in the activity levels of relevant gut microbe enzymes may be quite high, especially for beta-glucuroronidase (Rowland et al. 1986). Given the importance of microbial beta-glucuronidases in enterohepatic cycling, and the enrichment of related genes (i.e. those encoding hydrolases) in obese microbiomes (Turnbaugh et al. 2009a), it is important that we understand interindividual differences in enterohepatic cycling and how they may affect the risk of obesity or diabetes.

Conclusions and Future Research Needs

Microbial populations and/or metabolic capacities are known to differ in lean and obese subjects (and in Type II diabetes), yet we know surprisingly little about the effect of these differences on the body burden of obesogenic / diabetogenic chemicals. The ability to characterize and manipulate microbial populations in gnotobiotic mice, however, including humanizing of the rodent gut (Goodman and Gordon 2010), provide us with an unparalleled opportunity to begin exploring the impact of gut microbe variability on the disposition of environmental chemicals in humans. Future research in this area should quantify how interindividual variations in gut microbiota affect the body burden of environmental chemicals by altering: 1) these chemicals directly, 2) the level and activity of host Phase I and II enzymes, 3) enterohepatic circulation of environmental chemicals, 4) depletion of host detoxification capacity, and 5) alterations of gut barrier function. Studies should also identify biomarkers that are predictive of impaired obesogenic / diabetogenic chemical absorption, distribution, metabolism, and excretion, and assess the interaction between microbiota and developmental obesogens, including intergenerational effects. This approach will shed light on how variations in gut ecology affect the metabolism of obesogenic and diabetogenic chemicals and lead to more personalized approaches in the treatment and prevention of obesity and diabetes.

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