lipases. Importantly, these proteins had been previously implicated in regulating the plasma levels of lipids associated with VLDL particles. These data then set the stage for the authors to examine whether XBP1 knockout or ablation via silencing depresses the levels of plasma lipids. As hypothesized, decreased plasma TG and cholesterol were evident when XBP1 was absent. XBP1 deficiency also protected against hyperlipidemia in dyslipidemic mice. Based on these results, the authors propose XBP1 as a therapeutic target to reduce plasma lipid levels.

Together, the companion papers pinpoint the IRE1-XBP1 arm of the UPR as a target to suppress the production of circulating atherosclerosis-causing lipids, a goal that will ultimately affect a disease that results in one-quarter of all deaths in the United States. However, enthusiasm for this approach needs to be calibrated by potential adverse effects on one or more of the many targets of this pathway and by questions that arise from some of the disparate results in these studies. Most notably, in So et al. there was decreased lipogenesis and protection from steatosis in mice that were either insulin resistant or fed a lipogenic diet, but in Wang et al. there was mild steatosis even in chow-fed mice, which was exacerbated by a lipogenic diet. It is unclear why Wang et al. (2012) did not observe the decrease in lipogenesis reported in So et al. (2012) and Lee et al. (2008). This discrepancy will need to be resolved experimentally before clinical extensions can be contemplated, given that an agent that reduces plasma lipid levels while concurrently promoting steatosis is unlikely to gain U.S. Food and Drug Administration approval. More fundamentally, it will be interesting to pursue why neither study found adverse effects on general protein production by the liver despite the loss of this one arm of the UPR, which is a key quality control process in the ER for secretory proteins. Nonetheless, by identifying components of the UPR pathway that regulate the composition and plasma levels of VLDL lipids, these reports provide a wealth of provocative information that will enliven the field of lipoprotein metabolism from both basic science and therapeutic perspectives.

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Antibiotic Exposure Promotes Fat Gain

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Recent research suggests that obesity may be influenced not only by dietary and genetic risk factors, but also by the trillions of microorganisms inhabiting our gastrointestinal tract. Consistent with this notion, Cho et al. (2012) use mice to demonstrate that subtherapeutic antibiotic treatment promotes adiposity.

Humans and other mammals have coevolved with trillions of microorganisms that thrive in and on our bodies. The ensuing host-microbial interactions are often beneficial; however, disturbance of this delicate balance is thought to lead to an increased risk of disease (Dethlefsen et al., 2007). Recent efforts have extensively characterized the composition of the microbial communities found in multiple body habitats (Consortium, 2012), but we are only just beginning to discover the unintended consequences of a Western lifestyle on our microbial partners (Blaser and Falkow, 2009). In particular, orally administered broad-spectrum antibiotics have a clear potential to influence the microbes inhabiting our gastrointestinal tract (the gut microbiota).

But what are the functional consequences of the widespread use of antibiotics? One possible ramification is altered host energy balance. A number of studies have linked the gut microbiota to obesity (Bäckhed et al., 2004; Turnbaugh et al., 2006), and low-dose antibiotics have been used for decades to enhance growth and feed efficiency in farm animals (Jukes, 1971). The mechanisms through which antibiotics promote this phenomenon and whether the increasing use of antibiotics in children contributes

to a predisposition to obesity later in life are currently unknown. Tractable model systems are needed to tease apart the many confounding factors potentially influencing host energy balance. Recently, work done by Martin Blaser and colleagues (Cho et al., 2012) has made an important first step toward addressing these issues, exposing young mice to various low-dose antibiotic treatments for 7 weeks, resulting in increased adiposity (Figure 1).

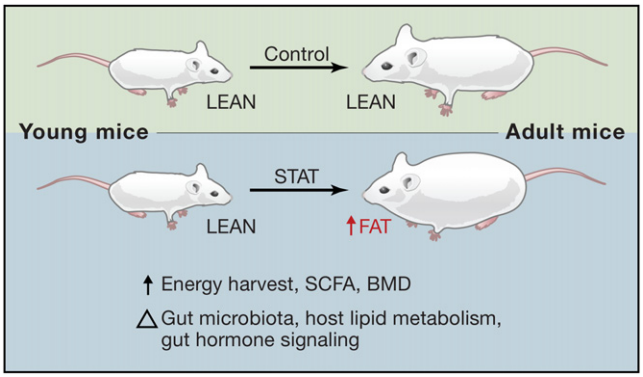

Figure 1. The Many Effects of Subtherapeutic Antibiotic Treatment on Young Mice

Relative to control mice, STAT results in similar total body weight but increased adiposity, dietary energy harvest, bone mineral density (BMD), and short-chain fatty acid (SCFA) levels. These phenotypic differences were associated with changes to the gut microbial community, host lipid metabolism, and gut hormone signaling. Future studies promise to elucidate the mechanistic basis for these changes.

The microbial response to *sub*therapeutic *a*ntibiotic *t*reatment (STAT) was evidenced by an altered community structure rather than total abundance. A shift toward an increased Firmicutes to Bacteroidetes ratio has been associated with an obese state (Ley et al., 2005; Turnbaugh et al., 2006). Surprisingly, this shift was evident after coadministration of penicillin and vancomycin, two primarily Gram-positive (i.e., Firmicutes) targeting compounds, and not after administration of either antibiotic alone. Chlortetracycline, another antimicrobial that acts through a different mechanism, also caused this shift. Given the different modes of action and range of these antimicrobials, this calls into question how these various antibiotics affect the microbial community and to what degree individual microbial taxonomic groups, or multiple groups working in concert, might directly contribute to the development of adiposity.

One possibility is that the disrupted gut microbiota following STAT has an enhanced ability to harvest energy from the diet, through microbial production of short-chain fatty acids (SCFAs), to provide substrates for storage into adipose tissue, as proposed for obesity (Turnbaugh et al., 2006). The authors observed that STAT led to increased overall caloric extraction from the diet, together with increased SCFA concentrations. Although the four treatment options had variable effects on microbial community structure and SCFA levels, all of these treatments resulted in some degree of increased adiposity, suggesting that there may be a common host response to multiple types of perturbations to the gut microbiota. To determine whether a link could be made between the gut microbiota, SCFA levels, and adiposity, changes in downstream host genes for hepatic lipid metabolism were quantified by microarray and quantitative PCR. Some genes coding for lipogenesis and triglyceride synthesis were upregulated, but no phenotypic signs of hepatic steatosis or differences in metabolic activity of visceral adipose tissues were observed. Increased serum levels of a secreted gastrointestinal hormone (GIP) may be an alternative contributor to adiposity. Further experiments to clarify these putative mechanisms and experiments in germ-free mice exposed to STAT are necessary to rule out any direct effects these compounds may have on host physiology.

Interestingly, unlike what is seen in livestock, STAT had a minimal effect on overall body weight and feed efficiency in mice. The authors did report an accelerated growth rate within the first week of STAT and an increase in bone mineral density after 3 weeks. It is possible that this initial accelerated growth toward maturity is sufficient to promote fat deposition.

This work raises many questions as to the potential links between antibiotic exposure and adiposity. Given that antibiotic use has a greater effect on growth in young animals than mature animals (Jukes, 1971), to what degree does the effect of STAT depend on the age of the mice? Is this phenomenon unique to early life? How much, if at all, does the effect depend on the specific antibiotics used and their relative proportions? Do antibiotics influence host energy balance through direct effects on the host, or are the observed phenotypes entirely dependent on the gut microbiota? To what degree does the prevalence and horizontal transfer of antibiotic resistance genes influence the increase in adiposity? How dependent are these effects on other factors known to influence the gut microbiota and host energy balance? For example, would consumption of a high-fat/high-sugar "Western" diet exacerbate the effects of STAT? Also, while much of the attention devoted to the role of the microbiota in regulating host metabolism is focused on an increase in dietary energy harvest, what impact could the microbiota have on the other side of the energy balance equation? Is there an effect of the gut microbiota on energy expenditure?

Perhaps most importantly, this work provides a tractable animal model to investigate these and many other burning questions regarding the energetic consequences of perturbations to the gut microbiota. Of note, another recent study performed by Blaser and colleagues supports the relevance of these findings to humans: infants exposed to antibiotics within the first 6 months of life had increased body mass later in life (Trasande et al., 2012). Together, these results underscore a view of human metabolism as a composite of our human and microbial genomes and the critical need for a better understanding of how these host-microbial interactions contribute to health and disease in the presence or absence of a wide range of orally administered therapeutics.

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PPAR-ϒ Pathway to Vascular Dysfunction

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Humans with dominant-negative (DN) mutations in PPAR-ϒ develop early onset hypertension. A recent study by Pelham et al. (2012) shows that loss of PPAR-ϒ repression of the Rho kinase pathway leads to altered vascular function via a smooth-muscle-dependent pathway that is independent of NO and oxidant stress.

PPAR-ϒ is a ligand-activated transcription factor that is targeted by the thiazolidinedione (TZD) class of antidiabetes medications and is known to be important in the regulation of adipogenesis and type II diabetes (Beyer et al., 2008). Activation of PPAR-ϒ by TZD drugs improves insulin sensitivity and lowers blood pressure in type II diabetics; however, individuals with dominant-negative (DN) mutations of PPAR-ϒ not only present with type II diabetes and severe insulin resistance, but also exhibit early onset hypertension via unknown mechanisms (Barroso et al., 1999). This early onset hypertension is mimicked in transgenic mice with a similar mutation. In this issue, Pelham et al. (2012) utilize one of these transgenic models to identify mechanisms by which a DN mutant form of PPAR-ϒ, expressed specifically in the smooth muscle, causes enhanced contraction and impaired vascular relaxation, leading to hypertension. The study reveals a pathway in which smooth muscle-specific PPAR-ϒ and Cullin-3, a member of the E3-ubiquitin ligase complex, participate in the regulation of vascular smooth muscle tone and, possibly, in the regulation of arterial blood pressure.

The authors find that mice with the DN PPAR-ϒ mutation exhibit increased vascular contraction, impaired relaxation, and elevated blood pressure similar to the phenotypes found in various forms of human hypertension; and that these alterations are associated with increases in RhoA and Rho-kinase activity. They further show that this upregulation of the Rho kinase pathway is related to reduced expression and decreased neddylation (conjugation of the ubiquitin-like protein Nedd8) to Cullin-3, which normally reduces the expression and activity of RhoA by promoting its degradation in the proteasome. Phenotypes similar to those in mice expressing the DN PPAR-ϒ mutation could be produced in wild-type mice by treating them with a pharmacological agent (MLN4924) that inhibits the neddylation of Cullin-3.

One gap in existing knowledge is the mechanism by which Cullin-3 is regulated by PPAR-ϒ. Cullin-3 is a cullin-RING E3 ubiquitin ligase complex scaffold protein that regulates the turnover of RhoA (Chen et al., 2009). The present study provides evidence that Cullin-3 is downregulated in mice with the DN PPAR-ϒ mutation, and that this occurs via suppression of RhoBTB1, a novel PPAR-ϒ target gene that may regulate the targeting of some Cullin-3 substrates, including RhoA, for proteasomal degradation via the Cullin-3 E3 RING ubiquitin ligase complex.

A particularly attractive feature of the study is the linking of this pathway to vascular regulation in intact blood vessels, in this case the aorta, rather than exclusive use of cultured cell models. The authors found that vascular relaxation in response to acetylcholine and the NO donor sodium nitroprusside were impaired in mice harboring the DN mutation of PPAR-ϒ. The investigators provide convincing evidence that altered vascular function in aortas of these mice is mediated by a mechanism other than changes in NO availability or elevated vascular oxidant stress. This finding is significant, given the multitude of studies showing the importance of NO and reactive oxygen species as major mediators of pathophysiological changes in multiple cardiovascular disorders. Other mechanisms of potential importance have seldom been sought.

The authors also show that systemic administration of MLN4924 leads to an elevated blood pressure in wild-type mice. This is a potentially important finding because it provides support for a whole-body effect of the Cullin-3 mechanism, a proof of concept that cannot be obtained with in vitro studies of molecular