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The Origins and Drivers of Insulin Resistance

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Obesity-induced insulin resistance is the major determinant of metabolic syndrome, which precedes the development of type 2 diabetes mellitus and is thus the driving force behind the emerging diabetes epidemic. The precise causes of insulin resistance are varied, and the relative importance of each is a matter of ongoing research. Here, we offer a Perspective on the heterogeneous etiology of insulin resistance, focusing in particular on the role of inflammation, lipid metabolism, and the gastrointestinal microbiota.

Introduction

Insulin resistance is the key etiologic defect that defines metabolic syndrome (Moller and Kaufman, 2005). After initiation of the insulin-resistant, metabolic syndrome state, many of these patients eventually develop β cell failure, which triggers the onset of type 2 diabetes mellitus (T2DM). The incidence of T2DM has risen dramatically in the past couple of decades, now affecting >300 million people worldwide, including an estimated 55 million in India, 25 million in the United States, and 80 million in China, with great human and financial costs. In parallel, there has been an even more dramatic epidemic of obesity, and because obesity is the most common cause of insulin resistance in man, it is the obesity epidemic that is driving the parallel T2DM epidemic. Indeed, >80% of people with T2DM in the United States are classed as overweight. Therefore, obesity-induced insulin resistance is the dominant factor underlying both metabolic syndrome and the rising tide of T2DM.

It is clear that T2DM is a heterogeneous disease, and the etiology of insulin resistance is equally complex (Moller and Kaufman, 2005). There are a number of known causes of insulin resistance, as well as competing views as to their relative importance. Our view is that insulin resistance is a multifactorial condition in which overnutrition triggers increased inflammation (Gregor and Hotamisligil, 2011), changes in lipid metabolism (Samuel and Shulman, 2012), and changes in the gastrointestinal (GI) microbiota (dysbiosis) (Kau et al., 2011; Nicholson et al., 2012), all of which are interconnected to varying degrees, leading to the final state of insulin resistance. In this Perspective, we will not highlight all areas implicated in insulin resistance, as we believe that inflammation, lipid metabolism, and the microbiota are the major interacting components and that other proposed factors ultimately work through one or more of these mechanisms. Nor will we attempt an exhaustive review of these three areas, as excellent and more detailed reviews have recently been published (Gregor and Hotamisligil, 2011; Kau et al., 2011; Lumeng and Saltiel, 2011; Nicholson et al., 2012; Saltiel, 2012; Samuel and Shulman, 2012). Instead, we will offer our view as to how inflammation, lipids, and dysbiosis work together to cause decreased insulin sensitivity and will discuss common misunderstandings, gaps in our knowledge, and challenges in the field. Finally, we will focus on current therapeutic strategies, as it is quite clear that our basic research and preclinical knowledge of these processes surpass our ability to use these insights for a better understanding of human disease and its treatments.

Inflammation-Mediated Insulin Resistance

Chronic, low-grade tissue inflammation is a major and well-known cause of obesity-induced insulin resistance (Gregor and Hotamisligil, 2011; Lumeng and Saltiel, 2011). Initial landmark observations in this area were made by Hotamisligil et al., who showed an increase in tumor necrosis factor α (TNF-α) concentration in obese adipose tissue and that TNF-α neutralization improves insulin sensitivity in obese rodent models (Hotamisligil et al., 1993, 1996). Later studies by Weisberg et al. and Xu et al. demonstrated that adipose tissue from obese humans and mice is characterized by a striking accumulation of macrophages. Indeed, up to 40% of all cells in obese adipose tissue are infiltrating macrophages (Weisberg et al., 2003; Xu et al., 2003). Subsequent studies have demonstrated that adipose tissue macrophages (ATMs) are highly activated, with increased expression of a large array of proinflammatory genes (Lumeng et al., 2007; Nguyen et al., 2007). These proinflammatory genes include a set of cytokines, particularly TNF-α, that are excessively secreted from activated macrophages and directly cause insulin resistance by acting on insulin target cells in local tissues through paracrine mechanisms (Hotamisligil et al., 1993, 1996). For example, TNF-α signaling activates intracellular kinases, such as c-Jun N-terminal kinase (JNK) and IκB kinase (IKK), which inhibit insulin receptor signaling by serine phosphorylation of insulin receptor substrate 1 (IRS-1). Furthermore, activation of the transcription factors AP-1 and NF-κB results in a feed-forward mechanism whereby proinflammatory cytokine production is exacerbated. If the magnitude of cytokine secretion is great enough, they can leak out of the tissue, raising circulating levels, to produce endocrine effects on distant organ systems, such as muscle and liver, exacerbating systemic insulin

resistance (Osborn and Olefsky, 2012). However, the adipose tissue concentrations of cytokines are much higher than circulating levels, and it is likely that, in most circumstances, the major effects of these secretory products are local rather than systemic (Hotamisligil et al., 1995).

Although increased adiposity causes insulin resistance, it has long been known that visceral adipose tissue has a much greater negative metabolic effect than subcutaneous adipose tissue. In obesity, increased macrophage accumulation and other signs of inflammation occur in the visceral adipose tissue, but not in the subcutaneous depots, consistent with the negative impact of visceral adipose expansion on insulin sensitivity.

Liver and Hypothalamic Inflammation in Obesity

Similar etiologic events can also occur in the liver (Gregor and Hotamisligil, 2011; Osborn and Olefsky, 2012). During obesity, there is a substantial increase in hepatic macrophages (Obstfeld et al., 2010). Liver macrophages consist of a resident macrophage population, termed Kupffer cells, and recruited hepatic macrophages (RHMs), which migrate into the liver under obese conditions (Obstfeld et al., 2010; Oh et al., 2012). Kupffer cells are bone marrow derived and form a stable long-lived population of resident macrophages in the liver (Klein et al., 2007). However, the exact cell type that gives rise to Kupffer cells remains unknown. RHMs are also bone marrow derived, migrate to the liver under the influence of liver-derived chemokines, particularly MCP-1, and are highly proinflammatory in the context of obesity (Obstfeld et al., 2010; Oh et al., 2012). Based on recent studies, 30%–70% of all hepatic macrophages in obesity are RHMs (Oh et al., 2012). These cells turn over in a matter of weeks and do not give rise to Kupffer cells. Chemically mediated depletion of Kupffer cells and RHMs by gadolinium (Neyrinck et al., 2009) or clodronate (Lanthier et al., 2010) treatment ameliorates hepatic insulin resistance, and thus, in obesity, both RHMs cells and Kupffer cells are responsible for the chronic hepatic inflammatory state. However, the relative contributions of these different cell types to hepatic inflammation and insulin resistance remain unresolved. It has also been shown that obesity is associated with hypothalamic inflammation and that the resulting local production of proinflammatory cytokines can cause central leptin resistance, a key feature of obesity. In addition, central nervous system (CNS) inflammation can contribute to systemic insulin resistance, particularly in the liver, via a brain-liver neuronal signal (Milanski et al., 2012). Thus, inflammation in the CNS during obesity may be a key determinant of weight gain and systemic insulin sensitivity.

Macrophages and Insulin Resistance

The diversity of ATMs, Kupffer cells, and RHMs indicates that tissue macrophages consist of heterogeneous populations with differential functions (Mosser and Edwards, 2008). Based on in vitro studies, macrophages can be divided into M1 and M2 classifications (Gordon and Martinez, 2010). M1 macrophages, also termed "classically activated macrophages," are highly proinflammatory, secreting the bulk of the cytokines that cause insulin resistance. M2 macrophages, also termed "alternatively activated macrophages," are not inflammatory and give rise to cytokines that exert anti-inflammatory effects, such as IL-10 and IL-4 (Gordon and Martinez, 2010). However, in the in vivo situation, macrophages do not conveniently fall into either exclusively M1 or M2 phenotypes and exist across a polarization spectrum from the most inflammatory to noninflammatory cells (Jenkins et al., 2011; Mosser and Edwards, 2008). The overall macrophage-induced inflammatory state of the tissue is determined by the balance between these different macrophage subpopulations. In the obese state, the balance is clearly tilted toward the proinflammatory macrophage phenotype (Gregor and Hotamisligil, 2011).

The role of these inflammatory macrophages in causing decreased insulin sensitivity has been repeatedly demonstrated. Most compelling are a large number of studies in which macrophage inflammatory pathways have been genetically manipulated through loss- or gain-of-function approaches to show that decreased or increased macrophage-mediated inflammation leads to insulin sensitization or insulin resistance, respectively (see Osborn and Olefsky [2012] for references). In addition, complementary findings have been generated in man, although in comparison to the studies in rodents, the body of work is by necessity largely descriptive and correlative. As examples, interventions that improve insulin sensitivity, such as weight loss (Forsythe et al., 2008) and exercise (Teixeira-Lemos et al., 2011), decrease inflammation, whereas manipulations that cause insulin resistance, such as lipid overload, increase inflammation (Tripathy et al., 2003). Again, however, these are correlative studies, and clear data from pharmacologic anti-inflammatory therapy for the treatment of insulin resistance has remained an elusive goal.

Nonmacrophage Immune Cells and Insulin Resistance

Macrophages are by no means the only immune cell type that participates in the process of inflammation-induced insulin resistance. Neutrophils (Talukdar et al., 2012) and mast cells (Liu et al., 2009) are increased in obese adipose tissue, and studies in mice have indicated that these two cell types can promote insulin resistance. Lymphocytes also play an important role. B lymphocytes (Winer et al., 2011), $CD8^+$ cytotoxic T lymphocytes (Nishimura et al., 2009), and $CD4^+$ Th1 cells (Winer et al., 2009) promote insulin resistance, whereas $CD4^+$ regulatory T (Treg) cells are anti-inflammatory (Cipolletta et al., 2012; Feuerer et al., 2009). Lymphocytes require the presentation of antigen and costimulation by macrophages/dendritic cells for their activation, and therefore, these immune cell populations are interreliant. The numbers of Tregs and Th2 cells are absolutely or relatively decreased in obese adipose tissue (Feuerer et al., 2009; Winer et al., 2009), and these cells serve an important function in modulating macrophage behavior. Treg and Th2 cells can inhibit polarization of proinflammatory macrophages and can also prevent their recruitment into tissues. Adipose tissue Treg cell numbers can be restored in mice fed a high-fat diet (HFD) by insulin-sensitizing Thiazolidinedione (TZD) therapy, and this likely forms part of the mechanism of action for these drugs (Cipolletta et al., 2012). Another checkpoint promoting the anti-inflammatory state is provided by eosinophils, and a deficiency of adipose tissue eosinophils has been reported to cause insulin resistance (Wu et al., 2011a). Lastly, deficiency of natural killer T (NKT) cells, which can respond to lipid antigen, reduces glucose tolerance in 18-week-old lean mice (Schipper et al., 2012), although a separate study found no effect in younger mice (Kotas et al., 2011). Absence of NKT cells has no effect

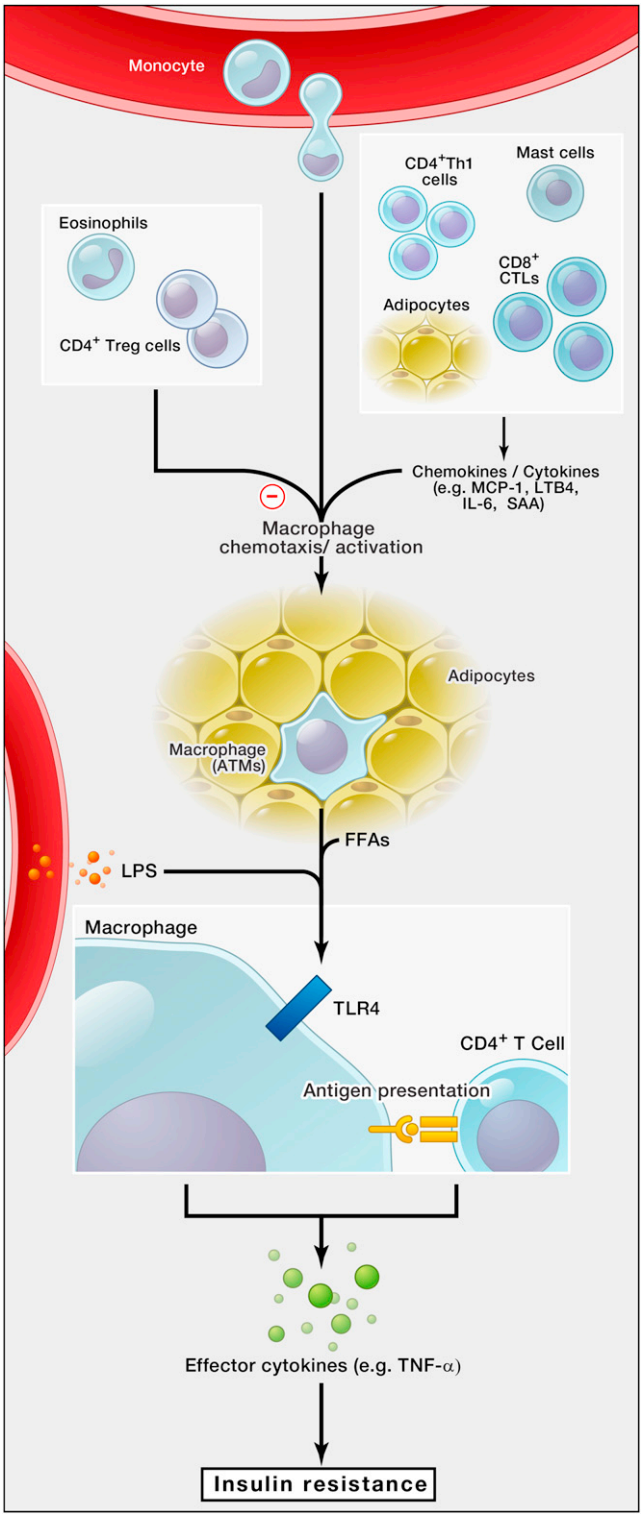

Figure 1. The Immune Response in Obese Adipose Tissue

Release of chemokines, such as MCP-1 and LTB4, facilitates the recruitment of inflammatory adipose tissue macrophages (ATMs) from blood monocytes. ATMs can sense "danger" signals, such as free fatty acids (FFAs) and lipopolysaccharide (LPS), and produce inflammatory cytokines, such as TNF-α. ATMs can also engage effector lymphocyte populations, CD4+ Th1 cells, and CD8+ cytotoxic T lymphocytes (CTLs), which act to enhance ATM recruitment and supplement effector cytokine production. ATM recruitment and inflammatory function can be inhibited by resident Treg cells.

on glucose tolerance in obese mice (Ji et al., 2012; Kotas et al., 2011; Mantell et al., 2011; Schipper et al., 2012).

It is of interest, and somewhat puzzling, that individual studies exist, supporting an important role for each of these cell types in the etiology of inflammation-mediated insulin resistance. In the in vivo situation, it is unlikely that these cell types work in isolation but, rather, are part of an ongoing interactive immune cell conversation (see Figure 1). It is possible that time-course studies could reveal insights into the primary and secondary events in adipose tissue immune cell interactions. Although detailed time-course data assessing each of the aforementioned immune cell types have not been published within a single study, it has been shown that neutrophils and M1-like macrophages accumulate in adipose tissue as early as 3–14 days after HFD feeding, plateauing at maximal accumulation several weeks later (Lee et al., 2011; Strissel et al., 2010; Talukdar et al., 2012; Winer et al., 2011) and that Th1 cells, Th2 cells, and B lymphocytes begin to accumulate at 4 weeks according to Winer et al. [2011] or at 22 weeks according to Strissel et al. [2010].

This immune cell cooperation would mean that inhibition of any component within this interlinked cellular network can disrupt the overall inflammatory state. Because macrophages are the most abundant proinflammatory immune cell type in adipose tissue and liver, it is likely that they represent the final effector cell secreting the predominance of the cytokines that cause insulin resistance. However, in vivo, it is most likely that several immune cell types work together in a more integrated fashion to achieve the common goal, meaning that, from a treatment point of view, there are many potential entry points for therapeutic intervention.

Lipids and Insulin Resistance

A variety of abnormalities in lipid metabolism have been described in insulin-resistant states, some of which may be the result of insulin resistance, whereas others may participate in causality. The concept of lipotoxicity holds that increased levels of circulating fatty acids and/or lipid accumulation in muscle and liver can lead to insulin resistance (Samuel and Shulman, 2012). In this way, lipotoxicity is a contributing factor to the pathophysiology of the metabolic derangements in obesity and T2DM, and in general, there are two overall ways in which lipids can negatively impinge on insulin action. First, circulating fatty acids can activate cell-signaling pathways, which interfere with insulin action (Glass and Olefsky, 2012). Second, metabolism of fats can lead to the accumulation of intracellular lipid products, which cause insulin resistance (Jornayvaz and Shulman, 2012) (see Figure 2). In T2DM, hyperglycemia can also have a detrimental impact on insulin signaling (glucotoxicity), and in the diabetic state, the negative effects of lipotoxicity and glucotoxicity might be additive.

Fatty Acid Signaling and Insulin Resistance

Circulating fatty acid levels are often elevated in obese and/or diabetic states, and saturated fatty acids (SFAs) can activate cellular proinflammatory pathways through Toll-like receptor 4

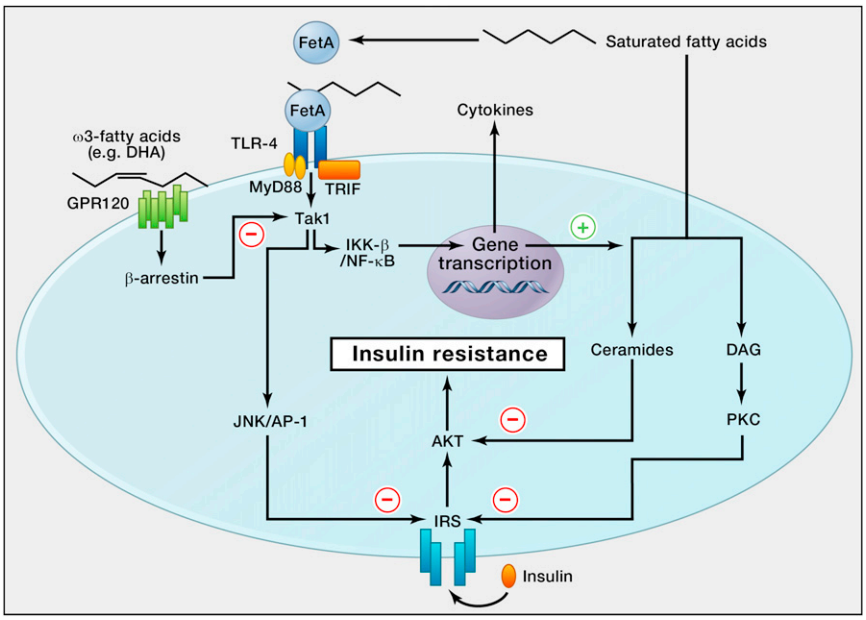

Figure 2. Pathways Leading to Lipid-Mediated Insulin Resistance

Fetuin-A (FetA)-bound saturated fatty acids (SFAs) can signal via TLR4 to induce proinflammatory responses and inhibitory IRS phosphorylation. Alternatively, SFAs can be metabolized to ceramides or diacylglycerol (DAG), which reduce insulin sensitivity via AKT inhibition or inhibitory IRS phosphorylation. TLR4 signaling can synergize with the ceramide pathway by promoting transcription of ceramide biosynthetic genes as well as cytokines. Conversely, some FFAs can also be anti-inflammatory. For example, ω-3 FAs signal via GPR120 and inhibit TLR4 signaling via sequestration of Tak1.

(TLR4)-dependent mechanisms, demonstrating the link between this form of lipotoxicity and inflammatory signaling (Lee et al., 2001; Shi et al., 2006). Stimulation of TLR4 by SFAs induces activation of JNK and IKK, which directly inhibit insulin signaling via serine phosphorylation of IRS-1 (Glass and Olefsky, 2012). Furthermore, activation of these kinases leads to NF-κB and AP-1 activation and changes in the expression of genes that influence insulin action, providing an additional mechanism for SFA-induced insulin resistance (Glass and Olefsky, 2012). Activation of the inflammasome can also be a consequence of SFA stimulation (Wen et al., 2012). The inflammasome mediates the caspase-1-driven cleavage of pro-IL-1β and pro-IL-18, leading to the secretion of the active forms of these cytokines; deficiency of inflammasome components (such as *Nlrp3*) ameliorates insulin resistance. Thus, SFA-induced inflammasome activation exacerbates inflammation, resulting in the inhibition of insulin action.

Although TLR4 is an important component of SFA-induced insulin resistance (Lee et al., 2001; Nguyen et al., 2007; Shi et al., 2006), recent work indicates that SFAs are not direct ligands for TLR4, raising the question of how they work. This question has recently been addressed by the studies of Pal et al. (2012), who demonstrated that Fetuin A, a circulating glycoprotein secreted from the liver, binds SFAs with high affinity, and, in turn, Fetuin A binds to and activates TLR4. In this way, Fetuin A provides the physical link between SFAs and TLR4 signaling. SFAs can also be incorporated into specific lipid raft domains of the plasma membrane, where they enhance TLR4 dimerization and c-Src recruitment (Holzer et al., 2011; Wong et al., 2009). This activates downstream stress signaling pathways (such as JNK/AP-1) that inhibit insulin action (Wong et al., 2009).

Certain classes of lipids can also exert anti-inflammatory effects. For example, fish oils or omega-3 fatty acids DHA and EPA exert potent anti-inflammatory effects (Serhan et al., 2008). Recently, it has been found that GPR120 on macrophages and adipocytes functions as the receptor or sensor for omega-3 fatty acids, activating a β-arrestin2/TAB1 signaling system that blocks intracellular proinflammatory pathways, preserving insulin sensitivity (Ichimura et al., 2012; Oh et al., 2010). Interestingly, the recent identification of a loss-of-function polymorphism in the human *Gpr120* gene associated with obesity suggests a role for this anti-inflammatory lipid pathway in man (Ichimura et al., 2012).

Tissue Lipid Metabolism and Insulin Resistance

Excessive accumulation of intracellular lipid products is another proposed mechanism for insulin resistance (Samuel and Shulman, 2012). A general concept related to this mechanism is that fat calories ingested in excess of caloric needs are stored in adipose tissue. This has led to the proposal that, in obesity, the excessive fat calorie load is so high that it exceeds the buffering capacity and efficiency of adipose tissue storage so that these excess fat calories "spill over" into other tissues like skeletal muscle and liver (Danforth, 2000; Shulman, 2000). Indeed, increased skeletal muscle triglyceride concentrations and hepatic steatosis are typical concomitant abnormalities of insulin resistance states (Samuel and Shulman, 2012). However, even though this "spillover" concept is straightforward, it is also an oversimplification and is unlikely to be correct in the typical sense of its use. As a case in point, if a given individual gains 50 pounds to become obese, it is very likely that he or she will have some degree of steatosis and increased intramuscular triglyceride (IMTG) levels. This "spillover" cannot be due to the fact that the adipose tissue compartment is saturated, as such individuals can easily gain 20, 50, or even 100 additional pounds. Furthermore, the amount of lipid accumulation in muscle and liver in obesity amounts to no more than a couple of pounds. Thus, the build-up of hepatic and muscle lipids must result from a more complex mechanism. One component could be a simple kinetic issue of lipid flux in which liver and muscle normally clear a certain percentage of the incoming fat load; in obesity, this fraction could be slightly increased and, over time, lead to the observed lipid accumulation. Another possibility is that, when liver and muscle become insulin resistant, lipid metabolism is reprogrammed with a proportional increase in fatty acid re-esterification or de novo lipogenesis. Most likely both and perhaps additional

mechanisms exist to account for tissue lipid accumulation outside of the adipose depots.

This raises the key question as to whether lipid accumulation in liver and muscle is the result or the cause of insulin resistance. Here there are strong proponents of both views. For example, there are a number of situations in which steatosis and increased IMTGs exist in the absence of decreased insulin sensitivity, arguing against a causal role for lipid accumulation in insulin resistance (Cohen et al., 2011). On the other hand, metabolism of excessive fatty acid loads can result in the generation of bioactive lipid products that can attenuate insulin signaling (Jornayvaz and Shulman, 2012). As is the case in most complex, interconnected metabolic abnormalities, both sides are probably partially correct. Clearly, tissue lipid accumulation can serve as a biomarker for the insulin-resistant state, as increased hepatic and IMTG content is a typical concomitant of insulin resistance, particularly in obesity. Good evidence for the lipotoxicity concept also exists, as both diacylglycerides (DAGs) and ceramides can lead to decreased insulin signaling. Thus, fatty-acid-derived DAGs stimulate protein kinase C (PKC) activity, which leads to inhibitory serine phosphorylation of insulin signaling components (such as IRS proteins), causing insulin resistance (Samuel and Shulman, 2012). Similarly, SFAs can be converted to ceramides that inhibit AKT activity and decrease insulin sensitivity (Stratford et al., 2004). Inflammatory pathway activation can also connect excessive tissue lipids and insulin resistance. Activation of inflammatory pathways stimulates the expression of a set of genes involved in ceramide biosynthesis (Holland et al., 2011). In this way, inflammatory signaling potentiates ceramide production, emphasizing the interplay between inflammation and lipid-mediated insulin resistance and providing an additional mechanism for inflammation-induced decreased insulin sensitivity. Excessive fatty acid influx can also overwhelm the mitochondrial capacity for β oxidation, resulting in a build-up of partially oxidized acylcarnitine intermediates, which might contribute to insulin resistance (Muoio and Neufer, 2012). This subject has been extensively reviewed (Samuel and Shulman, 2012), and despite many publications on the subject, a definitive, consensus view has yet to emerge. The two points of view are not mutually exclusive, and it is certainly possible that tissue lipid accumulation is both a biological marker that results from insulin resistance and a contributing factor under certain circumstances.

Models of Fat-induced Insulin Resistance

Feeding rodents high-fat diets (HFDs) causes weight gain with expansion of adipose tissue mass, and HFD-fed mice and rats are typically used as a model to study the detailed pathophysiologic consequences of obesity. In fact, HFD-fed mice are frequently referred to as diet-induced obese (DIO) animals. Alhough this is correct, normal chow diets typically contain 4%–6% fat, whereas HFDs are commonly 45%–60% fat, and so DIO rodents are also models of high fat intake. Thus, interpretation of such studies must always be mindful of the possibility that the excessive fat intake or composition, rather than the underlying obesity, may be responsible for some of the specific phenotypic features in this model. This is not the case with ob/ob or gold thioglucose mice, which become obese due to increased caloric intake of normal chow diets.

Another way to assess the metabolic effects of lipid overload is to acutely infuse lipid emulsions to abruptly raise circulating free fatty acid (FFA) levels, and this maneuver will cause decreased insulin sensitivity within a couple of hours (Boden et al., 2002). Acute lipid infusions and chronic HFD are often viewed as interchangeable models to create lipid overload for studies of metabolic sequelae. However, the mechanisms whereby acute elevations of FFAs and chronic HFD cause decreased insulin sensitivity may not be the same, and evidence has already been published to this effect (Lee et al., 2011). For example, lipid infusions present a large acute lipid load to tissues with abrupt lipotoxic effects. Chronic HFD leads to long-term, gradual lipid storage and obesity, and this model results in chronic tissue inflammation, changes in the GI microbiota (dysbiosis), and hyperinsulinemia, which are not features of acute lipid infusion. Therefore, one should not conflate these approaches from a mechanistic point of view because it is likely that they produce the end result of insulin resistance through somewhat different pathways.

Gut Microbial Influence on Obesity and Insulin Resistance

The microbiota of the gastrointestinal (GI) tract has a profound impact on host physiology (Blumberg and Powrie, 2012; Kau et al., 2011; Nicholson et al., 2012). The collective microbiota genome, termed the microbiome, can also be studied, and there is great interest in the contributions of the microbiota to energy metabolism and metabolic disease.

In mouse models of obesity, the composition of the microbiota is altered—a phenomenon referred to as dysbiosis (Hildebrandt et al., 2009; Ley et al., 2005; Turnbaugh et al., 2008). Specifically, a decrease in *Bacteroidetes* species and an increase in *Firmicutes* species are associated with the obese state. Gnotobiotic (germ-free) mice are resistant to obesity induced by a HFD (Bäckhed et al., 2007), and colonization of gnotobiotic mice with a conventional mouse microbiota increases adipose tissue mass (Bäckhed et al., 2004). This increased adiposity is exaggerated when gnotobiotic mice are colonized with the microbiota derived from obese animals, indicating that the composition of the microbiota can predispose to the development of obesity (Turnbaugh et al., 2006). In addition, increased levels of circulating bacteria or bacterial products, such as lipopolysaccharide (LPS), which are derived from the microbiota, have been associated with insulin resistance (Burcelin et al., 2012).

Cause versus Consequence

Whether microbial dysbiosis is a cause or consequence of obesity and/or insulin resistance is a matter of ongoing debate (Blumberg and Powrie, 2012), given that most studies describe associations of dysbiosis with metabolic disease rather than mechanistic connections (Ley et al., 2005; Serino et al., 2012). It is also possible that reverse mechanisms exist in which some aspect of obesity itself can cause dysbiosis. Intervention studies will be necessary to sort out these ''cause versus effect'' questions, and indeed some positive steps have been made in this regard. Transfer of a dysbiotic microbiota into a lean mouse recipient will induce increased adiposity or weight gain (Henao-Mejia et al., 2012; Turnbaugh et al., 2006). Additionally, in mouse studies, antibiotic, prebiotic, and probiotic intervention can

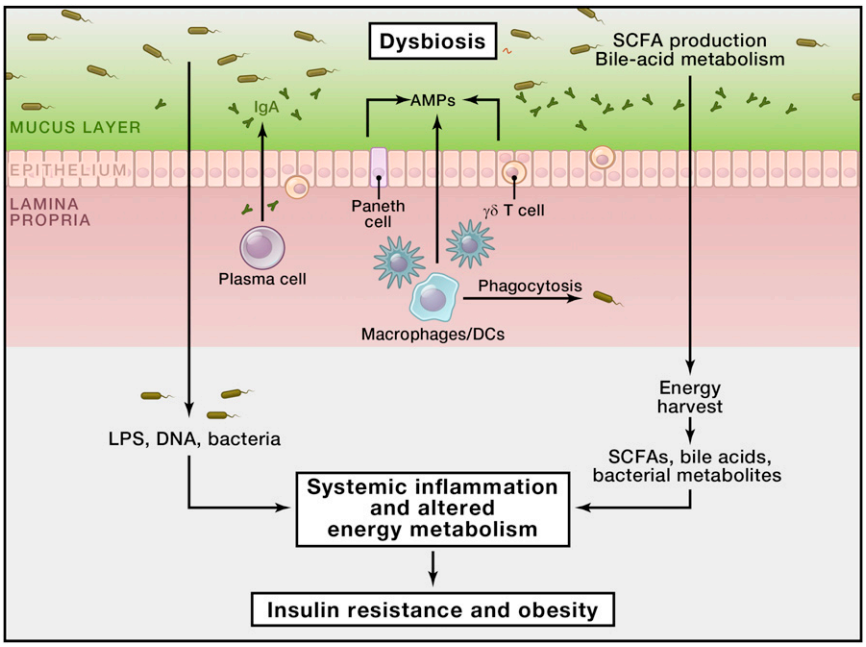

Figure 3. Microbial Contribution to Insulin Resistance

The intestinal immune response, such as IgA antibodies and antimicrobial peptides (AMPs), influence and are influenced by the gastrointestinal microbiota. Under homeostatic conditions, the intestinal immune system helps to preserve systemic ignorance of intestinal bacteria and also regulates its composition over time. A dysbiotic microbiota contributes to insulin resistance by increased energy harvest and the direct effect of altered bacterial metabolite production (e.g., short-chain fatty acids [SCFAs] and bile acid derivatives) on the liver and adipose tissue. In addition, gastrointestinal permeability caused by either intestinal inflammation or intestinal immune dysfunction results in penetration of bacteria or bacterial products (e.g., LPS, DNA), which can drive systemic inflammation.

improve insulin sensitivity (Amar et al., 2011a; Cani et al., 2008; Membrez et al., 2008).

Potential Mechanisms and Outstanding Questions

The microbiota influences energy harvest, energy metabolism, and the development of systemic inflammation (Nicholson et al., 2012). Bacteria can complement digestive function, such as fermentation of complex carbohydrates to short-chain fatty acids (SCFAs), a function predominantly assigned to Firmicutes. Indeed, energy stores are elevated following colonization of gnotobiotic mice with a conventional microbiota demonstrated by increases in adiposity and hepatic triglycerides (Bäckhed et al., 2004), and the obesity-associated microbiota has an enhanced capacity to harvest energy from the diet (Turnbaugh et al., 2006). Several studies have proposed that chronic low-grade intestinal inflammation in HFD-fed mice can cause intestinal permeability or "leakiness" (de La Serre et al., 2010; Ding et al., 2010; Li et al., 2008), and genetic ablation of certain host immune defense components (e.g., *MyD88*$^{-/-}$*Trif*$^{-/-}$ or *Asc*$^{-/-}$) can result in intestinal bacteria or bacterial products penetrating systemically (Henao-Mejia et al., 2012; Slack et al., 2009). Therefore, intestinal inflammation or intestinal immune dysfunction may cause GI permeability, leading to increased circulating LPS and bacterial DNA, which are key factors promoting systemic inflammation and insulin resistance in both mice and humans (Burcelin et al., 2012) (Figure 3).

Through their activity in the lumen, the microbiota also produce bioactive metabolites, including SCFAs, such as acetate, bile acid derivatives, and hydrogen species, all of which can reach the circulation (Nicholson et al., 2012). These products could influence systemic insulin sensitivity, inflammation, and energy metabolism either by signaling through G-protein-coupled receptors (GPCRs), nuclear hormone receptors, or posttranslational modification of host proteins (for example, lysine acetylation) (Bäckhed et al., 2004, 2007; Nicholson et al., 2012; Simon et al., 2012). For example, bile acids can signal through G-protein-coupled bile acid receptor (TGR-5) or the farnesoid X receptor (FXR). It should be kept in mind that these metabolites could also influence CNS satiety pathways.

The role of bile acids in the crosstalk between host metabolism and the microbiota is particularly intriguing. The microbiota has a profound influence on circulating bile acid profiles, and bile acids help to shape the GI microbiota (Nicholson et al., 2012). Interestingly, gastric bypass surgery has a significant impact on specific bile acid species, and it is possible that some of the beneficial effects of bariatric surgery are related to bile acid profile changes (Patti et al., 2009).

Whether dysbiosis can cause decreased insulin sensitivity independent of increased adiposity remains an open question. One study using fecal transplants from lean to obese individuals improved insulin resistance without altering body weight, fully consistent with this possibility (Vrieze et al., 2012). Similarly, antibiotic treatment of mice ameliorates insulin resistance even when compared to body-weight-matched controls (Membrez et al., 2008). However, further studies to establish this concept will need to more clearly document dysbiosis-induced insulin resistance independent of increased adiposity. Such studies should include direct assessment of tissue insulin signaling (liver, muscle, and fat) and quantitative measurements of in vivo insulin sensitivity (for example, glucose-clamp studies).

Another outstanding question is whether the effects of dysbiosis are due to the outgrowth of particular bacterial species or to overall functional changes within the collective microbiome. In support of the latter, the human microbiota shows a high level of variability in species composition across individuals; however, metagenomic studies suggest that genes within the microbiome that pertain to particular functions can be relatively conserved (Turnbaugh et al., 2009). Similarly, though a core group of species associated with dysbiosis has not been reproducibly identified in obese humans, metagenomic studies have indicated that the overall proportion of genes representative of

specific functions, such as increased microbial carbohydrate metabolism, are associated with obesity (Greenblum et al., 2012; Turnbaugh et al., 2009). Conclusive determination of microbiota compositions and metagenomic features associated with human obesity will require further in-depth study with larger sample sizes, an objective facilitated by the establishment of the Human Microbiome Project. The presence of specific species may be most relevant to microbiota-induced inflammation. While the majority of intestinal bacteria are restricted to the lumen, adherence to host cells or invasion beyond the intestine are important features of select species that promote inflammatory responses (Hooper et al., 2012). In HFD-fed mice, the profile of 16 s rRNA gene PCR products amplified from blood DNA was comparatively limited in its diversity compared to DNA from the cecum, suggesting that only a limited number of species are capable of reaching the circulation (Amar et al., 2011a). Similarly, sequencing of bacterial DNA from the blood of prediabetic individuals showed that >85% came from the *Proteobacteria* phylum (Amar et al., 2011b). Therefore, in the context of driving systemic inflammation and insulin resistance, specific species may well be dominant.

Translation to Man

In general, the data obtained from mouse studies have indicated that the intestinal microbiota plays an important role in the modulation of obesity and insulin sensitivity (Blumberg and Powrie, 2012; Kau et al., 2011; Nicholson et al., 2012). The effect of the microbiota in increasing adiposity may be due to the fact that obesity or HFD, or both, produce a dysbiosis associated with increased food energy harvest and altered energy metabolism. Independent of obesity, insulin sensitivity could be adversely effected by enhanced GI permeability, allowing bacterial products, such as LPS or bacteria themselves to enter the circulation, producing proinflammatory effects in insulin target tissues (Burcelin et al., 2012). Other mechanisms could also exist, as the microbiota produce bioactive metabolic products (e.g., bile acids and SCFAs) and reactive chemical species, such as H_2S, which may alter insulin sensitivity (Nicholson et al., 2012).

It remains to be established how well these processes or mechanisms observed in mice can be extrapolated to humans. Unlike mice, which are genetically homogeneous and eat the same artificially prepared diets, humans consume a range of diets and are genetically heterogeneous. As a result of this, there is substantial interindividual variation of microbiota composition in man, which has made it difficult to identify a core group of bacterial species that reproducibly define dysbiosis in obese subjects (Eckburg et al., 2005; Turnbaugh et al., 2009; Wu et al., 2011b). Despite these marked differences between mice and humans, some promising clinical investigative studies have emerged. For example, the blood levels of bacterial DNA are elevated in prediabetic patients and can be predictive of diabetes development in subsequent years, raising the possibility that blood bacterial DNA may be a biomarker for disease progression (Amar et al., 2011b). Furthermore, a recent clinical trial showed improved insulin sensitivity in obese individuals receiving fecal transplants from lean subjects, even though body weight was unaffected (Vrieze et al., 2012). Such studies offer hope that therapeutic ways to modify the GI microbiota may ultimately emerge.

An important issue that could affect any future therapeutic attempts is that, in humans, the intestinal microbiota tends to remain fairly stable within an individual over time, suggesting that, once established in early life, the host adapts to the resident microbiota in a way that favors the maintenance of the same, or similar, species composition (Gordon et al., 2012; Nicholson et al., 2012; Turnbaugh et al., 2009). Microbial ecology and host immune pathways are likely to predominate in these adaptive mechanisms (Hooper et al., 2012; Lemon et al., 2012). For example, the induction of adaptive immune responses (e.g., IgA antibody production) and the secretion of antimicrobial peptides (e.g., defensins) from GI epithelial cells, Paneth cells, and innate immune cells could create an environment permissive for only a select microbiota composition (Hooper et al., 2012). Supporting this hypothesis, dysbiosis can be induced in mice by genetic manipulation of immune components (Elinav et al., 2011; Vijay-Kumar et al., 2010). This might explain why therapeutic attempts to change microbiota composition with long-term probiotic therapy have shown little success (McNulty et al., 2011). This could also predict that antibiotics may not prove to be a useful treatment because without changing the host's adaptive mechanisms, eventually a similar intestinal microbiota will grow back. Thus, therapeutic manipulations of this sort might have to be long term. Furthermore, without knowing the core species associated with obesity or their antibiotic susceptibility, a targeted antibiotic regimen cannot be designed. These are important questions and will need resolution before the knowledge of the intestinal microbiota and metabolic disease can be harnessed for therapeutic purposes.

Treatment of Insulin Resistance: Translational Perspective

Given that obesity is the most common cause of insulin resistance in man and because the obesity epidemic underlies the increasing prevalence of T2DM, it is obvious that the most effective form of therapy would be to prevent or treat the underlying obesity. This has been an elusive therapeutic goal, but new anti-obesity drugs have recently received FDA approval, indicating progress in this area.

TZDs are PPARγ ligands that cause insulin sensitization by stimulating downstream PPARγ transcriptional programs. They were first introduced into the market in 1997, and two agents, pioglitazone and rosiglitazone, are still available, although their use has been curtailed in recent years due to side effects and potential toxicities. Although it is well known that these drugs are PPARγ ligands, despite many years of study, their exact mechanisms of action remain unknown (Tontonoz and Spiegelman, 2008). These drugs have direct effects in adipocytes, liver, macrophages, skeletal muscle, Treg cells, and the CNS, and all of these tissue-specific effects can contribute to overall systemic insulin sensitization (Tontonoz and Spiegelman, 2008). Although it is recognized that the improvement in insulin action is due to transcription of PPARγ target genes, the specific target genes responsible for insulin sensitization and how they work remain unclear despite many years of study. We know that TZDs can induce genes involved in insulin action in adipocytes, hepatocytes, and muscle. They also exert robust anti-inflammatory effects

in immune cells, promote the M2 polarization state of macrophages, and enhance Treg cell differentiation and function (Tontonoz and Spiegelman, 2008). However, a unified mechanism for how these drugs actually cause insulin sensitization in man has not yet emerged. It is possible that all of these different tissue-specific effects are additive in vivo and together account for the insulin-sensitizing effects of this category of drugs. One thing that is clear is that PPARγ itself does not cause insulin sensitization and that this effect is due to induction of its target genes. Indeed, Choi et al. have shown that phosphorylation of PPARγ at serine 273 is sufficient to activate a set of insulin-sensitizing genes in adipocytes (Choi et al., 2010), and agents that promote this phosphorylation state in the absence of PPARγ agonism can cause insulin sensitization (Choi et al., 2011). More in-depth study of the set of PPARγ target genes that are responsible for the insulin-sensitizing effects is warranted, as it is possible that one or more of these genes could prove to be a more precise and direct target for future drug discovery.

Having said this, it is probable that PPARγ-independent mechanisms will need to be exploited in order to generate a class of more effective insulin sensitizers. This is based on observations that are often overlooked. Although TZDs are potent insulin sensitizers, in the clinical setting, they only partially correct insulin resistance. Indeed, if one compares insulin resistant obese or T2DM patients to normals, then TZD therapy only corrects insulin resistance 20%–50% toward normal. This means that TZD-treated patients are still insulin resistant, just less so. And this is not often appreciated in the clinical setting. This also shows that a relatively modest effect to improve insulin sensitivity has a rather robust effect to lower glucose levels in T2DM subjects. The upside of this observation is that an even greater degree of glycemic improvement would occur if a treatment could more completely normalize insulin resistance.

Metformin is the most commonly used antidiabetic drug and is quite effective at reducing levels of HbA_{1c} (glycated hemoglobin), a measurement that reflects long-term blood glucose levels (Viollet et al., 2012). Although claims are made that Metformin produces insulin sensitization, the available data indicate that this is not likely to be the case. Though the mechanism of action of Metformin is still not fully known, this drug lowers glucose levels primarily by inhibiting hepatic glucose production. Because these effects are exerted in a cell-autonomous manner in hepatocytes and are independent of insulin, this is not insulin sensitization in the true sense of the word (Viollet et al., 2012). Therefore, although Metformin is an extremely important and efficacious antidiabetic agent, it should not properly be classified as an insulin sensitizer.

A number of additional targets have been pursued to develop treatments that reduce hepatic glucose production in T2DM. As this is the main mechanism of action of Metformin, this is a worthwhile and validated goal. One caveat should be kept in mind in translating the glucose-lowering efficacy from rodents to man. Rodents have much higher rates of basal glucose turnover, and their circulating glucose levels are much more dependent on hepatic glucose production than in man. In general, hepatic glucose production is 5- to 10-fold greater on a mg/kg basis in mice compared to man so that treatments that inhibit excessive hepatic glucose production are more potent in normalizing glucose levels in rodents than in humans.

Anti-Inflammatory Therapy and Insulin Sensitization

Anti-inflammatory treatments have obvious potential for insulin-sensitizing therapies. However, attempts at this have not been overly successful. For example, TNF-α blocking agents have marked effects to improve insulin sensitivity and lower blood glucose levels in rodents, but have been either ineffective or only modestly efficacious in humans (Bernstein et al., 2006; Dominguez et al., 2005). The reasons for this are unclear. One potential issue is that TNF-α exerts its effects in a paracrine fashion and interstitial adipose tissue levels are much higher than circulating TNF-α concentrations (Hotamisligil et al., 1995), raising the possibility that the TNF-α blocking agents have not sufficiently neutralized the relatively high concentrations of TNF-α at its tissue site of action. Nevertheless, one recent trial of anti-TNF-α treatment in obese individuals showed a modest reduction in blood glucose levels (Stanley et al., 2011) and treatment of rheumatoid arthritis patients with TNF-α inhibitors led to a decreased rate of progression to T2DM (Solomon et al., 2011). Therefore, therapeutic TNF-α blockade in T2DM could still be a potential goal. IL-1β inhibitors have also been used in the treatment of T2DM, again with only moderate success (Larsen et al., 2007; van Asseldonk et al., 2011). However, whether IL-1β actually plays an important role in the etiology of the tissue inflammation that leads to insulin resistance has not been established. On the other hand, inflammation in pancreatic islets generates IL-1β, which has detrimental effects on β cell function. Interestingly, the clinical studies with IL-1β inhibitors indicate that the major effects to improve glycaemia are related to improved insulin secretion rather than improved insulin sensitivity (van Asseldonk et al., 2011).

As mentioned above, TZDs exert anti-inflammatory effects, but in the in vivo state in man, it is hard to tease out to what degree this aspect of TZD action contributes to the overall insulin sensitization induced by these drugs. The salicylate prodrug Salsalate has showed promising results in this arena, and treatment of T2DM has demonstrated significant improvement in insulin sensitivity, as measured by glucose clamps studies, along with meaningful reductions in HbA_{1C} levels (Goldfine et al., 2010). The HbA_{1C} reductions are not as robust as typically seen with other classes of antidiabetic therapy, but perhaps more targeted and improved anti-inflammatory approaches might be more efficacious.

From a clinical perspective, any anti-inflammatory therapy must be closely scrutinized for unwanted immune suppression, which might lead to deleterious side effects. This would suggest that an anti-inflammatory approach targeted to those elements of the inflammation pathway most directly related to insulin resistance would be preferred. Indeed, until a specific anti-inflammatory agent shows robust efficacy in humans, the role of tissue inflammation in causing insulin resistance in obese and T2DM subjects remains incompletely established.

Given that pharmacologic therapy based on targets relevant to inflammation-induced insulin resistance is still in its early stages, it is difficult to predict how this field will evolve. An interesting point often discussed in this context is whether specific therapies, or targets, might be most effective in

particular subgroups of T2DM insulin-resistant patients. This type of clinical approach to personalized medicine is increasingly common in cancer therapeutics but has not yet reached the level of evidence-based treatment in T2DM. Perhaps anti-inflammatory treatment would be particularly efficacious in subgroups of patients with T2DM with large components of inflammation-induced insulin resistance. This would require specific biomarkers to identify subphenotypes within the spectrum of T2DM that predict responsiveness to particular therapeutic modalities.

Conclusions

It is clear that additional approaches are needed to develop new insulin-sensitizing compounds and that new treatment opportunities will be of great help in our ability to mitigate the current T2DM epidemic. The results of gastric bypass surgeries offer a hopeful outlook on this. It is now well recognized that bariatric surgery can lead to rapid improvements in insulin resistance and decreases in glycaemia in T2DM patients well in advance of the onset of significant weight reduction. So far, the mechanisms for this early amelioration of insulin resistance and hyperglycemia following bariatric surgery remain unknown. Whether it is due to postsurgical secretion of a new incretin or some other factor with beneficial effects or is due to reduced secretion of a diabetogenic factor is unknown. Possibly, the rearrangement of the gastrointestinal tract has major effects on the microbiota (Li et al., 2011; Zhang et al., 2009) or the pathophysiology of systemic inflammation. Regardless of the mechanism, the remarkable acute postbariatric surgery results indicate that it is possible to develop insulin-sensitizing therapies with much greater glucose-lowering efficacy then current treatments allow.

Insulin resistance is obviously a key pathogenic feature of T2DM with a complex, multifactorial etiology. Newer and more efficacious insulin-sensitizing therapies are badly needed, but despite the importance of this issue and intense efforts over the past decades, no new insulin sensitizers have been introduced into the clinical arena since the advent of TZDs in 1997. This is a rather remarkable fact and underscores the difficulties involved. As there are multiple interacting mechanisms of insulin resistance, many approaches to developing insulin-sensitizing therapeutics are possible, and likely several will be necessary. Yet, because of the recent progress in defining the interwoven influences of inflammation, lipid metabolism, and the microbiota, multiple new targets are emerging that offer the possibility, through a concerted and integrated effort, to confront and ameliorate the diabetes epidemic.

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