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Fat uses a TOLL-road to connect inflammation and diabetes

The link between inflammation and diabetes is well established, but the nature of the link is unresolved. Obesity, inflammation, and insulin resistance are major characteristics of diabetes. A study by Flier and colleagues (Shi et al., 2006) identifies Toll-like receptor 4 signaling activated by fatty acids as part of that link. This receptor mediates inflammatory events and insulin resistance in peripheral organs.

More than a century ago, Williamson and Lond reported the beneficial effects of sodium salicylate on the treatment of glycosuria and diabetes (Williamson and Lond, 1901). The interesting effect of an anti-inflammatory agent on diabetes was confirmed in 1957 when Reid and colleagues showed improvement in oral glucose tolerance in aspirin-treated diabetic patients (Reid et al., 1957). The potential relationship between inflammation and diabetes was silenced by the ground-breaking discovery of Randle's "Glucose Fatty-Acid Cycle" (Randle et al., 1963), which was the first mechanism that described fatty acid-induced insulin resistance. Further fueled by McGarry's insightful views that highlight the importance of lipid disorder in diabetes (McGarry, 1992), much of the late 20th-century diabetes research was directed to identifying adipocyte genes associated with lipid metabolism as the main causative factor of insulin resistance. This effort led to the discoveries of *obese* (ob) gene by Zhang and Friedman (Zhang et al., 1994) and a host of adipocyte-derived hormones, such as leptin, adiponectin, and resistin, that redefined the endocrine and metabolic role of adipose tissue. The latest effort is highlighted in an article from Shi and Flier (Shi et al., 2006), who report the key role of Toll-like receptor 4 on lipid-induced activation of inflammatory and metabolic signaling in insulin resistance and again raise the question on the link between obesity, innate immunity, and diabetes.

Studies investigating the relationship between obesity and insulin resistance unveiled two significant observations that generated an enormous interest in the role of inflammation in diabetes. First, in addition to energy-regulating hormones, adipose tissue releases proinflammatory cytokines, including TNF and IL-6. Lang observed that TNF causes insulin resistance in peripheral organs (Lang et al., 1992), and Hotamisligil and Spiegelman delineated a mechanism that involved TNF-α-mediated serine phosphorylation of IRS-1 (Hotamisligil and Spiegelman, 1994). Subsequent findings that adipocyte production of TNF-α was increased in obese subjects identified TNF-α as an important component of the obesity-diabetes link. IL-6 is another proinflammatory cytokine that is produced by adipocytes, elevated in obese subjects, and shown to alter glucose metabolism in peripheral organs (Kim et al., 2004).

Second, studies of the complex network of insulin signaling and observations of impaired insulin signaling in diabetic organs suggested that mechanisms other than the Glucose Fatty-Acid Cycle mediate FFA-induced insulin resistance. This search led to the discovery of at least three inflammation-associated proteins that are involved in the cross-talk between inflammatory and metabolic pathways. Shulman (Shulman, 2000) demonstrated that intracellular accumulation of lipid metabolites (fatty acyl CoAs, DAG, ceramides) activates PKC-θ, which causes serine phosphorylation of IRS-1 and insulin resistance in skeletal muscle. Also, FFAs activate IKK/NF-κB, and Yuan and Shoelson (Yuan et al., 2001) showed that salicylate-mediated inhibition of IKK attenuates lipid-induced defects in insulin signaling and insulin resistance. Their findings present a molecular explanation to the earlier observations of Williamson and Reid. Lastly, Hirosumi and Hotamisligil (Hirosumi et al., 2002) reported that c-Jun amino-terminal kinases (JNKs), which are activated by inflammatory cytokines and lipids, impair insulin signaling and mediate obesity-associated insulin resistance. Together, the findings support the triangular relationship between obesity, inflammation, and insulin resistance.

In the article of Shi and Flier (Shi et al., 2006), the investigators present Toll-like receptor 4 (TLR4) as the link between innate immunity, lipid, and insulin resistance. Toll-like receptors, a family of pattern-recognition receptors, play an important role in the innate immune system, and the authors show that TLR4 is expressed in many cell types including macrophages, adipocytes, liver and skeletal muscle. Binding of LPS to TLR4 recruits the adaptor protein myeloid differentiation factor 88 (MyD88) which leads to activation of NF-κB associated genes that encode inflammatory

cytokines, such as TNF-α and IL-6. Recent studies indicate that adipocytes in obese subjects manifest macrophage-like properties including cytokine production and activation of chronic inflammatory state that are partly due to infiltration of macrophages in adipose tissue (Wellen and Hotamisligil, 2005). In this regard, Shi and Flier hypothesized that TLR4 mediates lipid-induced activation of inflammatory signaling and insulin resistance in peripheral organs.

The authors demonstrate that fatty acids, particularly saturated fatty acids (C14:0, C16:0, and C18:0), activate the IKK/NF-κB pathway and stimulate macrophage production of TNF-α and IL-6, dependent on TLR4 signaling. 293T cells transfected with dominant-negative MyD88 or peritoneal macrophages isolated from TLR4$^{-/-}$ mice showed blunted cytokine production in response to FFA treatment. The authors also found elevated adipocyte expression of TLR4 in obese rodent models (*ob/ob*, *db/db*, and diet-induced obese mice). Moreover, FFA-induced expression of TNF-α and IL-6 was attenuated in the adipocytes of TLR4$^{-/-}$ mice. The authors tested their hypothesis in vivo by applying an acute lipid infusion to promote FFA-induced insulin resistance in peripheral organs and performing hyperinsulinemic-euglycemic clamps in TLR4$^{-/-}$ mice. Lipid infusion activated nuclear translocation of NF-κB and increased the production of TNF-α and IL-6 in wild-type adipocytes; these lipid-mediated effects were markedly blunted in TLR4$^{-/-}$ adipocytes. TLR4 deficiency attenuated lipid-induced serine^{307} phosphorylation of IRS-1 and normalized insulin-stimulated IRS-1 tyrosine phosphorylation. Physiological role of TLR4 was demonstrated using chronic high-fat feeding in female TLR4$^{-/-}$ mice. TLR4 deletion partly prevented diet-induced insulin resistance despite increased adiposity in the null mice, and these effects were associated with reduced expression of TNF-α, IL-6, SOCS3, MCP-1, and the macrophage marker F4/80 in adipose tissue and liver of high-fat fed TLR4$^{-/-}$ mice. Taken together, these findings strongly support the role of TLR4 as a mediator of lipid-induced activation of inflammation and insulin resistance.

It is clear that many factors mediate obesity-associated insulin resistance. Inhibition of TNF-α, IKK, PKC, JNK, and SOCS3 as well as a decrease in fatty acids all improve insulin sensitivity. A significant implication of Shi and Flier's findings is that TLR4 may be one gateway by which fatty acids impact inflammation and metabolism. In this scenario, increased fatty acids, as found in obese subjects, activate TLR4 signaling in macrophages, adipocytes, and liver. This directly activates IKK, JNK, PKC, and SOCS3, which leads to serine phosphorylation of IRS proteins and impaired insulin signaling and action. Lipid-induced activation of TLR4 and IKK/NF-κB also increases local production of TNF-α and IL-6 that indirectly attenuates insulin action in other organs (Figure 1). However, the authors' findings pose noteworthy questions. TLR4 deletion increased food intake, suggesting the potential role of CNS circuitry on metabolism. A direct versus indirect role as well as macrophage-independent effects of TLR4 on insulin resistance need to be addressed by generating tissue-specific KO mice. It is also puzzling that insulin sensitivity phenotypes are evident in female TLR4$^{-/-}$ mice but not in male null mice despite blunted diet-induced inflammatory gene expression.

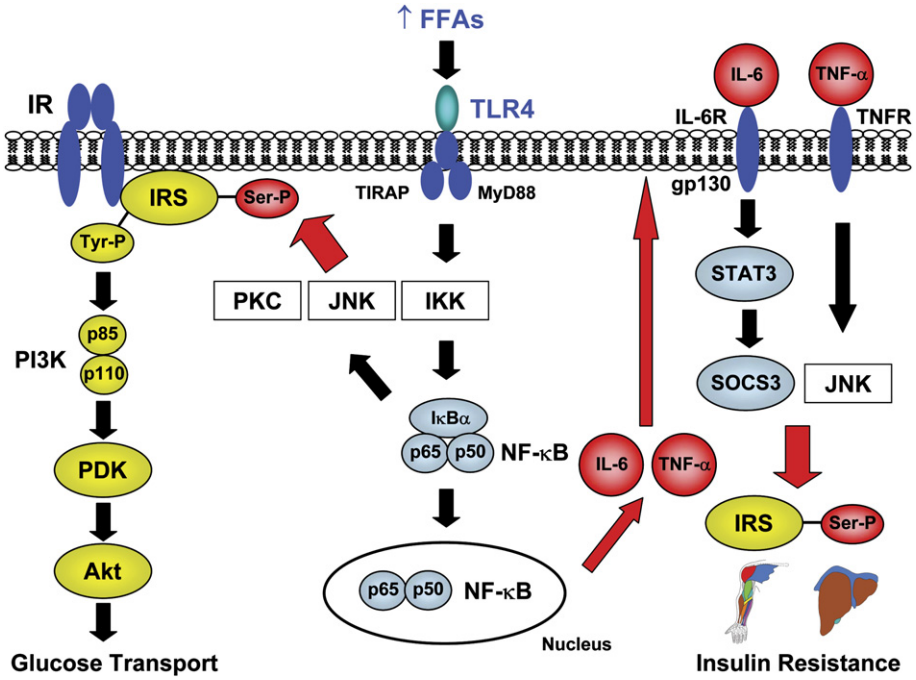

Figure 1. TLR4 mediates FFA-induced activation of inflammatory and metabolic signaling in insulin resistance

FFAs bind to TLR4 to activate IκB-kinase (IKK), which causes degradation of IκBα and stimulates nuclear translocation and expression of NF-κB associated genes such as IL-6 and tumor necrosis factor (TNF)-α. Activated IKK, c-Jun amino-terminal kinase (JNK), and protein kinase C (PKC) directly increase serine phosphorylation of insulin receptor substrate (IRS) that downregulates insulin signaling associated with IRS tyrosine phosphorylation, PI 3-kinase (PI3K), PDK, and Akt, resulting in reduced glucose transport into cells (insulin resistance). FFA-induced activation of TLR4/IKK/NF-κB and production of IL-6 and TNF-α further downregulate insulin signaling via suppressor of cytokine signaling 3 (SOCS3) and JNK and indirectly cause insulin resistance in peripheral organs.

For future targeting strategy, it will be important to identify a TLR4 inhibitor that affects the "bad" metabolic arm of signaling without impacting the "good" inflammation arm of the pathway. Overall, TLR4 is an appealing answer to a century-old question on the link between obesity, inflammation, and insulin resistance, and their findings will lead to uncovering new therapeutic targets to fight this old disease.

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Protein nutrition as therapy for a genetic disorder of bone?

Bone formation is controlled by a network of transcription factors and signaling molecules. In this issue, Elefteriou et al. (2006), studying the role of the transcription factor ATF4 in a new mouse model of neurofibromatosis type I skeletal defects, demonstrate striking effects of changing dietary protein on bone formation abnormalities.

Genetic analyses in humans and mice have uncovered transcription factors that control osteoblast differentiation and bone formation through enhancing (e.g., *Runx2* and *Osterix*) or diminishing (e.g., *Twist*) the expression of target genes (Karsenty and Wagner, 2002). Other genetic mutations pinpoint previously unrecognized pathways of control of bone formation. For example, activation of *Lrp5* (a component of the Wnt signaling complex) results in a rare high bone-mass syndrome; inactivation of *Lrp5* is accompanied by severe osteoporosis. Genetic inactivation of *SOST* results in another rare ''big-bone'' syndrome—*SOST* encodes the protein product sclerostin, which is localized in bone to the osteocyte and operates as a powerful inhibitor of bone formation.

Novel aspects of transcriptional control of bone development are reported in this issue of *Cell Metabolism* by Elefteriou et al. (2006), showing quite remarkably that the increased bone-mass phenotype resulting from neurofibromin (*Nf1*) deficiency can be rescued through nutritional restriction of protein intake. In addition, the opposite skeletal phenotype, *Atf4* deficiency, which is analogous to Coffin-Lowry syndrome, can be rescued by high protein feeding.

These authors had shown previously that mice null for either the transcription factor *Atf4* or its essential activating kinase, *Rsk2*, exhibited greatly impaired bone formation, with reduced numbers and thickness of bone trabeculae (Yang et al., 2004). The defect in bone formation continued postnatally, after having become obvious relatively late in fetal development. Type I collagen protein synthesis was decreased almost 10-fold in $Atf4^{-/-}$ osteoblasts, even though expression of *α(I) collagen* was unchanged. Notably, the defect in collagen production was corrected in vitro by addition of nonessential amino acids, indicating that the failed collagen production resulted from a posttranscriptional effect. This revealed in osteoblasts a regulatory role of ATF4 in amino acid transport that had been recognized in other cell types (Harding et al., 2003).

In the present work (Elefteriou et al., 2006), the authors used a mouse model to investigate the skeletal phenotype seen in patients with neurofibromatosis type 1, which results from deficiency of the *Nf1* protein product, a tumor-suppressor protein. Skeletal abnormalities in these patients include bowing of the long bones and pseudoarthroses and increase with age (Ruggieri et al., 1999). Since ablation of *Nf1* in mice is embryonic lethal and since *Nf1* was found to be amply expressed in osteoblasts, the authors chose to prepare mice in which *Nf1* was ablated specifically in osteoblasts.

In the $Nf1_{ob}^{-/-}$ mice, bone volume increased progressively starting soon after birth, with quantitative histomorphometry showing increases in osteoblast surface and number, osteoid amount, and bone formation rate (Figure 1). Additionally, bone resorption was increased, with increased osteoclasts in bone and excretion of a marker of bone resorption. Importantly, the enhanced osteoclast formation was not cell autonomous but required coculture of *Nf1*-deficient osteoblasts with wild-type hematopoietic cells. Thus, the phenotype appeared to be the opposite of that accompanying *Atf4* or *Rsk2* deficiency.

The critical clue, however, came from molecular studies in bone and osteoblasts: production of type I collagen protein was increased despite normal collagen mRNA in osteoblasts and bone of $Nf1_{ob}^{-/-}$ mice. Although the amount of ATF4 was not increased in the bones of mutant mice, its activation by its crucial kinase, RSK2, was enhanced, leading to the conclusion that NF1 acts in the osteoblast to limit phosphorylation of ATF4 by RSK2 and, in so doing, limits expression of osteocalcin as well as the transport of amino acids necessary for the synthesis of the most abundant protein in bone, type I collagen. Confirmation of the mechanism came by overexpressing *Atf4* in osteoblasts and generating mice that recapitulated the