nutrients act to more slowly alter a spectrum of signaling pathways and gene expression changes, ultimately leading to depolarization or hyperpolarization. Furthermore, POMC neurons are much less homogeneous than the NPY/AgRP neurons, with only a subset of cells depolarized by leptin (Williams et al., 2010). Data also suggest there may be both GABAergic and glutamatergic POMC cells (Hentges et al., 2009). Thus, electrical stimulation of POMC cells may produce a more mixed response than the pure inhibitory GABAergic drive from NPY/AgRP neurons or the potent anorexigenic response to α-MSH alone.

Nonetheless, optogenetics is a powerful new tool to apply to the analysis of the energy homeostasis circuitry, and the rapidly developing optogenetic armamentarium includes channels that can be used to hyperpolarize and inhibit neurons, channels that can be targeted to presynaptic nerve terminals, and light-activated molecules that couple to activation of G protein signaling pathways (Gradinaru et al., 2010). For illuminating the role of neurons that are too diffusely distributed to activate with a fiber-optic light stimulus, chemical-genetic tools, such as a G protein-coupled receptor capable of activating neurons in response to a pharmacologically inert, orally bioavailable drug, clozapine-N-oxide, have also been engineered (Alexander et al., 2009). Given the complexity of the circuitry involved in energy homeostasis, optogenetics and chemical-genetic tools may prove invaluable.

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# Endothelium-Dependent Delivery of Insulin to Muscle Interstitium

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**Insulin contributes to skeletal muscle glucose uptake by increasing blood flow and recruiting perfused capillaries. In this issue of *Cell Metabolism*, Kubota et al. (2011) show that deletion of IRS-2 in endothelial cells in mice causes impaired transcapillary insulin transport, decreased insulin-stimulated glucose uptake in muscle, and mild glucose intolerance.**

Insulin signaling in vascular endothelium produces at least two types of discrete actions. First, insulin modifies endothelial homeostasis in arteries, thereby making the vascular wall less susceptible to atherosclerosis (Rask-Madsen and King, 2007). Second, insulin may regulate its own delivery to skeletal muscle and other tissues (Barrett et al., 2009; Chiu et al., 2008). Whether this mechanism contributes significantly to systemic insulin sensitivity is not clear in spite of extensive investigation. In this issue, Kubota et al. (2011) report that endothelial insulin signaling through insulin receptor substrate-2 (IRS-2), a docking protein relaying insulin receptor activation to intracellular signaling, contributes to transcapillary insulin transport in muscle and affects glucose tolerance in mice. These results suggest that endothelial cell function may be a therapeutic target for improving peripheral insulin sensitivity.

The rate of insulin delivery from the blood to the interstitial space is limited by transport across the capillary wall in tissues where endothelial cells form tight junctions. After binding to its receptors, insulin can be transported across cultured endothelial cell monolayers by transcytosis (King and Johnson, 1985), but little is known about intracellular insulin signaling in this process (Wang et al., 2008). It is also unclear whether transcytosis (Barrett et al., 2009) rather than passive diffusion at cell junctions (Chiu et al., 2008) is responsible for transendothelial transport of insulin in vivo, which is important because transcytosis is more likely to be a regulated process which can be modified for therapeutic gain. Insulin resistance developing during high-fat feeding

of mice can be detected earlier in endothelium than in other tissues (Kim et al., 2008), suggesting that early reversal of endothelial insulin resistance could help prevent peripheral insulin resistance if there was a cause-and-effect relationship between the two.

Kubota et al. (2011) studied mice with obesity-associated insulin resistance induced by high-fat diet or the *ob*/*ob* mutation and found that IRS-1 and IRS-2 in endothelial cells were downregulated by 50% and 80%, respectively. Capillary blood volume increased 10 min after start of a euglycemic hyperinsulinemic clamp in control mice, followed by an increase in interstitial insulin concentrations after 60 min. However, in obese mice the increase in capillary blood volume in skeletal muscle was blunted at 10 min, and interstitial insulin concentrations were decreased at 60 min, suggesting that insulin delivery from the blood to the interstitial space was delayed by transport across the capillary wall during physiological conditions, and further delayed in obesity-associated insulin resistance. To study whether insulin activates insulin signaling to facilitate its own transendothelial transport, the authors then created and studied mice with knockout of IRS-1 or IRS-2 in endothelial cells (ETIrs1KO and ETIrs2KO mice, respectively). ETIrs1KO mice were not different than their controls with respect to endothelial cell insulin signaling, whole-body insulin sensitivity, or glucose tolerance, but ETIrs2KO mice had similar defects in increases of capillary blood volume and interstitial insulin concentrations as mice with obesity-associated insulin resistance. In addition, ETIrs2KO mice had glucose intolerance relative to their controls. If a similar mechanism exists in humans, it would be a new target for insulin-sensitizing drugs.

Insulin-stimulated increases in blood flow and increased recruitment of perfused capillaries are both dependent on nitric oxide (NO) production and may augment insulin delivery to the interstitium. Because NO has been shown to mediate insulin-stimulated capillary recruitment, Kubota et al. (2011) treated ETIrs2KO mice with beraprost sodium, a prostacyclin analog which increases eNOS expression. In ETIrs2KO mice, this treatment increased eNOS protein levels in endothelial cells and completely normalized capillary recruitment, interstitial insulin concentrations, and whole-body insulin sensitivity. Beraprost sodium had no effect in eNOS knockout mice, making it unlikely that this effect was mediated by effects of prostacyclin directly on skeletal muscle perfusion.

Glucose intolerance in ETIrs2KO mice was mild, similar to that described for eNOS knockout mice (Duplain et al., 2001), and much less severe than in mice fed a high-fat diet. In the study by Kubota et al. (2011), plasma glucose levels measured 30 min after glucose administration were approximately 180 mg/dl higher in high-fat-fed mice but only 40 mg/dl higher in ETIrs2KO mice, compared with controls. This was partly explained by a maintained ability in ETIrs2KO mice to reduce endogenous glucose production from the liver in response to insulin, an effect that was profoundly impaired in mice after high-fat feeding. This abnormality of whole-body glucose metabolism contrasted with a considerable impairment of glucose uptake in skeletal muscle during a euglycemic hyperinsulimic clamp. These experiments show that insulin signaling through IRS-2 in endothelial cells is important for insulin delivery to skeletal muscle interstitium and insulin-stimulated glucose uptake in muscle, but has little effect on whole-body glucose tolerance when insulin sensitivity in the liver is normal. Whether the contribution of endothelial insulin resistance to whole-body glucose intolerance is more pronounced when insulin signaling in the liver is impaired, as in human insulin resistance, remains to be determined.

The results of Kubota et al. (2011) are somewhat at odds with a previous study in mice with the insulin receptor deleted in endothelial cells (Vicent et al., 2003). These animals were glucose tolerant except when challenged with diets with abnormal sodium content. However, when cross-bred with atherosclerosis-susceptible apolipoprotein E (apoE) null mice, atherosclerotic lesion area in the aorta and carotid artery was increased by up to 2.9-fold (Rask-Madsen et al., 2010). Glucose and insulin tolerance, plasma lipids, and blood pressure were not different in the two groups, and the increased atherosclerosis susceptibility could not be recapitulated by transplantation of bone marrow from insulin receptor knockout mice (Rask-Madsen et al., 2010), showing that the phenotype was due to an isolated defect in endothelial cell insulin signaling. Therefore, loss of insulin action in endothelial cells in this model had a large impact on atherosclerosis development, but no effect on glucose tolerance (Rask-Madsen et al., 2010).

Kubota et al. (2011) speculate that selective impairment of the phosphatidylinositol 3-kinase (PI3K) pathway of insulin signaling, which is activated by IRS proteins, has a larger effect on insulin sensitivity in muscle than deletion of all insulin signaling (Vicent et al., 2003; Rask-Madsen et al., 2010) because activation of the PI3K and Erk pathway of insulin signaling can have opposite hemodynamic effects. In addition to this question, it is unresolved whether the blunted increase in interstitial insulin concentrations during hyperinsulinemia in ETIrs2KO mice is due to a blunted increase in capillary surface area, or a defect in a mechanism by which transendothelial insulin transport is regulated by endothelial cell insulin signaling, with impaired capillary recruitment as an associated (or contributing) factor. Future challenges include determining whether the mechanisms impairing insulin signaling in obesity and type 2 diabetes are the same in endothelial cells as in classical insulin-sensitive tissues like muscle. Discoveries in this field may provide targets for augmenting endothelial insulin sensitivity which may not only improve muscle insulin sensitivity but also help prevent long-term complications like atherosclerosis in people with the metabolic syndrome or diabetes.

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# New Powers of Brown Fat: Fighting the Metabolic Syndrome

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**An understanding of the full powers of brown adipose tissue (BAT) is only successively being accumulated. In a paper in *Nature Medicine*, Bartelt et al. (2011) add further impressive aspects to the potential powers of BAT in the combat against the metabolic syndrome by demonstrating its vast capacity for triglyceride clearance and glucose disposal.**

Until very recently, brown adipose tissue (BAT) seemed to attract interest from only a few hibernation researchers and mitochondrial bioenergeticists. This was understandable. BAT is not easily discernable: It has a color and texture that blends into both fat and muscle, and it is found in small depots spread out in different parts of the body, totally amounting to only a small percentage of total body weight (Figure 1A). Yet this organ has the power to be a main player in metabolism. In their recent study in *Nature Medicine*, Bartelt et al. (2011) reveal new major roles for BAT in blood triglyceride clearance and glucose disposal.

To generate heat for thermogenesis, BAT first uses its stored lipid as substrate (Figure 1B). This early phase of thermogenesis corresponds molecularly to norepinephrine released from the sympathetic nerves activating the release of free fatty acids from triglyceride droplets (Figure 1B). Some of these fatty acids activate UCP1 (uncoupling protein 1). The remaining fatty acids are imported into the mitochondria and combusted, releasing energy as heat, due to UCP1 action. However, with brown fat comprising such a small percent of total body weight, the stored lipid can sustain thermogenesis for only a short time, and further energy supplies must come from outside the tissue.

The study of Bartelt et al. (2011) reveals the ability of BAT to import and combust triglycerides from the circulation and demonstrates how this uptake delivers substrate for continued thermogenesis. By exposing mice to cold, Bartelt et al. optimized the acute activity of BAT, and this had dramatic effects on triglyceride levels in the blood. Specifically, plasma triglycerides (mainly in the form of chylomicrons, i.e., the dietary fat) practically disappeared from the circulation—not because fewer chylomicrons were formed, but because they were nearly all cleared by BAT; in fact, nearly half of the triglyceride from a meal ended up in BAT (Figure 1C). The clearance capacity of BAT could probably be even higher: prior to being exposed to cold, the mice had been living at normal environmental temperature and would thus have to also shiver in the cold (explaining the fatty acid uptake in the muscles). Had cold-acclimated mice with recruited BAT been used, the fraction entering BAT may have been even higher.

The data of Bartelt et al. lead to the following scenario for the high triglyceride clearance into BAT (Figure 1D). While norepinephrine activates the release of free fatty acids from triglyceride droplets within the brown fat cell, it also induces VEGF (Fredriksson et al., 2005) and lipoprotein lipase (LPL) gene expression (Mitchell et al., 1992; Bartelt et al., 2011). VEGF leads to increased capillary permeability, allowing plasma triglycerides to leave the capillaries (as elegantly video-recorded in vivo by Bartelt et al.). LPL degrades the triglycerides and allows fatty acids to become available for combustion in the tissue through the action of the plasma membrane transporter CD36; without CD36, the mice could not fight the cold.

In addition to BAT being a major lipid-combusting tissue, Bartelt et al. also point to BAT as being a major organ for glucose disposal, as (particularly in obese mice) a large fraction of ingested glucose is channeled to BAT (Figure 1E), where the glucose, just like lipids, will be combusted. This result reinforces the observation that BAT is an avid glucose uptake organ (Cawthorne, 1989). Indeed, the present realization that adult humans possess active BAT initially arose from the unexpected observations, made by radiologists using glucose uptake to search for tumors, of highly active glucose uptake into areas that turned out to be BAT (Hany et al., 2002). Most of the high