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ATVB IN FOCUS:
Receptor-Mediated Transcytosis

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Regulation of Insulin Transcytosis Across Endothelium in Metabolic Health and Disease

Chieko Mineo, Philip W. Shaul

ABSTRACT: The delivery of insulin to the skeletal muscle has a major influence on glucose disposal in muscle, where 80% of total body glucose disposal occurs. The skeletal muscle microvascular endothelial cells play a critical role in peripheral insulin sensitivity through their regulation of insulin delivery. Recent advancements in methodologies have provided in-depth views of the molecular mechanisms by which the endothelial cells regulate the delivery process. However, how the cellular machinery is modulated under physiological or pathological conditions remains largely unexplored. Conditions with estrogen deficiency and obesity are 2 situations that are closely associated with peripheral insulin resistance and type 2 diabetes in humans. It is of great interest to determine whether and how endothelial control of insulin delivery impacts the development of metabolic dysregulation under these and other conditions. This review aims to provide an overview of the molecular mechanisms governing insulin delivery to the skeletal muscle. The available evidence will be presented that the transcytosis of insulin across the endothelial cell monolayer in skeletal muscle plays a critical role in muscle insulin delivery, thereby having a major impact on overall glucose homeostasis. In vivo investigations with manipulation of mechanisms in endothelial cells will be summarized, and the current knowledge gaps will be presented. Interrogation of the role of the endothelium in insulin transport provides a paradigm in which insights are being gained about cellular actions of insulin, molecular transport by endothelial cells, and the intricacies of glucose homeostasis.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: endothelial cells ■ endothelium, vascular ■ hyperglycemia ■ insulin ■ muscle fibers, skeletal

According to the National Diabetes Statistics Report by the Centers for Disease Control and Prevention, diabetes affects 38.4 million people or 11.6% of the population in the United States. In addition, an estimated 97.6 million, or 38% of adults aged 18 years, have prediabetes (https://www.cdc.gov/diabetes/php/data-research/index.html). The majority of diabetes is type 2 diabetes (T2DM), which is primarily characterized by peripheral insulin resistance,[1] which is also a hallmark of prediabetes.[2] Microvascular function in the skeletal muscle is critical to maintaining insulin sensitivity in health, and its attenuation likely contributes to T2DM and prediabetes in multiple instances.[3] Following its secretion from pancreatic β cells, insulin must reach its target tissues, such as skeletal muscle, heart, adipose tissues, and brain; it must then cross the endothelial cell monolayer to reach the parenchymal cells where it binds to the IR (insulin receptor) to trigger intracellular processes.[4–6] The skeletal muscle is a major target tissue of insulin,[7] with 80% of whole-body glucose disposal occurring in the skeletal muscle.[8–10] Thus, the delivery of insulin to the skeletal muscle has a major impact on global glucose homeostasis. In vivo insulin delivery to skeletal muscle has been studied in humans and in preclinical animal models using a variety of methods, including lymph fluid sampling,[11–13] microdialysis,[14–16] and intravital

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Nonstandard Abbreviations and Acronyms

apoE3	apolipoprotein E3
apoE4	apolipoprotein E4
BBB	blood-brain barrier
BMEC	brain microvascular endothelial cell
BPS	beraprost sodium
E2	estradiol-17β
EndoIRKO	endothelial cell-specific deletion of IR
eNOS	endothelial nitric oxide synthase
ER	estrogen receptor
ETIRS2KO	endothelial cell-specific IRS-2 knockout
FcγRIIB	Fc γ receptor IIB
GLUT4	glucose transporter 4
HFD	high-fat diet
IGF1R	insulin growth factor 1 receptor
IgG	immunoglobulin G
IR	insulin receptor
IRS	insulin receptor substrate
PBK/Akt	protein kinase B or Akt
PI3 kinase	phosphoinositide 3-kinase
SNX5	sorting nexin 5
T2DM	type 2 diabetes

Highlights

- Endothelial insulin transcytosis is a rate-limiting step for insulin delivery to the skeletal muscle, and it is mediated by a distinctive vesicular transport system in endothelial cells.
- Defects in endothelial insulin transport result in peripheral insulin resistance both in humans and in preclinical animal models.
- Loss of estrogens both in men and women is associated with insulin resistance and type 2 diabetes.
- There is estrogen-estrogen receptor α promotion of endothelial cell insulin transcytosis, and it is dependent on the association of the vesicular trafficking protein SNX5 (sorting nexin 5) with the receptor and subsequent phosphoinositide 3-kinase (PI3 kinase) activation.
- In obesity and its associated type 2 diabetes, there is impaired insulin signaling in endothelial cells, resulting in attenuated capillary recruitment and insulin delivery to the skeletal muscle. In addition, obesity-induced alterations of glycan modifications of IgG (immunoglobulin G) suppress endothelial cell insulin transcytosis through the receptor for IgG, FcγRIIB (Fc γ receptor IIB).

microscopy.[17–20] These studies have provided insight into the processes normally regulating insulin access to skeletal muscle myocytes and those underlying the attenuation in muscle insulin delivery in disease states.[13,16,21–24] However, some key aspects of the cellular mechanisms for insulin transcytosis remain unsolved. This review will briefly summarize the current knowledge on endothelial insulin transport and its impact on glucose homeostasis.

INSULIN DELIVERY TO SKELETAL MUSCLE

The importance of insulin delivery to skeletal muscle metabolic function has been revealed in human studies. Insulin is distributed more slowly to the skeletal muscle than other tissues during hyperinsulinemic-euglycemic clamps, and insulin concentrations in the skeletal muscle, not in the circulation, correlate with peripheral glucose uptake after systemic insulin infusion.[7,25] Preclinical studies using an euglycemic clamp method in dogs demonstrated that transcapillary transport is likely the rate-limiting step in insulin action.[11,12] Furthermore, a direct injection of insulin into the interstitium of the skeletal muscle in dogs was followed by a rapid increase in muscle glucose uptake, without the delay in insulin action observed following a systemic infusion.[26] In individuals with obesity and T2DM, although insulin secretion to the plasma and delivery to the liver are comparable to what is observed in healthy individuals, the delivery of insulin to skeletal muscle is decreased, and insulin-induced glucose disposal is suppressed in the skeletal muscle.[27–29] Women with obesity without diabetes with postprandial hyperglycemia require higher circulating insulin levels than lean controls to attain similar interstitial insulin levels in adipose tissue and skeletal muscle, indicating an impaired transfer of insulin across the capillary endothelium in prediabetic humans.[30] These findings have collectively established that insulin delivery to the skeletal muscle by the microvascular endothelium is the rate-limiting step in the majority of total body insulin-induced glucose disposal.

Three insulin-induced mechanisms facilitate insulin delivery to the skeletal muscle and other tissues with tight endothelial monolayers (Figure 1): (1) an increase in capillary blood flow, (2) an increase in available capillary surface area via capillary recruitment, and (3) an increase in the transendothelial transport of insulin.[3,31–33] Insulin-induced blood flow promotes its own tissue delivery.[31] An attenuation of insulin-stimulated peripheral tissue blood flow results in lower glucose uptake both in humans and in animal models.[34–38] Using contrast-enhanced ultrasound or intravital microscopy both in humans and in animal models, many studies have demonstrated the induction of capillary recruitment in the skeletal muscle in response to an insulin infusion.[9,14,39–43] Regarding the third mechanism entailing transendothelial transport of insulin, it is relevant that the capillary endothelium is continuous in the skeletal muscle and in other tissues including adipose and the heart.[44,45] The consensus is that in these tissues,

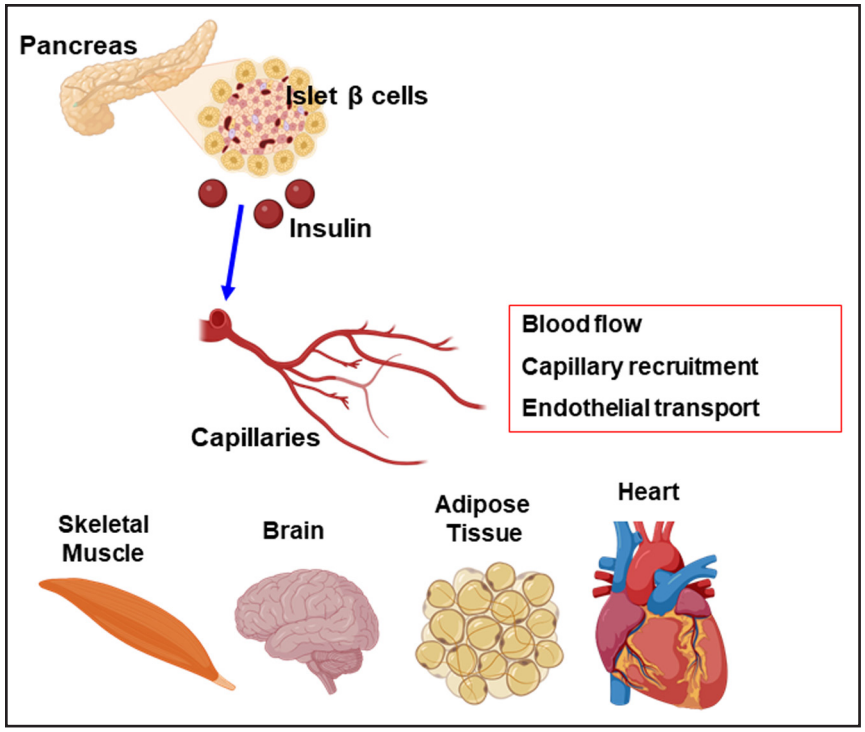

Figure 1. Insulin delivery to the relevant organs.
Following its secretion from pancreatic β cells, insulin reaches its target tissues, such as skeletal muscle, heart, adipose tissues, and brain. The delivery process is normally facilitated by 3 major mechanisms: (1) an increase in capillary blood flow, (2) an increase in available capillary surface area via capillary recruitment, and (3) an increase in the transendothelial transport of insulin. Created by BioRender.com.

particularly in skeletal muscle, the transendothelial insulin transport is transcellular and not paracellular, mediated by the vesicular transport process known as transcytosis.[33,46,47] Transcytosis is the composite intracellular process of endocytosis at the luminal cell surface, vesicular transport through the cytoplasm, and exocytosis at the basolateral surface, resulting in the release of insulin for interaction with and action in the subendothelial skeletal muscle myocytes.[33,46,47] Determining how insulin crosses the endothelium has been the focus of intense research, as impaired insulin transcytosis is a likely key contributing factor to peripheral insulin resistance that may be amenable to therapeutic intervention.[3,32,33,46,48]

ENDOTHELIAL CELL INSULIN TRANSCYTOSIS MECHANISM

Receptor-Mediated Saturable or Nonsaturable Endocytic Pathway

Opinions are divided as to whether endothelial cell insulin transcytosis occurs through receptor-mediated saturable endocytosis or a nonsaturable process such as fluid-phase endocytosis (Figure 2).[20,24,33,49] Studies in cultured endothelial cells found that labeled insulin transport across endothelial layers is saturable, suppressed by unlabeled insulin or antibodies against the IR.[50–53] Some studies have also suggested that the IGF1R

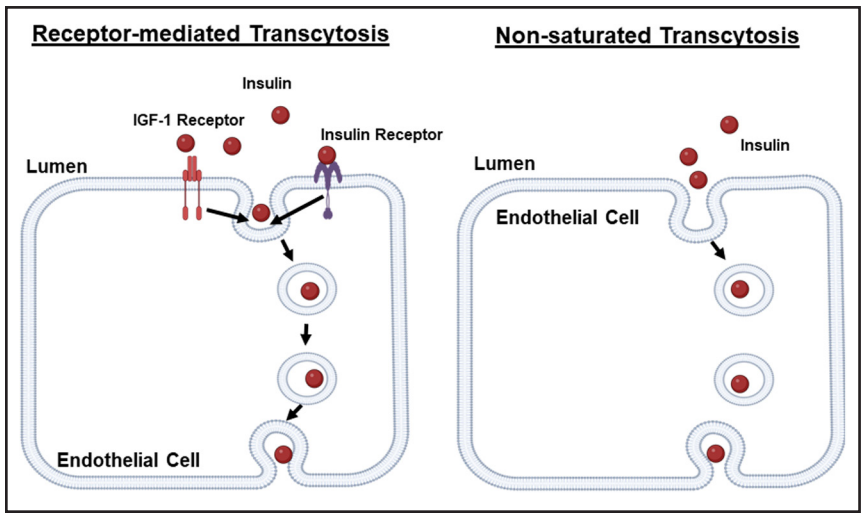

Figure 2. Receptor-mediated vs nonsaturated insulin transcytosis.
Insulin can be taken up by binding to endothelial cell surface receptors, such as insulin receptors or IGF1 (insulin growth factor 1) receptors, and transported via a saturable, receptor-mediated vesicular trafficking pathway. Alternatively, insulin can be internalized and transported via a nonsaturable vesicular pathway, such as pinocytosis or fluid-phase endocytosis. Created by BioRender.com.

(insulin growth factor 1 receptor) may participate.[54,55] This was supported by in vivo studies in rats, in which high concentrations of unlabeled insulin, delivered either continuously via hyperinsulinemic-euglycemic clamps or as a single bolus, attenuated ^{125}I-labeled insulin disposal in the skeletal muscle.[31,56] In contrast, Steil et al[57] performed euglycemic clamps in dogs and reported that the plasma-to-skeletal muscle interstitium ratio was markedly reduced at high, pharmacological compared with physiological levels of insulin, indicating that insulin transport is unlikely regulated by a saturable process. Another study in dogs by the same group used an insulin analog as a marker for transport. They found that a continuous infusion of pharmacological, saturating concentrations of insulin did not affect the rate of appearance of the insulin analog in the skeletal muscle interstitial fluid, consistent with a nonsaturable process.[58] Supporting that possibility, using intravital microscopy to measure the rate of fluorescent-labeled insulin efflux across the endothelium of skeletal muscle capillaries, Williams et al[20] reported that insulin crosses the capillary endothelium via a nonsaturable, fluid-phase endocytosis. It has been proposed that the differing conclusions regarding whether the transcytosis is receptor-mediated and saturable or nonsaturable and possibly fluid-phase endocytosis are due to the different concentrations of insulin used in the experimental models. More comprehensive reviews are available on the subject.[47,49]

IR Signaling and Transcytosis

It remains similarly controversial whether the IR and its signaling in the endothelium play a role in insulin transcytosis (Figure 3). A study in mice with endothelial cell–specific deletion of the IR, termed EndoIRKO,[59] demonstrated that in response to systemic insulin administration, the loss of endothelial IR caused a delay in the onset of insulin signaling in skeletal muscle, brown fat, and brain but not in liver where fenestrated endothelium allows insulin diffusion into the tissue.[44,45,60] The delay in insulin signaling in skeletal muscle, brown adipose tissue, and brain was ≈10 minutes. Considering that the time lag was longer than the half-life of insulin in the circulation, which is 4 to 7 minutes in mice,[61] the delay may contribute to the systemic insulin resistance and glucose intolerance that was also observed in the EndoIRKO mice. However, the kinetic delay had no impact on the maximum insulin signaling after systemic insulin administration.[59] A study using intravital microscopy with a fluorescent-labeled insulin probe showed that a peptide antagonist of the IR had no effect on transendothelial insulin efflux.[20] These results suggest involvement of additional receptors that mediate insulin transcytosis or signaling. The IGF1R may be such an alternative receptor[54,55] because IGF1R neutralizing antibodies have been shown to suppress transendothelial insulin transport in cultured endothelial cells.[53]

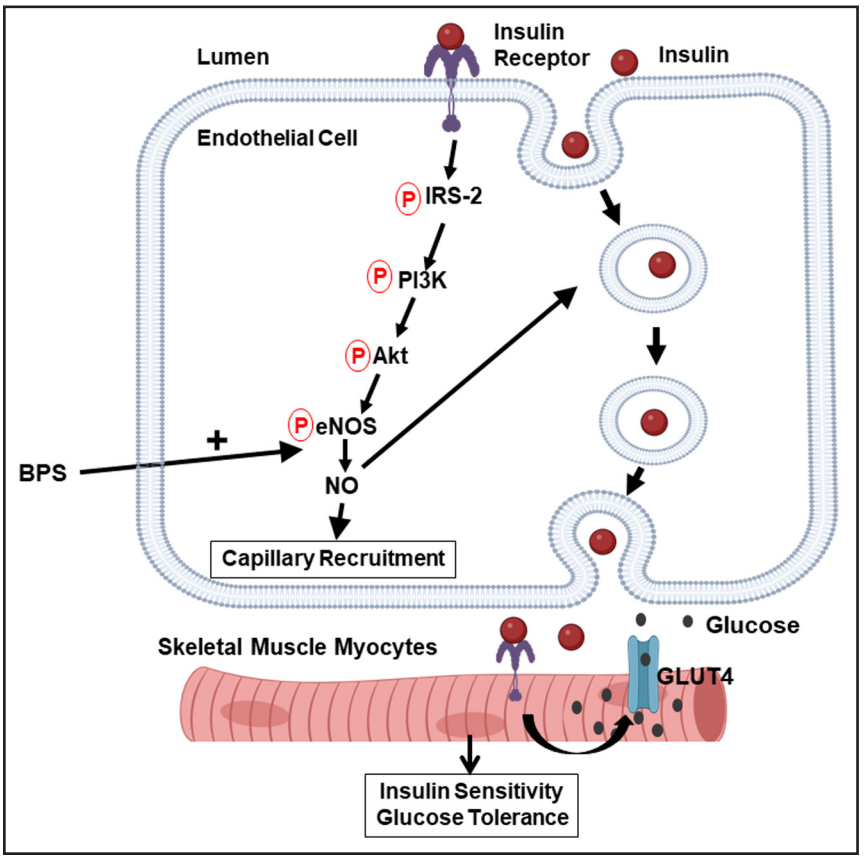

Figure 3. Insulin signaling promotes endothelial cell insulin transport and capillary recruitment in skeletal muscle.
Insulin activation of the insulin receptor stimulates NO production by eNOS (endothelial nitric oxide synthase) via IRS-2, PI3 kinase, and Akt. The NO production promotes both capillary recruitment and endothelial cell insulin transcytosis, enhancing insulin delivery to the skeletal muscle myocytes and glucose disposal involving glucose uptake by glucose transporter 4 (GLUT4). In this manner, insulin signaling in the skeletal muscle microvascular endothelium promotes insulin sensitivity and glucose tolerance. Created by BioRender.com.

Whether there is a requirement for endothelial IGF1R in insulin transcytosis in the skeletal muscle in vivo remains unclear.

In addition to possibly facilitating the internalization of insulin at the luminal surface of the endothelial cell, the IR initiates downstream signaling in the endothelium.[14,62–64] A cascade of kinase-driven phosphorylation events is triggered, involving IRSs (IR substrates), phosphoinositide 3-kinase (PI3 kinase), and protein kinase B or Akt (PKB/Akt), leading to activation of eNOS (endothelial nitric oxide synthase) and NO production.[65–69] Endothelium-derived NO mediates insulin-induced microvascular perfusion in skeletal muscle in preclinical animal models.[14,36,70,71] The eNOS knockout mouse has impaired capillary recruitment in the skeletal muscle,[14,36] and N-monomethyl-L-arginine, an NOS inhibitor, blocks muscle capillary recruitment in response to insulin.[70] In a pioneering study by Kubota et al,[14] in endothelial-cell–specific IRS-2 knockout (ETIRS2KO) mice insulin–induced eNOS phosphorylation, capillary recruitment and increases in interstitial insulin concentrations were impaired. Furthermore, decreased glucose uptake by the skeletal muscle was observed in the endothelial-cell–specific IRS-2 knockout mice after insulin infusion.[14] The upregulation of eNOS expression by the administration of beraprost sodium (BPS), a stable prostaglandin 2 analogue,[72–75] restored insulin-induced capillary recruitment, the interstitial increase in insulin concentrations, and glucose uptake in the skeletal muscle in endothelial-cell–specific IRS-2 knockout mice.[14] Through endothelial cell–specific loss-of-function approaches in vivo, this work demonstrated that insulin signaling via the IR and IRS-2 promotes eNOS stimulation through kinase activation, resulting in the promotion of capillary recruitment, insulin delivery, and glucose disposal in skeletal muscle (Figure 3). Attenuation of these mechanisms has been observed in high-fat diet (HFD)-fed mice, and BPS treatment partially restored insulin-induced phosphorylation of eNOS, capillary recruitment, and glucose uptake in the skeletal muscle.[14] Additional studies in HFD-fed rodents further support the key role of eNOS and NO in skeletal glucose uptake and protection against obesity-induced peripheral insulin resistance.[76–78] Importantly, inhibiting NOS diminishes insulin-induced capillary recruitment in the skeletal muscle in humans,[70,71,79] and the stimulation of NO production in T2DM subjects improves capillary recruitment.[80] Interestingly, in cultured aortic endothelial cells, the inhibition of IR kinase activity or downstream PI3 kinase has been shown to reduce the transendothelial transport of insulin[62] (Figure 3). Conversely, NO stimulates insulin transport across cultured endothelial cells, and the ability of insulin to stimulate cortical actin remodeling may be involved.[63,81] However, it is unknown whether NO regulates transcytosis in a similar manner in the microvascular endothelial cells in the skeletal muscle in vivo.[44–46]

IN VIVO INSIGHTS INTO DISTURBED INSULIN TRANSCYTOSIS IN ENDOTHELIAL CELLS AND TYPE 2 DIABETES

Estrogen, Estrogen Receptors, and Insulin Transcytosis

In addition to insulin itself, little was known about the processes that favor endothelial cell insulin transport until the possible effects of estrogens were entertained. Estradiol-17β (E2) plays a critical role in the regulation of energy balance and glucose homeostasis.[82,83] In female rodents and primates, ovariectomy leads to glucose intolerance and insulin resistance, particularly in the setting of diet-induced obesity, and these effects are negated by estrogen administration.[83–86] ERs (estrogen receptors) are members of the steroid hormone receptor superfamily that traditionally serve as transcription factors,[87] and the metabolic actions of E2 are mainly mediated by ERα.[83–86,88–93] Global deletion of ERα in mice leads to metabolic dysregulation, including increased body weight, enhanced adiposity, and impaired glucose homeostasis.[94,95] In women, surgical or natural menopause increases the age-adjusted odds ratio of developing diabetes compared with the rate in premenopausal women.[96] In postmenopausal women, hormone replacement therapy with estrogens lowers the risk of developing diabetes.[97,98] In men, individuals with a disruptive mutation in the *ERα* gene or deficiency in the aromatase gene, which produces estrogens by aromatizing androgens, have glucose intolerance, and in the latter category, E2 administration improves glucose tolerance.[99–101] In parallel, male aromatase null mice have glucose intolerance and insulin resistance that are reversed by E2.[102] Thus, estrogens and ERα play a major role in glucose metabolism in both males and females.

In endothelium, apart from its role as a transcription factor, there is a plasma membrane-associated subpopulation of ERα that mediates nonnuclear action of E2, activating downstream signaling pathways.[103–106] Until recently, it was unknown whether and how E2-ERα in the endothelium contributes to insulin action and glucose metabolism. Sacharidou et al[107] addressed this question using mice with selective endothelial ERα deficiency. Endothelial ERα deletion leads to systemic glucose intolerance and insulin resistance in both male and female mice. The metabolic derangement was not due to changes in adiposity or processes in adipose tissues, as endothelial ERα loss had minimal impact on adiposity or adipose inflammation. This finding was surprising, considering the well-acknowledged function of endothelial ERα in the regulation of inflammation and angiogenesis, which are essential for the healthy expansion of adipose tissue.[108–112] Instead, the study found that a loss of endothelial ERα impairs insulin transport

across the endothelium in the skeletal muscle, leading to attenuated glucose disposal and peripheral insulin resistance. Furthermore, the impaired insulin and glucose delivery was not related to the impact on insulin-induced capillary recruitment or blood flow in muscle, as the mice with endothelial ERα deletion and control mice showed comparable responses to insulin infusion assessed by the contrast-enhanced ultrasound method. The effect on insulin transcytosis was not related to changes in intracellular vesicles in the skeletal muscle microvascular endothelium, which is a potential basis for the insulin transport defect associated with obesity in mice.[19] The mechanism by which E2-ERα promotes insulin transcytosis was identified through nonbiased approaches that revealed the ERα interactome in cultured endothelial cells and its translatome in the skeletal muscle endothelium in vivo.[107] The strategy revealed that ERα promotion of insulin transport requires SNX5 (sorting nexin 5). SNX5 belongs to the SNX protein family of cytoplasmic and membrane-associated proteins that mediate the vesicular trafficking of plasma membrane proteins.[113–115] In cultured endothelial cells, SNX5 knockdown suppressed E2-stimulated insulin uptake and transcytosis. Additional experiments uncovered that both the transcriptional upregulation of SNX5 and nonnuclear E2-ERα–mediated PI3 kinase activation are required to drive plasma membrane recruitment of SNX5 to initiate transcytotic vesicular trafficking of insulin (Figure 4). In addition to broadening our understanding of metabolic actions of estrogen, these findings identified a physiological process that promotes insulin transport to the skeletal muscle to foster normal insulin sensitivity and glucose homeostasis.

Obesity and Insulin Transcytosis

Obesity is a major risk factor for insulin resistance and T2DM.[116] A major aspect of obesity-induced insulin resistance is impaired insulin action in the skeletal muscle, where the majority of whole-body glucose disposal normally occurs.[8,9,34,117,118] Studies in humans found that insulin delivery to the skeletal muscle is attenuated in subjects with obesity and T2DM.[29,119,120] In animal models of HFD-induced obesity, similar defects in insulin transport to the skeletal muscle have been reported.[14,19,21] Using intravital microscopy to assess transendothelial transfer of insulin in skeletal muscle, Williams et al[19] showed that male mice with diet-induced obesity have a reduction in insulin delivery compared with lean mice. Impaired insulin transport was associated with a decrease

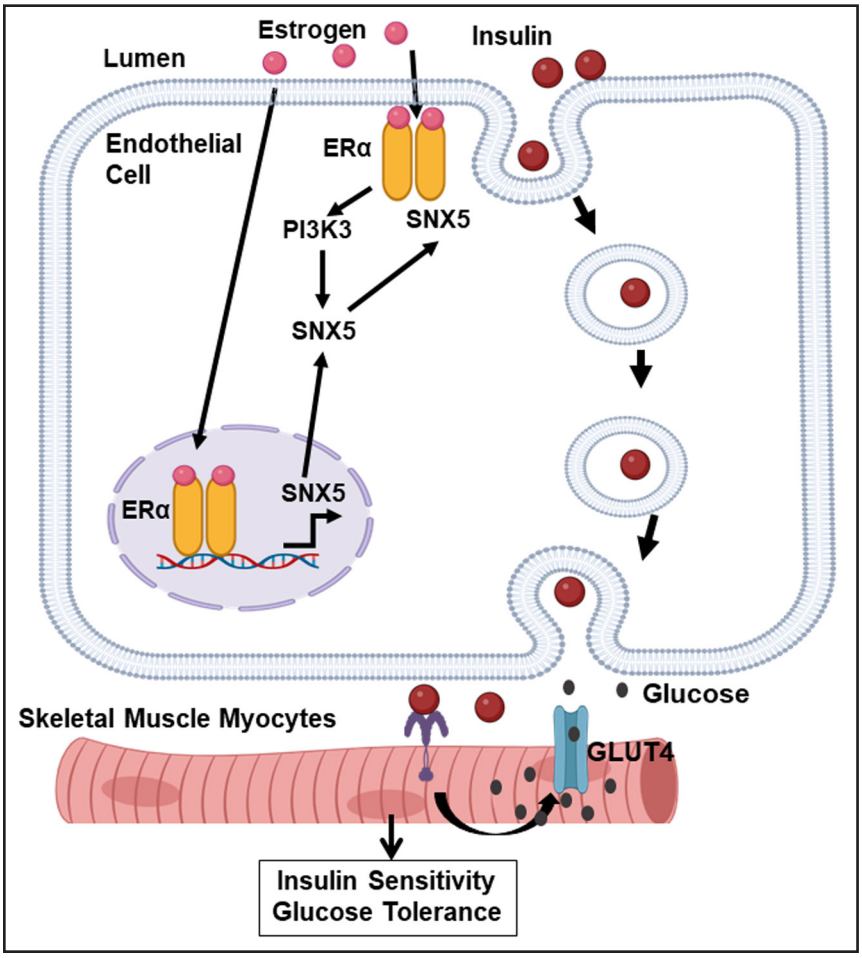

Figure 4. ERα activation promotes endothelial cell insulin transcytosis in the skeletal muscle microvasculature. Estrogens, such as estradiol, activate both ERα-dependent upregulation of SNX5 (sorting nexin 5) expression and nonnuclear ERα signaling, which causes PI3 kinase–dependent recruitment of SNX5 to the plasma membrane and to ERα. There is a resulting increase in endothelial cell insulin transcytosis that enhances insulin delivery to the skeletal muscle myocytes and glucose disposal, thereby promoting insulin sensitivity and glucose tolerance.[107] Created by BioRender.com.

in endothelial cell intracellular vesicles in the skeletal muscle, which were evaluated by electron microscopy.[19] Using primary human microvascular endothelial cells, Pillon et al[121] showed that treatment with the saturated fatty acid palmitate, mimicking in vivo exposure to HFD feeding, reduces insulin transcytosis in a toll-like receptor–dependent manner. However, how diet-induced obesity causes changes in endothelial insulin transcytosis remained unknown.

Obesity is frequently complicated by inflammation and activation of the adaptive immune system, including the production of IgG (immunoglobulin G) antibodies that can contribute to the disease pathogenesis.[122,123] A potential pathogenetic role of IgG in the development of insulin resistance first became apparent in an important report by Winer et al.[122] They revealed that HFD-fed obese mice lacking mature B cells and IgG production are resistant to insulin resistance, despite weight gain comparable to wild-type controls.[122] Furthermore, treatment with a B-cell–depleting CD20 antibody showed similar protective effects in wild-type obese mice. Importantly, the transfer of IgG from obese mice induced insulin resistance in B-cell–deficient recipients, whereas IgG from lean mice did not, implicating the pathogenic nature of HFD IgG.[122] This study provided the first evidence that alterations in IgG in the setting of HFD-induced obesity may cause insulin resistance. Tanigaki et al[124,125] uncovered a mechanism in endothelial cells in which the inhibitory IgG receptor–designated FcγRIIB (Fc γ receptor IIB) impairs glucose disposal in the skeletal muscle. Their studies revealed that FcγRIIB is expressed in the skeletal muscle microvascular endothelium, and that activation of endothelial FcγRIIB by C-reactive protein, another ligand for the receptor, causes the suppression of glucose delivery, leading to an insulin resistance phenotype in mice. However, these early results did not indicate whether FcγRIIB participates in obesity-induced insulin resistance.

IgG is a glycoprotein with a key conserved N-glycosylation site at asparagine 297 (Asn297) in the Fc domain, and modifications in the Fc glycan have a major impact on IgG binding to the Fcγ receptors that mediate cellular responses.[126–129] The IgG Fc glycans are biantennary structures with 2 N-acetylglucosamine and a trimannose core that is further extended by the addition of fucose, galactose, and sialic acid[130] (Figure 5, left). Changes in IgG glycosylation have been observed in several diseases including cancer, autoimmune disorders such as rheumatoid arthritis, and certain infectious diseases.[130,131] A higher proportion of sialic acid and galactose on the IgG glycan is associated with anti-inflammatory activity,[132] and proinflammatory IgG glycan features including hyposialylation have been observed in individuals with a higher BMI and with measures of central adiposity.[133–135] Tanigaki et al[136] addressed the question whether the hyposialylation of IgG contributes to T2DM development related to obesity, and if so, how. They found that HFD-fed obese mice lacking FcγRIIB selectively in the endothelium were protected from insulin resistance compared with control mice, despite comparable weight gain. It was primarily due to the preservation of insulin delivery to skeletal muscle and resulting maintenance of muscle glucose disposal, and IgG transfer experiments in B-cell–deficient mice implicated IgG as the pathogenetic ligand for endothelial FcγRIIB in obesity-induced insulin resistance[14,136] (Figure 5). Furthermore, the study showed that IgG transferred from patients with T2DM but not from metabolically healthy subjects caused

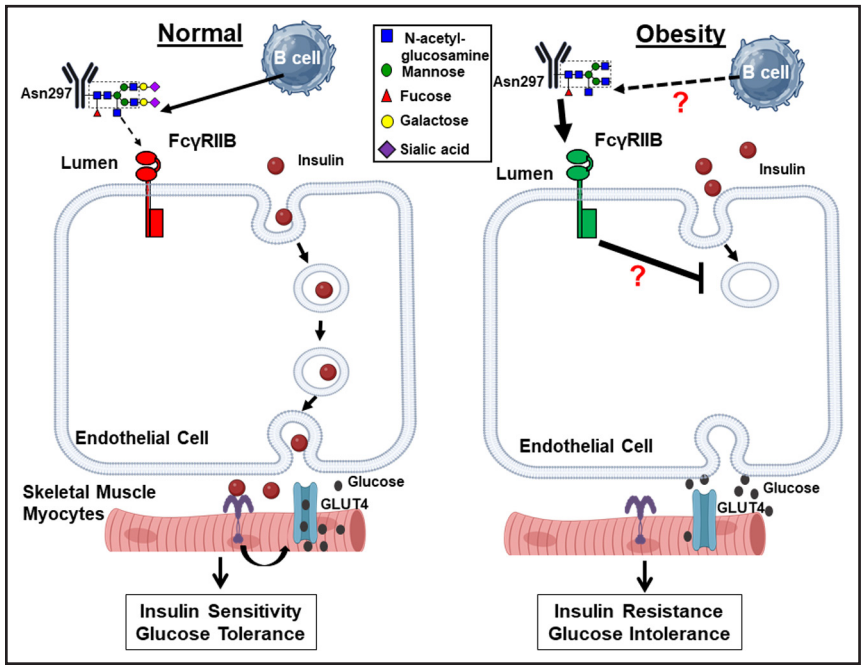

Figure 5. Effect of obesity on IgG glycosylation and the consequences for endothelial cell insulin transport. In contrast to normal conditions (**left**), in the setting of obesity (**right**), the IgG (immunoglobulin G) Fc glycan is hyposialylated, resulting in endothelial cell FcγRIIB (Fc γ receptor IIB) activation that inhibits insulin transcytosis. There is a decrease in insulin delivery to the skeletal muscle myocytes, causing a decline in glucose disposal, thereby promoting insulin resistance and glucose intolerance. Why obesity results in IgG hyposialylation and how the FcγRIIB activation inhibits transcytosis are currently unknown. Created by BioRender.com.

insulin resistance in B-cell–deficient mice via endothelial FcγRIIB, indicating that similar processes may be operative in T2DM in humans. These effects were attributed to hyposialylation of the Fc glycan in obese mice, and IgG from patients with T2DM was also hyposialylated. In HFD-fed mice, supplementation with the sialic acid precursor N-acetyl-D-mannosamine restored IgG sialylation and preserved insulin sensitivity without affecting weight gain. The activation of FcγRIIB by hyposialylated IgG from obese mice impaired endothelial cell insulin transcytosis in culture and in vivo; why obesity results in IgG hyposialylation and how the FcγRIIB activation inhibits transcytosis are yet to be determined. These results suggest that an attenuation of endothelial cell insulin transcytosis caused by IgG hyposialylation and endothelial FcγRIIB may represent a novel mechanism that links obesity with T2DM.

INSULIN DELIVERY TO THE CENTRAL NERVOUS SYSTEM AND HEART

The blood-brain barrier (BBB) is another site where insulin transport is critical. It is composed of brain microvascular endothelial cells (BMECs), and it serves as the physiological barrier between the blood and the central nervous system.[137–139] The BBB lacks fenestrations, and a tight cellular monolayer of BMEC limits the free entry of blood components into the brain. BMEC regulate the transport of circulating hormones and other bioactive molecules through receptor or transporter-mediated transcytosis via caveolae.[138,140,141] Insulin plays a major role in the central nervous system, and the mechanism by which the BBB regulates insulin transfer into the brain has been extensively studied.[49,142,143] The IR is expressed in BMEC, and in one report, endothelial cell–specific loss of the receptor delayed the onset of insulin signaling in specific brain regions, including the hypothalamus, hippocampus, and prefrontal cortex, leading to increased food intake and weight gain.[59] However, others have reported that insulin transport across the BBB is observed in the absence or upon inhibition of IRs in mice.[144] Regarding processes that retard BBB insulin transport, it has been found that apoE4 (apolipoprotein E4), compared with apoE3 (apolipoprotein E3), inhibits the transport. This may contribute to the increased risk of Alzheimer disease in individuals harboring the apoE4 allele because insulin impacts not just glucose utilization but also cell growth, autophagy, synaptic plasticity, and other important cellular processes in the central nervous system.[145] In mice, HFD feeding causes the suppression of key transporters in BMEC, including glucose transporter type 1.[146–148] However, it is unknown whether, in the setting of diet-induced obesity, the hyposialylation of IgG attenuates insulin transport across the BBB in a manner paralleling the observations in skeletal muscle.

Similar to the skeletal muscle microvasculature and BBB, the endothelium in the heart forms a tight, continuous layer, and impaired insulin actions in the cardiomyocytes contribute to the development of cardiac complications.[149] A recent study tested whether insulin transport across the endothelium is a rate-limiting step for insulin actions in the heart, using ex vivo insulin perfusion via the thoracic aorta into the myocardium of male mice.[48] It revealed that treatment with platelet-activating factor or vascular endothelial growth factor, which is known to increase vascular endothelial cell permeability, had no impact on Akt phosphorylation in myocytes induced by insulin infusion. Similarly, amiloride, an inhibitor of fluid-phase transport, did not impair insulin signaling in the myocytes. These data from the ex vivo studies suggest that the endothelial barrier may not be a rate limiting to insulin's action in the heart. However, future investigations are warranted to further clarify the mechanistic basis for endothelial insulin transport in the heart.

CONCLUSIONS AND PERSPECTIVES

Our knowledge of insulin production and secretion by pancreatic beta cells and its actions in target cells in virtually every organ has been expanding over more than a century. It is far more recent that it has been appreciated that the transport of insulin, particularly across the endothelium, is a major facet of insulin's impact on metabolism and other processes. The endothelial transport is likely particularly critical in tissues with contiguous endothelial cell layers in which paracellular transport occurs modestly if at all, such as the skeletal muscle, central nervous system, and heart. The basis of endothelial insulin transcytosis continues to be defined, with evidence favoring a receptor-mediated, saturable process involving IR or IGF1R or possibly another binding protein, or alternatively a mechanism consistent with a nonsaturable process. Challenges persist in the study of insulin transport in vivo because its accurate visualization is difficult, and the behavior of insulin differs greatly across the range of physiological to pharmacological concentrations. A limited number of studies have attempted to learn about endothelial insulin transport by the manipulation of mechanisms in the endothelium in vivo. Work in mice deficient in endothelial cell IRS-2 elegantly showed the importance of IR signaling, particularly that resulting in NO production, insulin delivery, and glucose disposal in the skeletal muscle.[14] The use of mice lacking ERα in the endothelium surprisingly revealed that antidiabetic actions of estrogens include the promotion of endothelial cell insulin transport to skeletal muscle.[107] Investigations in the setting of obesity have determined that unique actions of altered IgG glycosylation on endothelium via the Fc receptor FcγRIIB play a key role in obesity-related failures in skeletal muscle insulin delivery and glucose disposal.[136]

The remaining frontiers in our understanding of endothelial insulin transport are numerous. We know little about the mechanisms responsible for the transport under normal conditions. More recently, additional sets of molecules have been implicated in endothelial insulin transcytosis. One study found that endothelial selective deletion of apolipoprotein E receptor 2 impairs insulin transcytosis in skeletal muscle, resulting in systemic glucose intolerance and insulin resistance.[150] Mechanistically, the work revealed that apolipoprotein E receptor 2 stimulation by its ligand ApoE3 promotes insulin transport through the apolipoprotein E receptor 2 adaptor protein disabled homolog 2 and the scaffolding protein IQ motif containing GTPase activating protein 1. It is known to mediate insulin exocytosis in pancreatic β cells,[151] and the new finding suggests that endothelial insulin transcytosis may use a mechanism partially parallel to insulin secretion. Our knowledge gaps remain just as large about how endothelial insulin transport is perturbed in disease states. Recognizing how many disorders entail alterations in endothelial cell function, it is clear that we have a great deal more to learn. In addition, the basis of endothelial cell insulin transport is likely to be diverse in different tissues and anatomic locations. To date, most investigations of insulin biology have been focused on its production by the pancreatic β cell and its mechanisms of action in end-organ target cells. Further research on insulin transport outside of the beta cell is now warranted, and it has the potential to reveal new therapeutic targets in the battle against diabetes and other insulin-related disorders.

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The authors prepared the manuscript.

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Disclosures

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REFERENCES

1. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature*. 2019;576:51–60. doi: 10.1038/s41586-019-1797-8
2. Lawal Y, Bello F, Kaoje YS. Prediabetes deserves more attention: a review. *Clin Diabetes*. 2020;38:328–338. doi: 10.2337/cd19-0101
3. Wasserman DH, Wang TJ, Brown NJ. The vasculature in prediabetes. *Circ Res*. 2018;122:1135–1150. doi: 10.1161/CIRCRESAHA.118.311912
4. Vincent MA, Clerk LH, Rattigan S, Clark MG, Barrett EJ. Active role for the vasculature in the delivery of insulin to skeletal muscle. *Clin Exp Pharmacol Physiol*. 2005;32:302–307. doi: 10.1111/j.1440-1681.2005.04188.x
5. White MF, Kahn CR. The insulin signaling system. *J Biol Chem*. 1994;269:1–4.
6. Long YC, Zierath JR. Influence of AMP-activated protein kinase and calcineurin on metabolic networks in skeletal muscle. *Am J Physiol Endocrinol Metab*. 2008;295:E545–E552. doi: 10.1152/ajpendo.90259.2008
7. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237:E214–E223. doi: 10.1152/ajpendo.1979.237.3.E214
8. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes*. 1983;32:35–45. doi: 10.2337/diab.32.1.35
9. Dawson D, Vincent MA, Barrett EJ, Kaul S, Clark A, Leong-Poi H, Lindner JR. Vascular recruitment in skeletal muscle during exercise and hyperinsulinemia assessed by contrast ultrasound. *Am J Physiol Endocrinol Metab*. 2002;282:E714–E720. doi: 10.1152/ajpendo.00373.2001
10. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes*. 1981;30:1000–1007. doi: 10.2337/diab.30.12.1000
11. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest*. 1989;84:1620–1628. doi: 10.1172/JCI114339
12. Yang YJ, Hope ID, Ader M, Bergman RN. Importance of transcapillary insulin transport to dynamics of insulin action after intravenous glucose. *Am J Physiol*. 1994;266:E17–E25. doi: 10.1152/ajpendo.1994.266.1.E17
13. Kolka CM, Bergman RN. The barrier within: endothelial transport of hormones. *Physiology (Bethesda)*. 2012;27:237–247. doi: 10.1152/physiol.00012.2012
14. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, et al. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab*. 2011;13:294–307. doi: 10.1016/j.cmet.2011.01.018
15. Sjostrand M, Holmang A, Lonnroth P. Measurement of interstitial insulin in human muscle. *Am J Physiol*. 1999;276:E151–E154. doi: 10.1152/ajpendo.1999.276.1.E151
16. Sjostrand M, Holmang A, Strindberg L, Lonnroth P. Estimations of muscle interstitial insulin, glucose, and lactate in type 2 diabetic subjects. *Am J Physiol Endocrinol Metab*. 2000;279:E1097–E1103. doi: 10.1152/ajpendo.2000.279.5.E1097
17. McClatchey PM, Williams IM, Xu Z, Mignemi NA, Hughey CC, McGuinness OP, Beckman JA, Wasserman DH. Perfusion controls muscle glucose uptake by altering the rate of glucose dispersion in vivo. *Am J Physiol Endocrinol Metab*. 2019;317:E1022–E1036. doi: 10.1152/ajpendo.00260.2019
18. Akerstrom T, Goldman D, Nilsson F, Milkovich SL, Fraser GM, Brand CL, Hellsten Y, Ellis CG. Hyperinsulinemia does not cause de novo capillary recruitment in rat skeletal muscle. *Microcirculation*. 2020;27:e12593. doi: 10.1111/micc.12593
19. Williams IM, McClatchey PM, Bracy DP, Bonner JS, Valenzuela FA, Wasserman DH. Transendothelial insulin transport is impaired in skeletal muscle capillaries of obese male mice. *Obesity (Silver Spring)*. 2020;28:303–314. doi: 10.1002/oby.22683
20. Williams IM, Valenzuela FA, Kahl SD, Ramkrishna D, Mezo AR, Young JD, Wells KS, Wasserman DH. Insulin exits skeletal muscle capillaries by fluid-phase transport. *J Clin Invest*. 2018;128:699–714. doi: 10.1172/JCI94053
21. Broussard JL, Castro AV, Iyer M, Paszkiewicz RL, Bediako IA, Szczepaniak LS, Szczepaniak EW, Bergman RN, Kolka CM. Insulin access to skeletal muscle is impaired during the early stages of diet-induced obesity. *Obesity (Silver Spring)*. 2016;24:1922–1928. doi: 10.1002/oby.21562
22. Kolka CM, Bergman RN. The endothelium in diabetes: its role in insulin access and diabetic complications. *Rev Endocr Metab Disord*. 2013;14:13–19. doi: 10.1007/s11154-012-9233-5
23. Kolka CM, Harrison LN, Lottati M, Chiu JD, Kirkman EL, Bergman RN. Diet-induced obesity prevents interstitial dispersion of insulin in skeletal muscle. *Diabetes*. 2010;59:619–626. doi: 10.2337/db09-0839
24. Lee WL, Klip A. Endothelial transcytosis of insulin: Does it contribute to insulin resistance? *Physiology (Bethesda)*. 2016;31:336–345. doi: 10.1152/physiol.00010.2016
25. Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljenquist JE, Berman M, Andres R. A model of the kinetics of insulin in man. *J Clin Invest*. 1974;53:1481–1492. doi: 10.1172/JCI107697
26. Chiu JD, Richey JM, Harrison LN, Zuniga E, Kolka CM, Kirkman E, Ellmerer M, Bergman RN. Direct administration of insulin into skeletal muscle reveals that the transport of insulin across the capillary endothelium

limits the time course of insulin to activate glucose disposal. *Diabetes*. 2008;57:828–835. doi: 10.2337/db07-1444
27. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. *J Clin Invest*. 1986;78:472–481. doi: 10.1172/JCI112599
28. Turk D, Alzaid A, Dinneen S, Nair KS, Rizza R. The effects of non-insulin-dependent diabetes mellitus on the kinetics of onset of insulin action in hepatic and extrahepatic tissues. *J Clin Invest*. 1995;95:755–762. doi: 10.1172/JCI117723
29. Sjostrand M, Gudbjornsdottir S, Holmang A, Lonn L, Strindberg L, Lonnroth P. Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. *Diabetes*. 2002;51:2742–2748. doi: 10.2337/diabetes.51.9.2742
30. Sandqvist M, Strindberg L, Schmelz M, Lonnroth P, Jansson PA. Impaired delivery of insulin to adipose tissue and skeletal muscle in obese women with postprandial hyperglycemia. *J Clin Endocrinol Metab*. 2011;96:E1320–E1324. doi: 10.1210/jc.2011-0233
31. Barrett EJ, Wang H, Upchurch CT, Liu Z. Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *Am J Physiol Endocrinol Metab*. 2011;301:E252–E263. doi: 10.1152/ajpendo.00186.2011
32. Kubota T, Kubota N, Kadowaki T. The role of endothelial insulin signaling in the regulation of glucose metabolism. *Rev Endocr Metab Disord*. 2013;14:207–216. doi: 10.1007/s11154-013-9242-z
33. Williams IM, Wasserman DH. Capillary endothelial insulin transport: the rate-limiting step for insulin-stimulated glucose uptake. *Endocrinology*. 2022;163:bqab252. doi: 10.1210/endocr/bqab252
34. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest*. 1990;85:1844–1852. doi: 10.1172/JCI114644
35. Laakso M, Edelman SV, Brechtel G, Baron AD. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes*. 1992;41:1076–1083. doi: 10.2337/diab.41.9.1076
36. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, et al. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation*. 2001;104:342–345. doi: 10.1161/01.cir.104.3.342
37. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark AD, Clark MG. Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. *Diabetes*. 2002;51:3492–3498. doi: 10.2337/diabetes.51.12.3492
38. Halseth AE, Bracy DP, Wasserman DH. Limitations to basal and insulin-stimulated skeletal muscle glucose uptake in the high-fat-fed rat. *Am J Physiol Endocrinol Metab*. 2000;279:E1064–E1071. doi: 10.1152/ajpendo.2000.279.5.E1064
39. Clark MG. Impaired microvascular perfusion: a consequence of vascular dysfunction and a potential cause of insulin resistance in muscle. *Am J Physiol Endocrinol Metab*. 2008;295:E732–E750. doi: 10.1152/ajpendo.90477.2008
40. Barrett EJ, Eggleston EM, Inyard AC, Wang H, Li G, Chai W, Liu Z. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia*. 2009;52:752–764. doi: 10.1007/s00125-009-1313-z
41. Vincent MA, Dawson D, Clark AD, Lindner JR, Rattigan S, Clark MG, Barrett EJ. Skeletal muscle microvascular recruitment by physiological hyperinsulinemia precedes increases in total blood flow. *Diabetes*. 2002;51:42–48. doi: 10.2337/diabetes.51.1.42
42. Miles PD, Levisetti M, Reichart D, Khoursheed M, Moossa AR, Olefsky JM. Kinetics of insulin action in vivo. Identification of rate-limiting steps. *Diabetes*. 1995;44:947–953. doi: 10.2337/diab.44.8.947
43. Oezdemir I, Peng J, Ghosh D, Sirsi S, Mineo C, Shaul PW, Hoyt K. Multiscale and morphological analysis of microvascular patterns depicted in contrast-enhanced ultrasound images. *J Med Imaging (Bellingham)*. 2020;7:034001. doi: 10.1117/1.JMI.7.3.034001
44. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. *Circ Res*. 2007;100:174–190. doi: 10.1161/01.RES.0000255690.03436.ae
45. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res*. 2007;100:158–173. doi: 10.1161/01.RES.0000255691.76142.4a
46. Azizi PM, Zyla RE, Guan S, Wang C, Liu J, Bolz SS, Heit B, Klip A, Lee WL. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Mol Biol Cell*. 2015;26:740–750. doi: 10.1091/mbc.E14-08-1307
47. Fung KYY, Fairn GD, Lee WL. Transcellular vesicular transport in epithelial and endothelial cells: challenges and opportunities. *Traffic*. 2018;19:5–18. doi: 10.1111/tra.12533
48. Sanwal R, Khosraviani N, Advani SL, Advani A, Lee WL. The endothelial barrier is not rate-limiting to insulin action in the myocardium of male mice. *Endocrinology*. 2020;161:bqaa029. doi: 10.1210/endocr/bqaa029
49. Yazdani S, Jaldin-Fincati JR, Pereira RVS, Klip A. Endothelial cell barriers: transport of molecules between blood and tissues. *Traffic*. 2019;20:390–403. doi: 10.1111/tra.12645
50. King GL, Johnson SM. Receptor-mediated transport of insulin across endothelial cells. *Science*. 1985;227:1583–1586. doi: 10.1126/science.3883490
51. Dernovsek KD, Bar RS. Processing of cell-bound insulin by capillary and macrovascular endothelial cells in culture. *Am J Physiol*. 1985;248:E244–E251. doi: 10.1152/ajpendo.1985.248.2.E244
52. Schnitzer JE, Oh P, Pinney E, Allard J. Filipin-sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules. *J Cell Biol*. 1994;127:1217–1232. doi: 10.1083/jcb.127.5.1217
53. Wang H, Liu Z, Li G, Barrett EJ. The vascular endothelial cell mediates insulin transport into skeletal muscle. *Am J Physiol Endocrinol Metab*. 2006;291:E323–E332. doi: 10.1152/ajpendo.00047.2006
54. Li G, Barrett EJ, Wang H, Chai W, Liu Z. Insulin at physiological concentrations selectively activates insulin but not insulin-like growth factor I (IGF-I) or insulin/IGF-I hybrid receptors in endothelial cells. *Endocrinology*. 2005;146:4690–4696. doi: 10.1210/en.2005-0505
55. Yu Y, Kastin AJ, Pan W. Reciprocal interactions of insulin and insulin-like growth factor I in receptor-mediated transport across the blood-brain barrier. *Endocrinology*. 2006;147:2611–2615. doi: 10.1210/en.2006-0020
56. Majumdar S, Genders AJ, Inyard AC, Frison V, Barrett EJ. Insulin entry into muscle involves a saturable process in the vascular endothelium. *Diabetologia*. 2012;55:450–456. doi: 10.1007/s00125-011-2343-x
57. Steil GM, Ader M, Moore DM, Rebrin K, Bergman RN. Transendothelial insulin transport is not saturable in vivo. No evidence for a receptor-mediated process. *J Clin Invest*. 1996;97:1497–1503. doi: 10.1172/JCI118572
58. Hamilton-Wessler M, Ader M, Dea MK, Moore D, Loftager M, Markussen J, Bergman RN. Mode of transcapillary transport of insulin and insulin analog NN304 in dog hindlimb: evidence for passive diffusion. *Diabetes*. 2002;51:574–582. doi: 10.2337/diabetes.51.3.574
59. Konishi M, Sakaguchi M, Lockhart SM, Cai W, Li ME, Homan EP, Rask-Madsen C, Kahn CR. Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice. *Proc Natl Acad Sci USA*. 2017;114:E8478–E8487. doi: 10.1073/pnas.1710625114
60. Cogger VC, McNerney GP, Nyunt T, DeLeve LD, McCourt P, Smedsrod B, Le Couteur DG, Huser TR. Three-dimensional structured illumination microscopy of liver sinusoidal endothelial cell fenestrations. *J Struct Biol*. 2010;171:382–388. doi: 10.1016/j.jsb.2010.06.001
61. Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. *Endocr Rev*. 1998;19:608–624. doi: 10.1210/edrv.19.5.0349
62. Wang H, Wang AX, Liu Z, Barrett EJ. Insulin signaling stimulates insulin transport by bovine aortic endothelial cells. *Diabetes*. 2008;57:540–547. doi: 10.2337/db07-0967
63. Wang H, Wang AX, Barrett EJ. Insulin-induced endothelial cell cortical actin filament remodeling: a requirement for trans-endothelial insulin transport. *Mol Endocrinol*. 2012;26:1327–1338. doi: 10.1210/me.2012-1003
64. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev*. 2007;28:463–491. doi: 10.1210/er.2007-0006
65. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601–605. doi: 10.1038/21224
66. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest*. 1996;98:894–898. doi: 10.1172/JCI118871
67. Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation*. 2000;101:1539–1545. doi: 10.1161/01.cir.101.13.1539
68. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem*. 2002;277:1794–1799. doi: 10.1074/jbc.M103728200
69. Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, Panza JA. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation*. 1999;100:820–825. doi: 10.1161/01.cir.100.8.820
70. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab*. 2003;285:E123–E129. doi: 10.1152/ajpendo.00021.2003

71. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest*. 1994;94:2511–2515. doi: 10.1172/JCI117621
72. Kainoh M, Maruyama I, Nishio S, Nakadate T. Enhancement by beraprost sodium, a stable analogue of prostacyclin, in thrombomodulin expression on membrane surface of cultured vascular endothelial cells via increase in cyclic AMP level. *Biochem Pharmacol*. 1991;41:1135–1140. doi: 10.1016/0006-2952(91)90651-k
73. Niwano K, Arai M, Tomaru K, Uchiyama T, Ohyama Y, Kurabayashi M. Transcriptional stimulation of the eNOS gene by the stable prostacyclin analogue beraprost is mediated through cAMP-responsive element in vascular endothelial cells: close link between PGI2 signal and NO pathways. *Circ Res*. 2003;93:523–530. doi: 10.1161/01.RES.0000091336.55487.F7
74. Lievre M, Morand S, Besse B, Fiessinger JN, Boissel JP. Oral Beraprost sodium, a prostaglandin I(2) analogue, for intermittent claudication: a double-blind, randomized, multicenter controlled trial. Beraprost et Claudication Intermittente (BERCI) Research Group. *Circulation*. 2000;102:426–431. doi: 10.1161/01.cir.102.4.426
75. Galie N, Humbert M, Vachiery JL, Vizza CD, Kneussl M, Manes A, Sitbon O, Torbicki A, Delcroix M, Naeije R, et al; Arterial Pulmonary Hypertension and Beraprost European (ALPHABET) Study Group. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol*. 2002;39:1496–1502. doi: 10.1016/s0735-1097(02)01786-2
76. Ayala JE, Bracy DP, Julien BM, Rottman JN, Fueger PT, Wasserman DH. Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. *Diabetes*. 2007;56:1025–1033. doi: 10.2337/db06-0883
77. Chai W, Fu Z, Aylor KW, Barrett EJ, Liu Z. Liraglutide prevents microvascular insulin resistance and preserves muscle capillary density in high-fat diet-fed rats. *Am J Physiol Endocrinol Metab*. 2016;311:E640–E648. doi: 10.1152/ajpendo.00205.2016
78. Bonner JS, Lantier L, Hocking KM, Kang L, Owolabi M, James FD, Bracy DP, Brophy CM, Wasserman DH. Relaxin treatment reverses insulin resistance in mice fed a high-fat diet. *Diabetes*. 2013;62:3251–3260. doi: 10.2337/db13-0033
79. Baron AD, Tarshoby M, Hook G, Lazaridis EN, Cronin J, Johnson A, Steinberg HO. Interaction between insulin sensitivity and muscle perfusion on glucose uptake in human skeletal muscle: evidence for capillary recruitment. *Diabetes*. 2000;49:768–774. doi: 10.2337/diabetes.49.5.768
80. Jansson PA, Murdolo G, Sjogren L, Nystrom B, Sjostrand M, Strindberg L, Lonnroth P. Tadalafil increases muscle capillary recruitment and forearm glucose uptake in women with type 2 diabetes. *Diabetologia*. 2010;53:2205–2208. doi: 10.1007/s00125-010-1819-4
81. Wang H, Wang AX, Aylor K, Barrett EJ. Nitric oxide directly promotes vascular endothelial insulin transport. *Diabetes*. 2013;62:4030–4042. doi: 10.2337/db13-0627
82. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev*. 2013;34:309–338. doi: 10.1210/er.2012-1055
83. Hevener AL, Clegg DJ, Mauvais-Jarvis F. Impaired estrogen receptor action in the pathogenesis of the metabolic syndrome. *Mol Cell Endocrinol*. 2015;418 Pt 3:306–321. doi: 10.1016/j.mce.2015.05.020
84. Wagner JD, Thomas MJ, Williams JK, Zhang L, Greaves KA, Cefalu WT. Insulin sensitivity and cardiovascular risk factors in ovariectomized monkeys with estradiol alone or combined with nomegestrol acetate. *J Clin Endocrinol Metab*. 1998;83:896–901. doi: 10.1210/jcem.83.3.4628
85. Kumagai S, Holmang A, Bjorntorp P. The effects of oestrogen and progesterone on insulin sensitivity in female rats. *Acta Physiol Scand*. 1993;149:91–97. doi: 10.1111/j.1748-1716.1993.tb09596.x
86. Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, Gourdy P. Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. *Endocrinology*. 2009;150:2109–2117. doi: 10.1210/en.2008-0971
87. Osborne CK, Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. *J Clin Oncol*. 2005;23:1616–1622. doi: 10.1200/JCO.2005.10.036
88. Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, Kaplitt MG, Ogawa S. Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc Natl Acad Sci USA*. 2007;104:2501–2506. doi: 10.1073/pnas.0610787104
89. Bryzgalova G, Lundholm L, Portwood N, Gustafsson JA, Khan A, Efendic S, Dahlman-Wright K. Mechanisms of antidiabetogenic and body weight-lowering effects of estrogen in high-fat diet-fed mice. *Am J Physiol Endocrinol Metab*. 2008;295:E904–E912. doi: 10.1152/ajpendo.90248.2008
90. Ropero AB, Alonso-Magdalena P, Quesada I, Nadal A. The role of estrogen receptors in the control of energy and glucose homeostasis. *Steroids*. 2008;73:874–879. doi: 10.1016/j.steroids.2007.12.018
91. Barros R, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ER{alpha} and ER{beta} in glucose homeostasis in skeletal muscle and white adipose tissue. *Am J Physiol Endocrinol Metab*. 2009;297:E124. doi: 10.1152/ajpendo.00189.2009
92. Davis KE, Neinast D, Sun K, Skiles M, Bills D, Zehr A, Zeve D, Hahner D, Cox W, Gent M, et al. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation, and fibrosis. *Mol Metab*. 2013;2:227–242. doi: 10.1016/j.molmet.2013.05.006
93. Ribas V, Drew BG, Le JA, Soleymani T, Daraei P, Sitz D, Mohammad L, Henstridge DC, Febbraio MA, Hewitt SC, et al. Myeloid-specific estrogen receptor {alpha} deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. *Proc Natl Acad Sci USA*. 2011;108:16457–16462. doi: 10.1073/pnas.1104533108
94. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci USA*. 2000;97:12729–12734. doi: 10.1073/pnas.97.23.12729
95. Bryzgalova G, Gao H, Ahren B, Zierath JR, Galuska D, Steiler TL, Dahlman-Wright K, Nilsson S, Gustafsson JA, Efendic S, et al. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. *Diabetologia*. 2006;49:588–597. doi: 10.1007/s00125-005-0105-3
96. Heianza Y, Arase Y, Kodama S, Hsieh SD, Tsuji H, Saito K, Shimano H, Hara S, Sone H. Effect of postmenopausal status and age at menopause on type 2 diabetes and prediabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 17 (TOPICS 17). *Diabetes Care*. 2013;36:4007–4014. doi: 10.2337/dc13-1048
97. Gurka MJ, Vishnu A, Santen RJ, DeBoer MD. Progression of metabolic syndrome severity during the menopausal transition. *J Am Heart Assoc*. 2016;5:e003609. doi: 10.1161/JAHA.116.003609
98. Szmuilowicz ED, Stuenkel CA, Seely EW. Influence of menopause on diabetes and diabetes risk. *Nat Rev Endocrinol*. 2009;5:553–558. doi: 10.1038/nrendo.2009.166
99. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994;331:1056–1061. doi: 10.1056/NEJM199410203311604
100. Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M, Clyne CD, Davis S, Simpson ER, Carani C. Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *J Clin Endocrinol Metab*. 2004;89:61–70. doi: 10.1210/jc.2003-030313
101. Rochira V, Madeo B, Zirilli L, Caffagni G, Maffei L, Carani C. Oestradiol replacement treatment and glucose homeostasis in two men with congenital aromatase deficiency: evidence for a role of oestradiol and sex steroids imbalance on insulin sensitivity in men. *Diabet Med*. 2007;24:1491–1495. doi: 10.1111/j.1464-5491.2007.02304.x
102. Takeda K, Toda K, Saibara T, Nakagawa M, Saika K, Onishi T, Sugiura T, Shizuta Y. Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. *J Endocrinol*. 2003;176:237–246. doi: 10.1677/joe.0.1760237
103. Chambliss KL, Shaul PW. Estrogen modulation of endothelial nitric oxide synthase. *Endocr Rev*. 2002;23:665–686. doi: 10.1210/er.2001-0045
104. Levin ER. Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol*. 2005;19:1951–1959. doi: 10.1210/me.2004-0390
105. Lantin-Hermoso RL, Rosenfeld CR, Yuhanna IS, German Z, Chen Z, Shaul PW. Estrogen acutely stimulates nitric oxide synthase activity in fetal pulmonary artery endothelium. *Am J Physiol*. 1997;273:L119–L126. doi: 10.1152/ajplung.1997.273.1.L119
106. Chambliss KL, Yuhanna IS, Mineo C, Liu P, German Z, Sherman TS, Mendelsohn ME, Anderson RG, Shaul PW. Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ Res*. 2000;87:E44–E52. doi: 10.1161/01.res.87.11.e44
107. Sacharidou A, Chambliss K, Peng J, Barrera J, Tanigaki K, Luby-Phelps K, Ozdemir I, Khan S, Sirsi SR, Kim SH, et al. Endothelial ERalpha promotes glucose tolerance by enhancing endothelial insulin transport to skeletal muscle. *Nat Commun*. 2023;14:4989. doi: 10.1038/s41467-023-40562-w
108. Gu P, Xu A. Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. *Rev Endocr Metab Disord*. 2013;14:49–58. doi: 10.1007/s11154-012-9230-8

109. Scalia R. The microcirculation in adipose tissue inflammation. *Rev Endocr Metab Disord*. 2013;14:69–76. doi: 10.1007/s11154-013-9236-x
110. Chambliss KL, Wu Q, Oltmann S, Konaniah ES, Umetani M, Korach KS, Thomas GD, Mineo C, Yuhanna IS, Kim SH, et al. Non-nuclear estrogen receptor alpha signaling promotes cardiovascular protection but not uterine or breast cancer growth in mice. *J Clin Invest*. 2010;120:2319–2330. doi: 10.1172/JCI38291
111. Lu Q, Schnitzler GR, Ueda K, Iyer LK, Diomede OI, Andrade T, Karas RH. ER alpha rapid signaling is required for estrogen induced proliferation and migration of vascular endothelial cells. *PLoS One*. 2016;11:e0152807. doi: 10.1371/journal.pone.0152807
112. Nilsson BO. Modulation of the inflammatory response by estrogens with focus on the endothelium and its interactions with leukocytes. *Inflamm Res*. 2007;56:269–273. doi: 10.1007/s00011-007-6198-z
113. Teasdale RD, Loci D, Houghton F, Karlsson L, Gleeson PA. A large family of endosome-localized proteins related to sorting nexin 1. *Biochem J*. 2001;358:7–16. doi: 10.1042/0264-6021:3580007
114. Worby CA, Dixon JE. Sorting out the cellular functions of sorting nexins. *Nat Rev Mol Cell Biol*. 2002;3:919–931. doi: 10.1038/nrm974
115. Haft CR, de la Luz SM, Barr VA, Haft DH, Taylor SI. Identification of a family of sorting nexin molecules and characterization of their association with receptors. *Mol Cell Biol*. 1998;18:7278–7287. doi: 10.1128/mcb.18.12.7278
116. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature*. 2006;444:881–887. doi: 10.1038/nature05488
117. Rabinowitz D, Zierler KL. Forearm metabolism in obesity and its response to intra-arterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. *J Clin Invest*. 1962;41:2173–2181. doi: 10.1172/JCI104676
118. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*. 2009;32:S157–S163. doi: 10.2337/dc09-S302
119. Gudbjornsdottir S, Sjostrand M, Strindberg L, Wahren J, Lonnroth P. Direct measurements of the permeability surface area for insulin and glucose in human skeletal muscle. *J Clin Endocrinol Metab*. 2003;88:4559–4564. doi: 10.1210/jc.2003-030434
120. Bergman RN. Insulin action and distribution of tissue blood flow. *J Clin Endocrinol Metab*. 2003;88:4556–4558. doi: 10.1210/jc.2003-031431
121. Pillon NJ, Azizi PM, Li YE, Liu J, Wang C, Chan KL, Hopperton KE, Bazinet RP, Heit B, Bilan PJ, et al. Palmitate-induced inflammatory pathways in human adipose microvascular endothelial cells promote monocyte adhesion and impair insulin transcytosis. *Am J Physiol Endocrinol Metab*. 2015;309:E35–E44. doi: 10.1152/ajpendo.00611.2014
122. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med*. 2011;17:610–617. doi: 10.1038/nm.2353
123. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*. 2017;127:1–4. doi: 10.1172/JCI92035
124. Tanigaki K, Vongpatanasin W, Barrera JA, Atochin DN, Huang PL, Bonvini E, Shaul PW, Mineo C. C-reactive protein causes insulin resistance in mice through Fcgamma receptor IIB-mediated inhibition of skeletal muscle glucose delivery. *Diabetes*. 2013;62:721–731. doi: 10.2337/db12-0133
125. Tanigaki K, Chambliss KL, Yuhanna IS, Sacharidou A, Ahmed M, Atochin DN, Huang PL, Shaul PW, Mineo C. Endothelial Fcgamma receptor IIB activation blunts insulin delivery to skeletal muscle to cause insulin resistance in mice. *Diabetes*. 2016;65:1996–2005. doi: 10.2337/db15-1605
126. Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, et al. Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. *Proc Natl Acad Sci USA*. 2011;108:12669–12674. doi: 10.1073/pnas.1108455108
127. Anthony RM, Wermeling F, Ravetch JV. Novel roles for the IgG Fc glycan. *Ann N Y Acad Sci*. 2012;1253:170–180. doi: 10.1111/j.1749-6632.2011.06305.x
128. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol*. 2008;8:34–47. doi: 10.1038/nri2206
129. Damelang T, Brinkhaus M, van Osch TLJ, Schuurman J, Labrijn AF, Rispens T, Vidarsson G. Impact of structural modifications of IgG antibodies on effector functions. *Front Immunol*. 2023;14:1304365. doi: 10.3389/fimmu.2023.1304365
130. Kristic J, Lauc G. The importance of IgG glycosylation-what did we learn after analyzing over 100,000 individuals. *Immunol Rev*. 2024;328:143–170. doi: 10.1111/imr.13407
131. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol*. 2018;333:65–79. doi: 10.1016/j.cellimm.2018.07.009
132. Vattepu R, Sneed SL, Anthony RM. Sialylation as an important regulator of antibody function. *Front Immunol*. 2022;13:818736. doi: 10.3389/fimmu.2022.818736
133. Nikolac Perkovic M, Pucic Bakovic M, Kristic J, Novokmet M, Huffman JE, Vitart V, Hayward C, Rudan I, Wilson JF, Campbell H, et al. The association between galactosylation of immunoglobulin G and body mass index. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:20–25. doi: 10.1016/j.pnpbp.2013.08.014
134. Russell AC, Kepka A, Trbojevic-Akmacic I, Ugrina I, Song M, Hui J, Hunter M, Laws SM, Lauc G, Wang W. Increased central adiposity is associated with pro-inflammatory immunoglobulin G N-glycans. *Immunobiology*. 2019;224:110–115. doi: 10.1016/j.imbio.2018.10.002
135. Liu D, Li Q, Dong J, Li D, Xu X, Xing W, Zhang X, Cao W, Hou H, Wang H, et al. The association between normal BMI with central adiposity and pro-inflammatory potential immunoglobulin G N-glycosylation. *Diabetes Metab Syndr Obes*. 2019;12:2373–2385. doi: 10.2147/DMSO.S216318
136. Tanigaki K, Sacharidou A, Peng J, Chambliss KL, Yuhanna IS, Ghosh D, Ahmed M, Szalai AJ, Vongpatanasin W, Mattrey RF, et al. Hyposialylated IgG activates endothelial IgG receptor FcgammaRIIB to promote obesity-induced insulin resistance. *J Clin Invest*. 2018;128:309–322. doi: 10.1172/JCI89333
137. Solar P, Zamani A, Lakatosova K, Joukal M. The blood-brain barrier and the neurovascular unit in subarachnoid hemorrhage: molecular events and potential treatments. *Fluids Barriers CNS*. 2022;19:29. doi: 10.1186/s12987-022-00312-4
138. Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin Immunopathol*. 2009;31:497–511. doi: 10.1007/s00281-009-0177-0
139. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. 2010;37:13–25. doi: 10.1016/j.nbd.2009.07.030
140. Bernacki J, Dobrowolska A, Nierwinska K, Malecki A. Physiology and pharmacological role of the blood-brain barrier. *Pharmacol Rep*. 2008;60:600–622.
141. Andreone BJ, Chow BW, Tata A, Lacoste B, Ben-Zvi A, Bullock K, Deik AA, Ginty DD, Clish CB, Gu C. Blood-brain barrier permeability is regulated by lipid transport-dependent suppression of caveolae-mediated transcytosis. *Neuron*. 2017;94:581–594.e5. doi: 10.1016/j.neuron.2017.03.043
142. Ohtsuki S. Insulin receptor at the blood-brain barrier: transport and signaling. *Vitam Horm*. 2024;126:113–124. doi: 10.1016/bs.vh.2024.05.001
143. Pomytkin I, Costa-Nunes JP, Kasatkin V, Veniaminova E, Demchenko A, Lyundup A, Lesch KP, Ponomarev ED, Strekalova T. Insulin receptor in the brain: mechanisms of activation and the role in the CNS pathology and treatment. *CNS Neurosci Ther*. 2018;24:763–774. doi: 10.1111/cns.12866
144. Rhea EM, Rask-Madsen C, Banks WA. Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. *J Physiol*. 2018;596:4753–4765. doi: 10.1113/JP276149
145. Akhtar A, Sah SP. Insulin signaling pathway and related molecules: role in neurodegeneration and Alzheimer's disease. *Neurochem Int*. 2020;135:104707. doi: 10.1016/j.neuint.2020.104707
146. Gray SM, Aylor KW, Barrett EJ. Unravelling the regulation of insulin transport across the brain endothelial cell. *Diabetologia*. 2017;60:1512–1521. doi: 10.1007/s00125-017-4285-4
147. Jais A, Solas M, Backes H, Chaurasia B, Kleinridders A, Theurich S, Mauer J, Steculorum SM, Hampel B, Goldau J, et al. Myeloid-cell-derived VEGF maintains brain glucose uptake and limits cognitive impairment in obesity. *Cell*. 2016;165:882–895. doi: 10.1016/j.cell.2016.03.033
148. Ogata S, Ito S, Masuda T, Ohtsuki S. Changes of blood-brain barrier and brain parenchymal protein expression levels of mice under different insulin-resistance conditions induced by high-fat diet. *Pharm Res*. 2019;36:141. doi: 10.1007/s11095-019-2674-8
149. Liu J, Jahn LA, Fowler DE, Barrett EJ, Cao W, Liu Z. Free fatty acids induce insulin resistance in both cardiac and skeletal muscle microvasculature in humans. *J Clin Endocrinol Metab*. 2011;96:438–446. doi: 10.1210/jc.2010-1174
150. Sacharidou A, Chambliss KL, Peng J, Tanigaki K, Islam MN, Sirsi S, Lemoff A, Hoyt K, Mineo C, Shaul PW. Apolipoprotein E receptor 2 in endothelium promote glucose tolerance by mediating insulin delivery to skeletal muscle. *Mol Metab*. 2025;101:102238. doi: 10.1016/j.molmet.2025.102238
151. Hedman AC, Smith JM, Sacks DB. The biology of IQGAP proteins: beyond the cytoskeleton. *EMBO Rep*. 2015;16:427–446. doi: 10.15252/embr.201439834