Published in final edited form as:
Rev Endocr Metab Disord. 2013 March ; 14(1): 21–27. doi:10.1007/s11154-012-9232-6.

The Endothelial Cell: an "Early Responder" in the Development of Insulin Resistance

Eugene J. Barrett and **Zhenqi Liu**
Division of Endocrinology and Metabolism, Department of Medicine, University of Virginia School of Medicine

Abstract

Vascular endothelium is an important insulin target and plays a pivotal role in the development of metabolic insulin resistance provoked by the Western lifestyle. It acts as a "first-responder" to environmental stimuli such as nutrients, cytokines, chemokines and physical activity and regulates insulin delivery to muscle and adipose tissue and thereby affecting insulin-mediated glucose disposal by these tissues. In addition, it also regulates the delivery of insulin and other appetite regulating signals from peripheral tissues to the central nervous system thus influencing the activity of nuclei that regulate hepatic glucose production, adipose tissue lipolysis and lipogenesis, as well as food consumption. Resistance to insulin's vascular actions therefore broadly impacts tissue function and contribute to metabolic dysregulation. Moreover, vascular insulin resistance negatively impacts vascular health by affecting blood pressure regulation, vessel wall inflammation and atherogenesis thereby contributing to the burden of vascular disease seen with diabetes and metabolic syndrome. In the current review, we examined the evidence that supports the general concept of vascular endothelium as a target of insulin action and discussed the biochemical and physiological consequences of vascular insulin resistance.

Overview

It is manifestly apparent that the "Western" lifestyle is a major driver for the development of insulin resistance and that the impact of this varies across different racial and ethnic groups due to variation in genetic susceptibility. Changes in both physical activity and nutrition are encompassed in this "Western" umbrella. It will be predictably complex to segregate out specific components (there will be multiple) among macronutrient selection (e.g. saturated or total fat, total carbohydrate or fructose), micronutrients and food additives (1) as well as differences in exercise or daily activity intensity, duration and frequency that occur over time.

Here we will focus on the vascular endothelium as a pivotal link in the development of metabolic insulin resistance provoked by the Western lifestyle. Several considerations drive this focus. First, the vascular endothelial cell is the first cell type to encounter blood-borne nutrients and pro-inflammatory factors as they pass through the systemic circulation. Second, the endothelial cell responds rapidly to insulin (2) and changes in shear stress as occur with activity/exercise (3–5). Third, it is clearly sensitive to inflammatory factors like free fatty acids (6–8), C-reactive protein (9), tumor necrosis factor (TNF)-α, and others. Fourth, studies of the time course of development of insulin resistance in response to high fat feeding suggest that the response by the endothelial cell precedes that of other insulin target tissues (11). Finally, vascular insulin resistance negatively impacts perfusion in

Corresponding author: Eugene J Barrett, M.D., Ph.D., University of Virginia Health System, P.O. Box 801410, 450 Ray C. Hunt Drive, Charlottesville, VA 22908, Telephone: 434-924-1175, Fax: 434-924-1284, ejb8x@virginia.edu.

important insulin sensitive target tissues like skeletal muscle and adipose tissue and this appears directly linked to impairments of insulin-mediated glucose disposal i.e. metabolic insulin resistance (12–14).

This review hypothesizes that the vascular endothelium acts as a “first-responder” to environmental stimuli (including nutrients, cytokines, chemokines and physical activity). Recent studies have begun to clarify how vascular insulin resistance, largely attributable to impaired insulin-mediated nitric oxide production, can link vascular and metabolic insulin resistance (15). This work builds on extensive investigations published over the last 30–40 years. The findings support considering the endothelium as an important insulin target tissue. When viewed from this perspective one can provide a rational role of the vasculature in regulating insulin delivery to muscle and adipose tissue and thereby affecting insulin-mediated glucose disposal by these tissues. In addition, there is a potential role of the endothelial cell in regulating the delivery of insulin (16) and other appetite regulating signals (17) from peripheral tissues to the central nervous system (CNS) and influencing the activity of nuclei that regulate hepatic glucose production (18), adipose tissue lipolysis and lipogenesis (19), as well as food consumption (20). Resistance to insulin’s vascular actions will impact function within each of these tissues and thereby contribute to the metabolic dysregulation that is a major component of the insulin resistance syndrome. Moreover, vascular insulin resistance can negatively impact vascular health by affecting blood pressure regulation, vessel wall inflammation and atherogenesis (21; 22) thereby contributing to the burden of vascular disease seen with diabetes and metabolic syndrome.

To develop this hypothesis, we begin by considering data that support the general concept that the vasculature is a target for insulin action and discuss the biochemical and physiological consequences of insulin’s vascular action. To provide a linkage between vascular and metabolic insulin action, we will consider the case of insulin delivery to muscle being a rate-limiting step for muscle insulin action and discuss how it is impaired by insulin resistance. We will then discuss insulin delivery to the CNS in vivo and the evidence that indicates that this delivery is impaired in states of insulin resistance. Finally, we will outline some newer techniques and approaches that may, over the coming years, allow us to more fully unravel the linkage between vascular insulin resistance, metabolic dysfunction, and the increased vascular disease burden that is experienced by individuals with insulin resistance and type 2 diabetes.

The vasculature - an insulin target tissue with physiologic consequences

Insulin receptors were demonstrated on cultured endothelial cells nearly 30 years ago (23; 24). Inasmuch as the endothelial cell does not express insulin-sensitive glucose transporters, the function of these receptors was initially uncertain. Early work suggested a role for insulin receptors in insulin uptake by and transport across the endothelial cell. Studies in the late 1990s demonstrated that insulin enhanced the activity of endothelial nitric oxide synthase (eNOS) in a calcium independent fashion (25). This effect depended on activation of protein kinase B (PKB or Akt) which phosphorylates eNOS at serine 1177 (26). In addition, studies in vitro demonstrated a nitric oxide-dependent vasodilator effect of insulin on arterioles from skeletal muscle (27). Insulin also enhanced the expression of the potent vasoconstrictor endothelin-1 by endothelial cells in vitro (28) and in vivo (29). The latter process appeared dependent on activation of the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway while eNOS activation proceeded via the phosphatidylinositol-3 kinase (PI3-kinase)/Akt pathway. These findings, combined with findings that insulin resistance in isolated endothelial cells, intact aorta and microvessels was selective for the PI3-kinase/Akt/eNOS pathway, led to the hypothesis that insulin might have a vasoconstrictive effect in the setting of vascular insulin

resistance (30). In aggregate, these and other studies provide convincing evidence that the endothelial cell is a target for insulin action and that insulin plays a physiological role in the regulation of vascular function.

The endothelial cell also expresses insulin-like growth factor 1 (IGF-1) as well as insulin/IGF-1 hybrid receptors (31) which have been found to be expressed at 5–10-fold greater abundance than insulin receptor. Furthermore, overexpression of the IGF-1 receptor leads to vascular insulin resistance, perhaps in part by promoting hybrid receptor formation and decreasing insulin receptor availability (32). The hybrid receptors, like the IGF-1 receptors do not respond to physiological concentrations of insulin (33). Unfortunately, the normal physiologic function of endothelial cell IGF-1 and Insulin/IGF-1 hybrid receptors remains obscure.

In considering the action of insulin on cultured endothelial cells, it is important to keep in mind that these cells do not necessarily mimic precisely endothelial cells in vivo. A significant difficulty with in vivo exploration of biochemical pathways of insulin action arises in part from the fact that the function of endothelial cells varies within different parts of the vasculature (arterial, venous, microvascular etc.) and from the reality that the endothelial cell is only a minor component of most tissues (e.g. muscle, heart, brain, adipose tissue etc). Therefore, traditional approaches to tissue homogenization and metabolite or protein assay are often not viable options for examining endothelial cell specific responses to insulin or to insulin resistance within an organ like muscle or fat. Other approaches have been to use large vessels (e.g. aorta, umbilical vein) or easily accessible arterioles (e.g. mesentery) and assume that this endothelium will reflect the behavior of the endothelium within metabolically active tissues. This is likely an oversimplification (34). The principal exception to this is the lung where the endothelial cell composes a significant fraction of the total tissue mass, however lung endothelium may be more specialized to its particular physiological role and not be representative of endothelium in other insulin responsive tissues.

With these limitations in mind, investigators have shown that within the endothelial cell insulin activates the canonical signaling pathways that have been described in classical insulin target tissues. This includes tyrosine phosphorylation of insulin receptor substrate (IRS) proteins (10; 35; 36) with subsequent signaling through PI3-kinase and activation of Akt and eNOS. The eNOS is a substrate for both Akt and AMP-kinase as well as protein kinase A and other kinases (37). The demonstration that insulin activation of Akt in endothelial cells enhanced nitric oxide production clarified an important aspect of insulin action to cause vascular relaxation and enhance blood flow which had been demonstrated by multiple clinical studies in the early 1990s (38). Such clinical studies had reported diminished vasorelaxation in response to insulin in insulin resistance states like obesity, hypertension, and type 2 diabetes.

Insulin also increases the endothelin-1 expression by endothelial cells (28). This occurs at insulin concentrations that mimic the characteristic dose response of insulin binding to the insulin receptor. The production of endothelin-1 is inhibited by blockade of insulin signaling through the MAP-kinase pathway. It is also known that circulating concentrations of endothelin-1 are modestly increased in insulin resistant states and blockade of endothelin receptors, in the presence or absence of added insulin, enhances glucose disposal within human forearm tissue (39). These findings argue for a significant physiological role for insulin-regulated endothelin-1 production in regulation of vasoconstrictor tone.

The endothelial cell uptake of glucose is rapid, is glucose transporter (GLUT)-4 independent (GLUT-1 appears to be the predominant glucose transporter) and occurs in proportion to the

plasma glucose concentration (40). As a result, the intracellular glucose concentration during hyperglycemia feeds substrate into glycolytic pathways which may account for increased production of reactive oxygen species that contribute to endothelial injury, dysfunction, and the microvascular pathology of diabetes (40).

Little is known with regard to insulin's actions to modulate the intracellular metabolism of glucose within the endothelial cell. Endothelial cells have very little glycogen, and appear to have limited ability to convert carbohydrates into fat. High concentrations of glucose appear capable of provoking glycosylation of eNOS on the same residue (serine 1177) that is a target for insulin-activated Akt (41). This may in part explain the ability of acute increases in circulating glucose concentrations to provoke endothelial dysfunction (42) (manifest as impaired flow-mediated dilation) and increase circulating cytokine concentrations within 2–3 hrs.

The enzyme fatty acid synthase is expressed in endothelial cells and appears to play an interesting role in sustaining the activity of eNOS by selectively supporting palmitoylation of eNOS in the endoplasmic reticulum and thereby promoting its targeting to the plasma membrane. In cultured cell and mouse models, decreasing fatty acid synthase activity causes a phenotypic change to a more pro-inflammatory endothelium. The endothelial cell responds differently to the palmitate synthesized within the endothelial cell compared to exogenous supplied palmitate or other fatty acids. The latter by supplying excessive nutrient that is oxidized by the mitochondrial electron transport chain promotes excess production of superoxide, decreases eNOS activity and the expression of prostacyclin (43) and promotes endothelial cell insulin resistance at least in part by decreasing endothelial cell IRS1 and IRS2 contents (35). Impaired insulin signaling within the endothelial cell may also decrease the activity of acetyl-CoA carboxylase preventing inhibition of carnitine palmitoyltransferase I (CPT1) by malonyl-CoA thereby adding a permissive effect on enhanced fatty acid oxidation. In clinical studies excess FFA levels secondary to lipid infusion (8) as well as an acute high fat meal can cause endothelial dysfunction. A high fat meal can also acutely increase plasma endotoxin concentrations (44), again supporting the endothelium as an early sensor and responder to factors that provoke insulin resistance. Thus, excess nutrient supply (of either carbohydrate or fat) adversely affects the endothelial cell.

The transport of insulin - cellular studies

Early studies from the laboratories of George King (23; 45) and Robert Bar (46–48) explored the "barrier function" of the endothelium with regard to insulin. They demonstrated that endothelial cells bind insulin via the insulin receptor, internalize the insulin and that insulin receptors were involved in the movement of insulin across the endothelial barrier (49). Though there has been some controversy as to whether in vivo insulin's transport into target tissues is a saturable process, the majority of data indicate that in both muscle (50) and brain (16) insulin entry is via a saturable transport process. The exact mechanism by which the endothelial cell moves insulin from the luminal to the anti-luminal surface thereby making it available to the target tissue may differ between tissues as the structure of the endothelium varies widely in different organs. Liver has a discontinuous endothelium which allows free access of relatively large molecules like insulin. Tissues with "relatively" tight continuous endothelial barriers like muscle and fat pose a more significant barrier to insulin access. The extremely tight endothelium of the blood-brain barrier provides an even more challenging issue. We have reviewed elsewhere evidence that the access of insulin to skeletal muscle interstitium is a saturable process (15). Extensive work from several laboratories has likewise demonstrated that movement of insulin into cerebrospinal fluid follows saturation kinetics (16) and it is slowed by insulin resistance evoked by high fat diet

(51) or dexamethasone. Because insulin action in muscle, fat, and CNS is critical to normal metabolic function, we have pursued further the cellular mechanisms by which insulin may cross a tight endothelium. Interesting findings over the past several years suggest that insulin associates with specialized lipid raft domains in the endothelial cell called caveolae. These structures form invaginations on both the luminal and anti-luminal membrane of the endothelial cell and can apparently migrate between the two surfaces allowing exchange of intravesicular contents with the surrounding media. While a number of investigators had postulated such a mechanism might mediate movement of macromolecules between plasma and the interstitial compartment of various tissues, progress in actually identifying the agents responsible has been slow.

Results of studies of albumin transport across lung microvascular endothelium provide convincing evidence for a role of caveolae (52; 53). More recently, we have shown that knock down or deletion of the principal structural protein of caveolae (caveolin-1)(54), or disruption of caveolae using cholesterol-binding detergents (55) substantially blocks insulin uptake by and transport across endothelial monolayers. Imaging studies have also clearly demonstrated a spatial relationship between caveolin-1 and insulin receptors within endothelial cells (55). In aggregate, these data strongly support a working hypothesis that insulin movement out of the vasculature to the interstitial compartment in tissues containing continuous endothelial lining involves a vesicular transport process. Details of how this vesicular machinery supports insulin transport and how it is impacted by insulin resistance are only beginning to emerge. It does appear that insulin must act on the endothelial cell in order to promote its own uptake and transfer (56). Inhibiting either the PI3-kinase or the MAP-kinase pathways of insulin action interferes with insulin transport by bovine aortic endothelial cells. Likewise, interfering with SRC-kinase (56) or with actin polymerization disrupts insulin transport (57). SRC-kinase is known to phosphorylate tyrosine 14 of caveolin-1 and this modification appears necessary for caveolar transport function (52). We have recently observed that inhibition of insulin-induced nitric oxide generation by the endothelial cell is sufficient to block insulin transport (Wang and Barrett, unpublished observation). It appears that a network of signaling pathways downstream of the insulin receptor participate in the regulation of insulin transport. Unraveling the details of these processes will require further investigation to address specifically the role of insulin in the regulation of caveolae transport by the endothelial cell. Initial studies have suggested that insulin resistance (simulated by the addition of relatively low concentrations of TNF-α or IL-6) interfere with both endothelial cell insulin signaling and insulin transport (56). Most of this work has been performed in cultured endothelial cells. Just how this might reflect the in vivo insulin transport in important tissues like skeletal muscle, adipose tissue, and brain is not known at this time. These effects of TNF-α or IL-6 require only 8–24 hrs to be manifest, indicating that the endothelial cell can respond very quickly to agents that provoke insulin resistance. Along this line, interesting studies from Kim et al. report that in vivo aortic tissue becomes “insulin resistant” as judged by impaired insulin-induced Akt phosphorylation much earlier than liver, muscle or fat tissue in animals fed a high fat diet (11). However, others have observed rapid peripheral insulin resistance following short term overfeeding (58), so this temporal relationship remains controversial.

The endothelium control of Insulin delivery is rate limiting for muscle insulin action

These findings occurred near simultaneous with the demonstration that in humans insulin could increase limb blood flow and that enhancement of flow was blocked by inhibition of eNOS (59). Not only can insulin increase total blood flow to skeletal muscle, but at even lower concentrations (60) and significantly more promptly (61). Insulin expands the volume of microvasculature perfused within skeletal muscle and adipose tissue (62). This expanded

microvascular volume occurs even under conditions where total blood flow is unchanged. Inasmuch as the endothelial surface area is (along with blood flow and endothelial permeability) an important determinant of the transfer of insulin and nutrients to skeletal muscle, it appeared that normal insulin action promoted its own delivery to muscle tissue. We have elsewhere discussed in detail data which supports the important role for insulin delivery to skeletal muscle interstitium as a regulator of muscle glucose disposal (15). Support for this includes studies of whole body insulin kinetics that demonstrate a slow exchange of insulin between plasma and skeletal muscle which strongly correlates with insulin mediated glucose disposal as measured during the euglycemic insulin clamp procedure (63). This is further supported by data demonstrating an excellent correlation between insulin-mediated glucose disposal (again under clamp conditions) and interstitial muscle insulin concentration as assessed by sampling lymphatic drainage (64; 65). Finally, studies using microdialysis demonstrate both a significant plasma to muscle interstitial insulin concentration gradient during steady-state hyperinsulinemia throughout the physiologic range of plasma insulin concentrations (66; 67) and a slow rate of transfer of insulin from plasma to the muscle interstitium (68). These consistent findings across several different methodologies strongly support the conclusion that insulin delivery to muscle interstitium is an important determinant of glucose disposal. Nearly all of the studies addressing this have used the insulin clamp procedure. In these studies a steady-state of hyperinsulinemia is maintained for up to 4 hours to allow estimates of the rate of insulin transfer. It can be anticipated that under more physiologic conditions (oral glucose or meal tolerance testing) where plasma insulin increases only transiently, the barrier imposed by slow delivery of insulin to muscle will only more dramatically impact muscle glucose disposal. Peterson et al. clearly summarized how muscle insulin resistance by promoting hyperinsulinemia in the setting of postprandial hyperglycemia would lead to excessive stimulation of hepatic glucose uptake and metabolism supplying substrate for triglyceride production and contributing to the dyslipidemia characteristic of the insulin resistance syndrome (69).

The endothelium controls insulin delivery to the CNS

Insulin transport into the CNS poses a particularly interesting challenge. A number of early studies had demonstrated a significant gradient between concentrations of insulin in circulating plasma and in the cerebrospinal fluid (CSF), with the latter being typically only 5–10% of that in circulating plasma. Moreover, insulin transport into the CSF appeared to occur via a saturable process, much like that responsible for its transfer into muscle and intriguingly the rate of insulin's movement into the CSF is impaired by obesity (51) or glucocorticoids concordantly with the development of insulin resistance. However, it is not certain that insulin concentrations in the CSF necessarily reflect insulin concentration in specific areas of the brain where insulin may be having significant metabolic effects. As has been pointed out by Pardridge, the CSF fluid may not reflect the milieu bathing specific nuclei where insulin may have important actions on either appetite/satiety, thermogenesis, sympathetic tone or other functions (70). In some areas of the brain like the Nucleus of the Solitary Tract (NTS) and area postrema and perhaps areas of hypothalamus the blood brain barrier is thought to be less "tight" and may form less of an obstacle to insulin movement. However, studies directly examining this are lacking.

Recent studies have suggested a very important role for insulin acting at hypothalamic nuclei in the control of hepatic glucose output (71) and of lipolysis in adipose tissue (19). However, this area of investigation is also not without controversy (72). In particular the concentrations of insulin utilized in most of the studies that involve its intrathecal instillation far exceed those achieved in CSF during systemic insulin infusion. As a result, the physiologic significance of observed changes in hepatic glucose production and adipose

lipolysis regulation secondary to insulin delivery to the third ventricle of laboratory rodents remains an open question. Currently there really is no information available regarding the rates of insulin transfer from plasma to selective CNS nuclei. It is very clear that multiple areas of the brain express high levels of insulin receptors (73). The function of these receptors is beginning to be unraveled through the use of knockout models that are specific for particular nuclei within the CNS (20). While such studies likely allow painstaking construction of a map of the CNS indicating specific insulin-modulated functions of particular nuclei, it is not clear that they will directly bear on the question of the kinetics of insulin access to these potential target sites.

Methodological limitations

We have recently discussed some of the limitations for measuring insulin's rate of entry into skeletal muscle (15). While techniques of microdialysis, lymphatic sampling and direct arterial/venous difference measurements have been used, each has significant limitations (74). Limitations to assessing the kinetics of insulin's CNS actions, particularly in clinical studies are substantially more severe, especially efforts to look at specific CNS nuclei. However, given the apparently important role for trans-endothelial insulin transport in determining the temporal response to systemic increases in plasma insulin, efforts to better understand both the molecular processes involved and the sites for potential therapeutic intervention are clearly warranted. Perhaps in the not distant future techniques like positron emission scanning or blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) will provide useful approaches to examine the delivery of proteins like insulin to important target tissues.

Summary

We have tried to show how resistance to insulin's vascular actions at any level of the arterial tree may lead to clinically evident disease processes. At the level of the conduit vessels it accelerates atherosclerosis. At the level of resistance vessels it enhances the risk for hypertension. In small arterioles it can diminish the nutrient exchange surface thereby limiting insulin access to the tissue and promoting metabolic insulin resistance. In healthy individuals acute changes in nutritional intake rapidly impact function within the vasculature as a result of targeting the endothelial cell. The consequence of this is to acutely change vascular function. These effects are transient following a single meal. However, if these changes are recurring over time secondary to a persistent nutrient surfeit, particularly in those genetically pre-disposed, it may be the earliest contributor both to metabolic abnormalities and to vascular injury that plagues those with metabolic syndrome and type 2 diabetes.

Acknowledgments

This work was supported by NIH grants R01DK057878 and R01DK073759 (to E.J.B) and R01HL094722 (to Z.L.), and American Diabetes Association grants 1-11-BS-06 (to E.J.B) and 1-11-CR-30 and 9-09-NOVO-11 (to Z.L.).

References

1. Corkey BE. Banting lecture 2011: hyperinsulinemia: cause or consequence? Diabetes. 2012; 61:4–13. [PubMed: 22187369]
2. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular Actions of Insulin. Endocr Rev. 2007
3. Gill JM, Al-Mamari A, Ferrell WR, Cleland SJ, Packard CJ, Sattar N, Petrie JR, Caslake MJ. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. J Am Coll Cardiol. 2004; 44:2375–2382. [PubMed: 15607401]

4. Vincent MA, Clerk LH, Lindner JR, Price WJ, Jahn LA, Leong-Poi H, Barrett EJ. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. Am J Physiol Endocrinol Metab. 2006; 290:E1191–1197. [PubMed: 16682488]

5. Weiss EP, Arif H, Villareal DT, Marzetti E, Holloszy JO. Endothelial function after high-sugar-food ingestion improves with endurance exercise performed on the previous day. Am J Clin Nutr. 2008; 88:51–57. [PubMed: 18614723]

6. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD. Elevated circulating free fatty acid levels impair endothelium- dependent vasodilation. J Clin Invest. 1997; 100:1230–1239. [PubMed: 9276741]

7. Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J, Baron AD. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. Diabetes. 2000; 49:1231–1238. [PubMed: 10909983]

8. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes. 2003; 52:2882–2887. [PubMed: 14633847]

9. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000; 102:2165–2168. [PubMed: 11056086]

10. Kim F, Gallis B, Corson MA. TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells. Am J Physiol Cell Physiol. 2001; 280:C1057–1065. [PubMed: 11287317]

11. Kim F, Pham M, Maloney E, Rizzo NO, Morton GJ, Wisse BE, Kirk EA, Chait A, Schwartz MW. Vascular inflammation, insulin resistance, and reduced nitric oxide production precede the onset of peripheral insulin resistance. Arterioscler Thromb Vasc Biol. 2008; 28:1982–1988. [PubMed: 18772497]

12. Rattigan S, Clark MG, Barrett EJ. Acute insulin resistance in rat skeletal muscle in vivo induced by vasoconstriction. Diabetes. 1999; 48:564–569. [PubMed: 10078557]

13. 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. [PubMed: 19283361]

14. 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. [PubMed: 21047922]

15. 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:E252–263. [PubMed: 21610226]

16. Porte D Jr, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes. 2005; 54:1264–1276. [PubMed: 15855309]

17. Banks WA, DiPalma CR, Farrell CL. Impaired transport of leptin across the blood-brain barrier in obesity. Peptides. 1999; 20:1341–1345. [PubMed: 10612449]

18. Pocai A, Obici S, Schwartz GJ, Rossetti L. A brain-liver circuit regulates glucose homeostasis. Cell Metab. 2005; 1:53–61. [PubMed: 16054044]

19. Scherer T, O'Hare J, Diggs-Andrews K, Schweiger M, Cheng B, Lindtner C, Zielinski E, Vempati P, Su K, Dighe S, Milsom T, Puchowicz M, Scheja L, Zechner R, Fisher SJ, Previs SF, Buettner C. Brain insulin controls adipose tissue lipolysis and lipogenesis. Cell Metab. 2011; 13:183–194. [PubMed: 21284985]

20. Myers MG Jr, Olson DP. Central nervous system control of metabolism. Nature. 2012; 491:357–363. [PubMed: 23151578]

21. Tsuchiya K, Tanaka J, Shuiqing Y, Welch CL, DePinho RA, Tabas I, Tall AR, Goldberg IJ, Accili D. FoxOs integrate pleiotropic actions of insulin in vascular endothelium to protect mice from atherosclerosis. Cell Metab. 2012; 15:372–381. [PubMed: 22405072]

22. Rask-Madsen C, Li Q, Freund B, Feather D, Abramov R, Wu IH, Chen K, Yamamoto-Hiraoka J, Goldenbogen J, Sotiropoulos KB, Clermont A, Geraldes P, Dall'Osso C, Wagers AJ, Huang PL, Rekhter M, Scalia R, Kahn CR, King GL. Loss of insulin signaling in vascular endothelial cells

accelerates atherosclerosis in apolipoprotein E null mice. Cell Metab. 2010; 11:379–389. [PubMed: 20444418]

23. Jialal I, King GL, Buchwald S, Kahn CR, Crettaz M. Processing of insulin by bovine endothelial cells in culture. Internalization without degradation. Diabetes. 1984; 33:794–800. [PubMed: 6378702]
24. Dernovsek KD, Bar RS, Ginsberg BH, Lioubin MN. Rapid transport of biologically intact insulin through cultured endothelial cells. J Clin Endocrinol Metab. 1984; 58:761–763. [PubMed: 6365948]
25. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. Journal of Clinical Investigation. 1996; 98:894–898. [PubMed: 8770859]
26. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature. 1999; 399:597–601. [PubMed: 10376602]
27. Chen YL, Messina EJ. Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. Am J Physiol. 1996; 270:H2120–2124. [PubMed: 8764264]
28. Oliver FJ, de la Rubia G, Feener EP, Lee ME, Loeken MR, Shiba T, Quertermous T, King GL. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. J Biol Chem. 1991; 266:23251–23256. [PubMed: 1744120]
29. 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. [PubMed: 10458717]
30. Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Invest. 1999; 104:447–457. [PubMed: 10449437]
31. Chisalita SI, Arnqvist HJ. Insulin-like growth factor I receptors are more abundant than insulin receptors in human micro- and macrovascular endothelial cells. Am J Physiol Endocrinol Metab. 2004; 286:E896–901. [PubMed: 14722023]
32. Johansson GS, Chisalita SI, Arnqvist HJ. Human microvascular endothelial cells are sensitive to IGF-I but resistant to insulin at the receptor level. Mol Cell Endocrinol. 2008; 296:58–63. [PubMed: 18708119]
33. 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. [PubMed: 16099860]
34. Chi JT, Chang HY, Haraldsen G, Jahnsen FL, Troyanskaya OG, Chang DS, Wang Z, Rockson SG, van de Rijn M, Botstein D, Brown PO. Endothelial cell diversity revealed by global expression profiling. Proc Natl Acad Sci U S A. 2003; 100:10623–10628. [PubMed: 12963823]
35. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, Kumagai K, Kawai T, Hashimoto S, Kobayashi T, Sato M, Tokuyama K, Nishimura S, Tsunoda M, Ide T, Murakami K, Yamazaki T, Ezaki O, Kawamura K, Masuda H, Moroi M, Sugi K, Oike Y, Shimokawa H, Yanagihara N, Tsutsui M, Terauchi Y, Tobe K, Nagai R, Kamata K, Inoue K, Kodama T, Ueki K, Kadowaki T. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. Cell Metab. 2011; 13:294–307. [PubMed: 21356519]
36. 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. [PubMed: 10747347]
37. Mount PF, Kemp BE, Power DA. Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. J Mol Cell Cardiol. 2007; 42:271–279. [PubMed: 16839566]
38. Baron A. Hemodynamic actions of insulin. American Journal of Physiology. 1994; 267:E187–E202. [PubMed: 8074198]

39. Shemyakin A, Salehzadeh F, Bohm F, Al-Khalili L, Gonon A, Wagner H, Efendic S, Krook A, Pernow J. Regulation of glucose uptake by endothelin-1 in human skeletal muscle in vivo and in vitro. J Clin Endocrinol Metab. 2010; 95:2359–2366. [PubMed: 20207830]
40. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414:813–820. [PubMed: 11742414]
41. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest. 2001; 108:1341–1348. [PubMed: 11696579]
42. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, Motz E. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation. 2002; 106:1211–1218. [PubMed: 12208795]
43. Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. J Clin Invest. 2006; 116:1071–1080. [PubMed: 16528409]
44. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, Dandona P. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. Diabetes Care. 2009; 32:2281–2287. [PubMed: 19755625]
45. Hachiya HL, Takayama S, White MF, King GL. Regulation of insulin receptor internalization in vascular endothelial cells by insulin and phorbol ester. J Biol Chem. 1987; 262:6417–6424. [PubMed: 3106355]
46. Dernovsek KD, Bar RS, Ginsberg BH, Lioubin MN. Rapid transport of biologically intact insulin through cultured endothelial cells. J Clin Endocrinol Metab. 1984; 58:761–763. [PubMed: 6365948]
47. Dernovsek KD, Bar RS. Processing of cell-bound insulin by capillary and macrovascular endothelial cells in culture. Am J Physiol. 1985; 248:E244–251. [PubMed: 3881990]
48. Bar RS, Boes M, Sandra A. Vascular transport of insulin to rat cardiac muscle. Central role of the capillary endothelium. J Clin Invest. 1988; 81:1225–1233. [PubMed: 3280603]
49. King GL, Johnson SM. Receptor-mediated transport of insulin across endothelial cells. Science. 1985; 227:1583–1586. [PubMed: 3883490]
50. Majumdar S, Genders A, Inyard A, Frison V, Barrett E. Insulin entry into muscle involves a saturable process in the vascular endothelium. Diabetologia. 2012; 55:450–456. [PubMed: 22002008]
51. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes. 2000; 49:1525–1533. [PubMed: 10969837]
52. Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. Am J Physiol Lung Cell Mol Physiol. 2003; 285:L1179–1183. [PubMed: 14604847]
53. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006; 86:279–367. [PubMed: 16371600]
54. Wang H, Wang AX, Barrett EJ. Caveolin-1 is required for vascular endothelial insulin uptake. American Journal of Physiology - Endocrinology And Metabolism. 2011; 300:E134–E144. [PubMed: 20959538]
55. 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:E323–332. [PubMed: 16569759]
56. Wang H, Wang AX, Liu Z, Chai W, Barrett EJ. The trafficking/interaction of eNOS and caveolin-1 induced by insulin modulates endothelial nitric oxide production. Mol Endocrinol. 2009; 23:1613–1623. [PubMed: 19608646]
57. 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. [PubMed: 22734037]
58. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. Diabetes. 2001; 50:2786–2791. [PubMed: 11723062]

59. Steinberg HO, Brechtel G, Johnson A, Fineberg F, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. J Clin Invest. 1994; 94:1172–1179. [PubMed: 8083357]
60. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, Barrett EJ. Insulin sensitivity of muscle capillary recruitment in vivo. Diabetes. 2004; 53:447–453. [PubMed: 14747297]
61. 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. [PubMed: 11756321]
62. Sjøberg KA, Rattigan S, Hiscock N, Richter EA, Kiens B. A new method to study changes in microvascular blood volume in muscle and adipose tissue: real-time imaging in humans and rat. American Journal of Physiology - Heart and Circulatory Physiology. 2011; 301:H450–H458. [PubMed: 21622816]
63. 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. [PubMed: 4856884]
64. Castillo C, Bogardus C, Bergman R, Thuillez P, Lillioja S. Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. J Clin Invest. 1994; 93:10–16. [PubMed: 8282776]
65. Yang YJ, Hope I, Ader M, Poulin RA, Bergman RN. Dose-response relationship between lymph insulin and glucose uptake reveals enhanced insulin sensitivity of peripheral tissues. Diabetes. 1992; 41:241–253. [PubMed: 1733816]
66. Jansson PA, Fowelin JP, von Schenck HP, Smith UP, Lonnroth PN. Measurement by microdialysis of the insulin concentration in subcutaneous interstitial fluid. Importance of the endothelial barrier for insulin. Diabetes. 1993; 42:1469–1473. [PubMed: 8375586]
67. Sjostrand M, Holmang A, Lonnroth P. Measurement of interstitial insulin in human muscle. Am J Physiol. 1999; 276:E151–154. [PubMed: 9886961]
68. 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. [PubMed: 12196467]
69. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL, Shulman GI. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A. 2007; 104:12587–12594. [PubMed: 17640906]
70. Pardridge WM. Drug transport across the blood-brain barrier. J Cereb Blood Flow Metab. 2012; 32:1959–1972. [PubMed: 22929442]
71. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med. 2002; 8:1376–1382. [PubMed: 12426561]
72. Edgerton DS, Lautz M, Scott M, Everett CA, Stettler KM, Neal DW, Chu CA, Cherrington AD. Insulin's direct effects on the liver dominate the control of hepatic glucose production. J Clin Invest. 2006; 116:521–527. [PubMed: 16453026]
73. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000; 289:2122–2125. [PubMed: 11000114]
74. Barrett EJ, Eringa EC. The vascular contribution to insulin resistance: promise, proof, and pitfalls. Diabetes. 2012; 61:3063–3065. [PubMed: 23172953]