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# Pull the Trigger, it Fires: The Critical Role of Insulin-Stimulated Caveolin-1 Tyrosine 14 Phosphorylation in Regulation of Insulin Trans-Endothelial Transport

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## Editorial

In order for insulin to exert its biological actions on target cells in peripheral tissues like muscle and adipose tissues, Insulin must pass through the endothelial barrier into the interstitium.Insulin's trans-endothelial transport (TET), particularly in muscle where capillaries are lined by a continuous endothelium, determines tissue insulin levels, and thereby critically determines insulin's metabolic effects [1-6]. This process is significantly impaired in insulin resistance states such as obesity and type 2 diabetes [2,7-9]. Current evidence obtained by us and others indicate that insulin TET is transcellular process and mediated by transporting caveolae that contain or associate with multiple structural and signaling molecules including the insulin receptor (IR), IGF-1receptor, caveolin-1, dynamin-2, actin filaments and eNOS [10-18]. Among these components, caveolae and its key structural protein caveolin-1 have been shown to serve as the center to organize the molecular transcytotic machinery mediating insulin TET [13]. We have demonstrated that insulin, through its signaling pathways in the endothelium, facilitates its own movement across the endothelial cells [15]. Very recently, we reported that eNOS and its activity play a critical role in regulation of insulin uptake and TET as inhibition of eNOS activity completely eliminates endothelial insulin uptake and TET [16]. Next critical question we would ask is how insulin intracellular signaling stimulates and coordinates the assembling of the molecular machinery for insulin trans-endothelial transport? A study just published by us has provided a clue to this puzzle, i.e. insulin stimulated caveolin-1 tyrosine 14 phosphorylation severs a trigger to possibly initiate insulin TET [19].

Caveolae are abundant in microvasculature [20] and represent >95% of the endothelial cell vesicles [21]. Caveolin-1 is the main structural unit and biological marker of endothelial caveolae. Caveolin-1 is required for the formation of caveolae in vascular endothelium as either targeted deletion of the gene for caveolin-1 (Cav-1-/-) [22] or knockdown of caveolin-1 expression using specific siRNA [23] results in the loss of caveolae.In contrast, expression of exogenous caveolin-1 in lymphocytes (which are normally devoid of caveolae) induces the de novo formation of caveolae, and the level of caveolin-1 expression directly correlated to the number of caveolae [24]. Early studies have shown that binding of protein macromolecules to their receptors on the cell surface induced a caveolae-mediated endocytosis whereby caveolae, as a transporting carrier, can efficiently mediate the transcellular transport of a variety of plasma proteins through the endothelial barrier [25-27]. While it would seem desirable to examine the role of caveolin-1 in mediating insulin TET using caveolin-1 (-/-) knockout mice, lack of caveolin-1 has been shown to induce a paracellular leak of macromolecules attributed to the loss of the tonic caveolin-1 inhibition of eNOS activity that restrains paracellular pathway permeability [28,29]. Alternatively, we have observed that siRNA-mediated knockdown of caveolin-1 expression in vascular endothelial cells reduced FITC-insulin uptake by 67%, whereas over-expression of wild-type caveolin-1 increased insulin uptake [13]. Consistently, in the same study, we also observed that aortic endothelial cells from caveolin-1 (-/-) knockout mice failed to take up FITC-insulin [13]. Moreover, caveolae are enriched in insulin receptors [30] and are known to concentrate and organize a variety of signaling molecules at the plasma membrane through the caveolin-1 scaffolding domain to facilitate intracellular signaling transduction [31]. Knockdown of caveolin-1 significantly reduced both insulin receptor protein level and insulin-stimulated Akt1 phosphorylation [13]. On the other hand, early studies have demonstrated that caveolin-1 is a major tyrosine-phosphorylated substrate of Src kinase [32-34]. There are total 9 tyrosine residues in the caveolin-1 molecule but only Tyr14 undergoes phosphorylation by cSrc kinase [34]. In 3T3L1 adipocytes insulin stimulates caveolin-1 phosphorylation at Try14, which is dramatically inhibited by inhibition of Src kinase [35]. However, blocking direct interaction between caveolin-1 and the insulin receptor by mutation of caveolin-1 scaffolding domain prevents insulin-stimulated caveolin-1 Tyr14 phosphorylation in adipocytes [36]. In studies of albumin TET, binding of albumin to its receptor (gp60) was found to activate cSrc kinase and induce caveolin-1 Tyr14 phosphorylation. The resultant increase in endothelial albumin uptake was prevented by inhibition of protein tyrosine kinase [37] and by expression of dominant negative Src [38]. Recently, it has been reported that cSrc activation by a Ca2+ and nitric oxide-dependent mechanism stimulates caveolin-1Tyr14 phosphorylation in response to albumin stimulation in human umbilical vein endothelial cells [39]. Moreover, EM immunocytochemistry has shown that increased vascular endothelial Tyr14 caveolin-1 phosphorylation after inhibition of protein tyrosine phosphatase induces caveolar vesicles moving away from the cell surface into the cytoplasma [40]. Very recently, we have demonstrated that insulin stimulates caveolin-1 Tyr14 phosphorylation through activating cSrc during vascular endothelial insulin uptake, and this insulin-stimulated Tyr14 caveolin-1 phosphorylation is insulin receptor or its activity-dependent [19]. Inhibition of insulin-stimulated Tyr14 caveolin-1 phosphorylation by either inhibition of cSrc activity or mutation of caveolin-1 tyrosine 14 to phenylalanine significantly inhibits endothelial insulin uptake [19]. These data strongly support the important role of Tyr14 caveolin-1 phosphorylation in the regulation of endothelial caveolae-mediated insulin transport. Although the exact mechanism by which Tyr14 caveolin-1phosphorylation regulates insulin TET is not clear, Src-mediated Tyr14 caveolin-1 phosphorylation has been shown to affect the caveolin-1 association with and the caveolae targeting of both

signaling molecules (e.g. eNOS, dynamin-2) and kinases such as active cSrc,which is required by assembling of endothelial transcytotic machinery [39,41,42]. The increased interaction of Tyr14 phosphorylated caveolin-1 and eNOS mediated by cSrc activation is determined by Tyr14 phosphorylation of caveolin-1 but not by the phosphorylation of eNOS at Ser1177 [39] suggesting that Tyr14 phosphorylated caveolin-1 may serve as a specific form of scaffold to recruit and organize multiple molecular components of endothelial transcytotic machinery in caveolae [19].

Taken together, current evidence strongly indicates that insulin-stimulated Tyr14 caveolin-1 phosphorylation is a critical step linking insulin signaling and insulin TET. Given the critical role of insulin TET in regulation of systemic/muscle insulin action, further studies are warranted to uncover the detailed regulatory mechanisms of this process. New discoveries may suggest new intervention sites to mitigate systemic/muscle insulin resistance. These discoveries will also suggest potential translational therapies for metabolic diseases through manipulating these pathways.

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