ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 7 & 8, 2005

Forum Review

The Role of Reactive Oxygen Species in Insulin Signaling in the Vasculature

GERALD D. FRANK,[1] SATORU EGUCHI,[2] and EVANGELINE D. MOTLEY[3]

ABSTRACT

Although there is an abundance of evidence suggesting that insulin resistance plays a significant role in the vasculature, the precise mechanistic role involved still remains unclear. In this review, we discuss the current background of insulin resistance in the context of insulin signaling and action in the vasculature. Also, studies suggest that insulin resistance, diabetes, and cardiovascular disease all share a common involvement with oxidative stress. Recently, we reported that lysophosphatidylcholine, a major bioactive product of oxidized low-density lipoprotein, and angiotensin II, a vasoactive hormone and a potent inducer of reactive oxygen species (ROS), negatively regulate insulin signaling in vascular smooth muscle cells (VSMCs). In endothelial cells, insulin stimulates the release of nitric oxide, which results in VSMC relaxation and inhibition of atherosclerosis. Other data suggest that angiotensin II inhibits the vasodilator effects of insulin through insulin receptor substrate-1 phosphorylation at Ser^{312} and Ser^{616}. Moreover, ROS impair insulin-induced vasorelaxation by neutralizing nitric oxide to form peroxynitrite. Thus, evidence is growing to enable us to better understand mechanistically the relationship between insulin/insulin resistance and ROS in the vasculature, and the impact they have on cardiovascular disease. *Antioxid. Redox Signal.* 7, 1053–1061.

INTRODUCTION

THE PREVALENCE OF DIABETES and insulin resistance is fast approaching epidemic levels throughout the Western world. Insulin, the primary metabolic regulatory hormone that controls glucose uptake by cells, is the major player in diabetes and diabetes-related conditions/diseases. It exerts multiple biological responses in targets cells, which include pleiotropic actions on cell proliferation, migration, apoptosis, differentiation, and metabolism (35, 94). Insulin resistance is defined as a condition that occurs as a result of increased insulin concentration and decreased insulin sensitivity, but essentially, the critical aspect of insulin resistance is the alteration of insulin signaling linked to other intracellular pathways regulating the metabolic effects of insulin (76, 91).

The subsequent hyperinsulinemia that results from insulin resistance has been recognized as an important risk factor in development of cardiovascular diseases, particularly atherosclerosis, coronary artery disease, hypertension, restenosis, and heart failure (10, 22, 37, 81). Therefore, it is not surprising that other pathophysiological roles of insulin resistance include dyslipidemia where triglycerides in plasma are elevated, high-density lipoprotein cholesterol is decreased, and low-density lipoprotein (LDL) particles are increased (17). Together these abnormal conditions are referred to as the insulin resistance syndrome, the metabolic syndrome, or syndrome X. Although there is an abundance of evidence suggesting that insulin plays a significant role in the abovementioned diseases, the precise mechanistic role involved, especially in the vasculature, still remains unclear.

In general, cardiovascular diseases are among the most detrimental in terms of morbidity and mortality (81), and all the complications derived from diabetes and insulin resistance are heavy contributors that serve only to magnify the risk of cardiovascular morbidity and mortality (72). Specifically, atherosclerosis is the most common and serious compli-

[1]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN.
[2]Cardiovascular Research Center, Temple University School of Medicine, Philadelphia, PA.
[3]Department of Physiology, Meharry Medical College, Nashville, TN.

cation of diabetes and insulin resistance. In fact, these conditions adjust the function of various cell types, including smooth muscle cells, endothelium, and platelets, indicating the extent of vascular disturbance involved with this disease (2). Oxidized LDLs play a major role in the development of atherosclerosis and diabetic patients are known to have a greater susceptibility to LDL oxidation (31, 89, 92).

Reactive oxygen species (ROS) are strongly linked to the diabetic/cardiovascular syndromes. In fact, several studies provide evidence that insulin resistance, diabetes, and cardiovascular disease all share a common involvement with oxidative stress (14, 26). Furthermore, hyperglycemia leads to hydrogen peroxide (H_2O_2) production within the cell (69). However, the relationship between insulin resistance and oxidative stress, particularly in "the vasculature," is not well defined.

There has been an increase in knowledge regarding ROS, particularly, the role these molecules play in both normal and abnormal cellular function. ROS are reduction/oxidation molecules derived from molecular oxygen that include superoxide anion ($O_2^{\bullet-}$), H_2O_2, and hydroxyl radical ($OH^{\bullet}$). They are produced by a variety of extracellular stimuli such as growth factors, G protein-coupled receptor agonists, cytokines, ultraviolet radiation, increased osmolarity, and other cellular stresses (15, 50). Important to note, ROS are widely recognized as prominent players in the pathophysiology of cardiovascular diseases such as hypertension, atherosclerosis, and restenosis after vascular injury (6, 29). The current prevailing thought is that ROS induce cardiovascular diseases by three major mechanisms, which include oxidation of LDL to produce oxidized LDL; inhibition of nitric oxide (NO) function through $O_2^{\bullet-}$ and NO interaction leading to peroxynitrite ($ONOO^-$) formation; and activation of intracellular signal transduction pathways by acting as second messengers (6, 18, 27, 29, 57). However, another very plausible concept is that cross-talk between ROS and insulin signaling result in insulin resistance and lead to cardiovascular diseases, especially at the vasculature composed of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). Recent studies show that both of these cell types are major targets of insulin and that the dysfunction of insulin action in the vasculature contributes to the pathophysiology of cardiovascular diseases.

Therefore, the focus in this review will be on the current findings and thoughts of signal transduction research that lead to our understanding of the exact pathophysiological function(s) of insulin in the vasculature and the mechanism of ROS in mediating insulin resistance in vascular cells that promote cardiovascular diseases.

INSULIN ACTION IN VSMCs

Although VSMCs are affected in a variety of ways by insulin, the mechanisms of action, as well as the functional significances of insulin in VSMCs, have recently started to become identified. Under insulin resistance and hyperinsulinemia, insulin-signaling pathways could be differentially regulated in VSMCs resulting in proatherogenic pathways within VSMCs. VSMC growth and migration play major roles in the development of atherosclerosis and other cardiovascular diseases. Insulin has been shown to be a modest promoter of VSMC growth (9, 39, 71, 79, 83). Insulin stimulates DNA synthesis in VSMCs through extracellular signal-regulated kinase 1/2 (ERK1/2), which also leads to transcription factor Elk-1 activation (24, 93). Along similar lines is the fact that S6 kinase is activated by insulin through insulin receptors (IRs) (84). Activation of S6 kinase has been associated with both protein synthesis and proliferation (8, 21, 70). Moreover, it has been shown that insulin promotes VSMC migration as measured by α-smooth muscle actin biomarker (91). Insulin-induced VSMC migration appears to be mediated via a mitogen-activated protein kinase (MAPK)-dependent pathway (91). Although, it remains controversial because the animal source of VSMCs, along with the time and concentration of insulin used for stimulation, indicates varied outcomes of migration (23, 66).

In contrast, Jacob *et al.* found that insulin inhibits platelet-derived growth factor-induced migration of VSMCs partly by inactivating MAPKs through the induction of a MAPK phosphatase, MKP-1, expression, which inactivates MAPKs by dephosphorylation. NO and guanosine 3′,5′-cyclic monophosphate (cGMP) signaling was shown to mediate the insulin-induced MKP-1 expression (42). Further support of this notion is provided by the findings of Zhang *et al.* that demonstrate that insulin-stimulated cGMP inhibits VSMC migration by inhibiting Ca^{2+}/calmodulin-dependent protein kinases II (98). Other studies by the same research group indicated that in VSMCs insulin increases lactate, which leads to increased $O_2^{\bullet-}$ (from activated NADH oxidase) that is converted to H_2O_2 by superoxide dismutase, and cGMP is increased by this H_2O_2 (47, 49). Determining the significance of each insulin function in VSMCs that modulate vascular diseases such as atherosclerosis requires careful interpretation. For example, the above information indicates that insulin stimulates or inhibits VSMC migration and that insulin-induced H_2O_2 formation, as well as growth of VSMCs, may rather promote atherosclerosis. Roles of insulin signals/functions in regard to prevention (protection) of atherosclerosis, as well as to promotion of atherosclerosis, are listed in Table 1. However, there is recent accumulating evidence that highlights the benefits of vascular insulin functions.

Insulin induces vasorelaxation by mechanisms that include stimulation of NO production and decreases in VSMC Ca^{2+} concentration and Ca^{2+}-myosin light chain sensitization in VSMCs (80). The NO production seems to involve both inducible nitric oxide synthase (iNOS) induction and endothelial NOS (eNOS) activation in VSMCs. Although the primary site of NO generation is ECs, VSMC-activated NOSs and subsequent generation of NO may have important functional consequences. Several reports indicate that VSMCs express iNOS (4, 45, 77, 78) that is activated by insulin and results in NO production, as well as cGMP (46, 47, 48). Interestingly, eNOS has also been shown to be expressed in VSMCs (68, 88), and this expression and activity along with $O_2^{\bullet-}$ release are enhanced by insulin stimulation. In human VSMCs, insulin-activated Ca^{2+}-dependent eNOS leads to increased cGMP and cAMP generation (88). These findings further suggest that the insulin-induced relaxation of vessels is not completely due to endothelial mechanisms, but involves NO production in VSMCs. In addition, the availability of NO and

TABLE 1. VARIOUS EFFECTS OF INSULIN ACTIONS IN VSMCs

	Signaling	*Response*	*References*
Antiatherogenic	cGMP/MKP-1	Inhibition of migration	42, 48, 98
	PI3K/Akt/NOS/cGMP	Vasorelaxation	4, 5, 46, 88
	PI3K/Akt	Inhibition of apoptosis	25
Atherogenic	ERK	DNA synthesis	24, 93
	ERK	Migration	91
	PI3K/Akt/NFκB	Growth/inflammation*	
	ROS	Growth/inflammation*	
	ROS	Inhibition of NO*	

*These insulin functions are speculated.

increased ROS in VSMCs seems to alter the level of cGMP in tissue from diabetic pancreatectomized rats, suggesting that ROS may have a significant impact on NO availability in diabetic VSMCs (68). Some of the vasodilatory effects of insulin on VSMCs are mediated by phosphatidylinositol 3-kinase (PI3K) activation and downstream signaling pathways. In this regard, it has been reported that insulin-induced MKP-1 expression is mediated by PI3K-initiated signals, leading to the induction of iNOS and elevated cGMP levels that stimulate MKP-1 expression (5). Also, insulin can reduce Ca^{2+} concentration by activating the Na^+,K^+-ATPase pump in VSMCs, a process known to be dependent on PI3K/Akt signaling (30, 58, 87).

The apoptosis of VSMCs has been implicated in vascular remodeling by accelerating phenotypic change of VSMCs. Goetze *et al.* demonstrated that tumor necrosis factor-α (TNF-α) inhibits insulin-induced antiapoptotic signal transduction in VSMCs (25). This seems to be a direct consequence of preventing the association of the IR substrate-1 (IRS-1)/PI3K complex by TNF-α because it has been previously demonstrated that protecting cells against apoptosis induced by insulin depends on activation of the IRS-1/PI3K/Akt pathway (35, 44, 95). Taken together, insulin seems to have both proatherogenic and antiatherogenic functions through ERK/MAPK/S6K-dependent hypertrophic/mitogenic effects and PI3K/Akt-dependent NO/cGMP production, as well as prevention of apoptosis, respectively (Fig. 1). The role of ERK in stimulating atherosclerosis is supported by the findings that activated ERK1/2 is highly expressed in atherosclerotic lesions of cholesterol-fed rabbits and that there is an increased migratory/proliferative ability of VSMCs derived from these lesions (40). Furthermore, Izumi *et al.* demonstrated that gene transfer of a dominant-negative mutant of ERK prevents neointimal formation in balloon-injured rat artery (41). Quite interestingly, under insulin resistance conditions, the ERK pathway is generally enhanced, whereas the PI3K/Akt pathway is down-regulated (22, 39). Therefore, prevention of insulin resistance in VSMCs could be beneficial in reducing the incidences of cardiovascular disease.

Although no direct evidence has been provided, the PI3K/Akt cascade activation by insulin may alternatively promote vascular diseases such as atherosclerosis. For example, prevention of apoptosis could enhance abnormal VSMC proliferation. In fact, causal roles of Akt in VSMC proliferation under response to injury (82) and hypertension (36) have been reported. In addition, Akt has been shown to stimulate nuclear factor-κB (NFκB) activity in many cells (73), including VSMCs (33), thereby possibly promoting inflammatory responses under atherosclerosis. Thus, further studies are obviously needed to understand the precise roles of PI3K/Akt cascade activation by insulin in VSMCs in regard to its significance in modulating cardiovascular diseases.

Recently, we reported that protein kinase C (PKC) is a potent negative regulator of the insulin signal in the vasculature (63) and that lysophosphatidylcholine, a major bioactive substance of oxidized LDL, negatively regulates insulin signaling in VSMCs at the point of IRS-1 through the specific PKC-α isotype, possibly explaining the association of hyperlipidemia with hyperinsulinemia in cardiovascular diseases (64). Another study showed that angiotensin II (AngII), a vasoactive hormone and a potent ROS inducer, impairs insulin stimulation of IRS-1 tyrosine phosphorylation and coupling of the insulin receptor pathway to PI3K in cultured VSMCs (16). We have further demonstrated that AngII inhibits insulin-induced Akt activation in VSMCs through the PKC-α isotype (65) (Fig. 2). These findings are in line with clinical evidence that AngII receptor blockers and angiotensin-con-

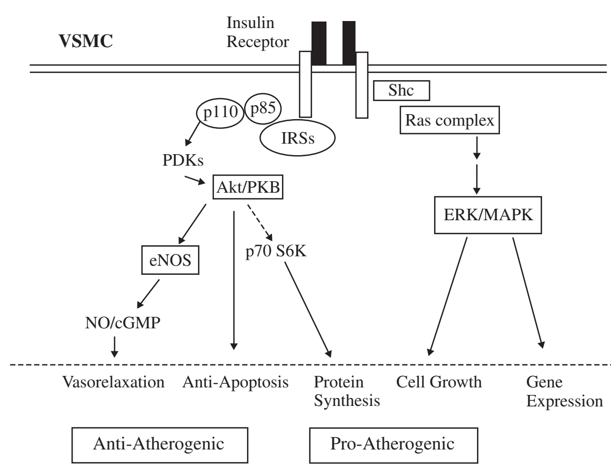

FIG. 1. Multiple insulin signaling pathways lead to proatherogenic and antiatherogenic functional responses in VSMCs through mainly the Akt/PI3K- and ERK/MAPK-mediated signals.

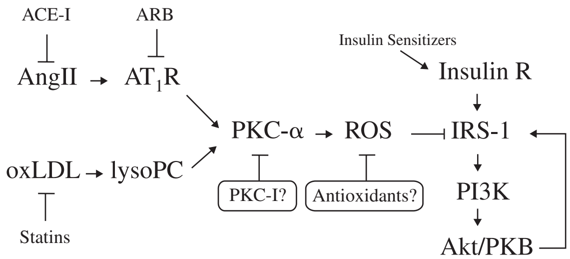

FIG. 2. AngII and lysophosphatidylcholine (lysoPC) inhibit insulin signals through PKC-α/ROS in VSMCs at the point of IRS-1, and several clinical agents can inhibit at other key signaling points. These agents belong to categories such as angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARB), statins, PKC inhibitors (PKC-I), and antioxidants.

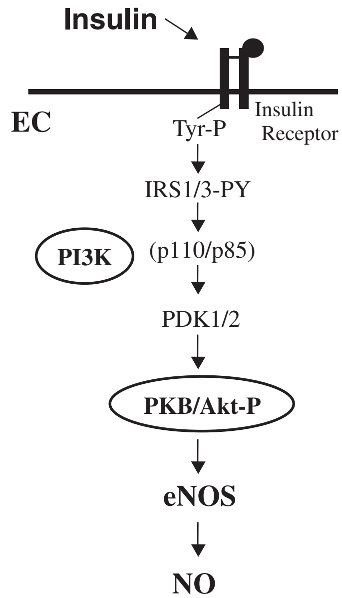

FIG. 3. Insulin signaling in ECs is mediated through the IR to the phosphorylation of IRS-1/3, which leads to the activation of the PI3K/PDK-1/2/Akt complex initiating NO production via eNOS.

verting enzyme inhibitors have preferential effects against insulin resistance (59).

Again, there seems to be some discrepancies regarding these findings because we and others have shown that AngII activates Akt in VSMCs (13, 65, 90). The AngII-induced Akt activation requires ROS, and H_2O_2 can activate Akt in VSMCs as well (90). As pretreatment of AngII effectively blocks Akt activation by insulin in VSMCs (65), the activation mechanism of Akt by AngII may be overridden by the inhibitory mechanism of Akt by AngII if followed by insulin stimulation.

INSULIN ACTION IN ECs

The endothelium plays an important role in mediating insulin's action through IRs, which are found on ECs in the blood vessels (43). One of the major vascular protective effects of the endothelium is attributed to its ability to produce NO. Insulin stimulates the release of NO from ECs, which results in VSMC relaxation and inhibition of atherosclerosis (12, 35). eNOS, which is responsible for NO production, is regulated by insulin at the level of expression and activity (56, 97).

The signaling pathways activated by insulin in ECs include IR tyrosine kinase, PI3K, and Akt, which are essential for activation of eNOS, leading to the production of NO in vascular endothelium (96, 97). Montagnani *et al.* demonstrated that phosphorylation of eNOS at Ser^{1179} by Akt is necessary for its activation by insulin (61). IRS-1 and phosphoinositide-dependent kinase-1 (PDK-1) are essential upstream components of the pathway (62), and this pathway is independent of the classical calcium-dependent eNOS activation pathway (Fig. 3). The PI3K/Akt pathway also mediates an antiapoptotic effect in ECs (11, 35, 56).

A recent study showed that AngII, through AngII type 1 receptor, inhibits NO production in human umbilical vein ECs by increased site-specific serine phosphorylation on IRS-1 (1). AngII increased c-Jun N-terminal kinase (JNK) and ERK1/2 activity, which was associated with a concomitant increase in IRS-1 phosphorylation at Ser^{312} and Ser^{616}, respectively. Inhibition of JNK and ERK1/2 activity reversed the negative effects of AngII on insulin-stimulated NO production. These findings suggest that AngII inhibits the vasodilator effects of insulin through IRS-1 phosphorylation at Ser^{312} and Ser^{616}, representing endothelial insulin resistance that leads to endothelial dysfunction. It should be noted that AngII is a potent inducer of ROS in both VSMCs and ECs and that ROS could activate ERK/JNK and PKC isoforms in these cells (18, 19, 27–29, 38, 54, 55). The possibility of ROS-dependent inhibition of vascular insulin signaling will be further discussed.

Multiple mechanisms, which are interrelated, also contribute to endothelial dysfunction in conditions of insulin resistance. A component of the metabolic syndrome that contributes to alterations in endothelial function is increased small dense LDL (the moiety of LDL cholesterol that is highly susceptible to oxidation). In addition, oxygen-derived free radials impair endothelium-dependent relaxation because NO is neutralized by $O_2^{\bullet-}$ to form $ONOO^-$ (3). This endothelial dysfunction could involve AngII signaling as briefly mentioned earlier. Further along these lines, ROS generation is enhanced in blood vessels in hypertensive animal models and in atherosclerotic lesions in both animals and humans (34, 52, 60). An essential cofactor for the catalytic activity of eNOS is tetrahydrobiopterin (BH_4) (51, 85). This cofactor is synthesized *de novo,* as mediated by dihydropterin reductase, and is depleted during states of oxidative stress because of excessive oxidation (51, 85). A depletion of BH_4 causes eNOS to uncouple and results in a decrease in NO production. These multiple mechanisms leading to endothelial dysfunction in the metabolic syndrome with regard to insulin resistance are illustrated in Fig. 4.

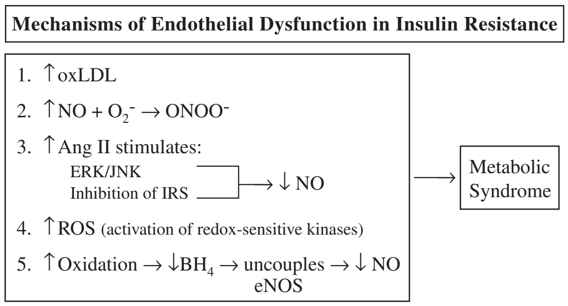

FIG. 4. Multiple mechanisms of ROS-related endothelial dysfunction in insulin resistance are responsible for the metabolic syndrome.

GENERAL MECHANISM BY WHICH ROS BLOCKS INSULIN SIGNALING

The complete understanding of ROS and its role in insulin signaling is still quite limited, but represents an increasing field of interest. In nonvascular but insulin target cells, H_2O_2 and other oxidative stress impair insulin-induced stimulated glucose transporter-4 (GLUT4) translocation, PI3K activation, and Akt activation (74, 75, 86). Hansen *et al.* showed in two fibroblast cell lines and 3T3-L1 adipocytes that micromolar concentrations of H_2O_2 strongly inhibit insulin responses by inhibiting IR kinase-mediated downstream signaling (IRS-1 phosphorylation, PI3K activation) (32). Furthermore, they speculated that the effect they observed with H_2O_2 could be via a tyrosine phosphatase and PKC mechanism (an IR-specific phosphatase) because orthovanadate prevented the inhibitory effect of H_2O_2, as did a PKC inhibitor. This is very plausible because, in general, H_2O_2 is known to inhibit phosphatases and PKC is widely believed to be activated by ROS (18, 53–55).

Agents that induce insulin resistance in insulin target cells activate particular sets of IRS kinases that induce serine/threonine phosphorylation of IRS, resulting in inhibition of insulin-induced IRS-1 tyrosine phosphorylation by dissociation of IR/IRS interaction (99). Some of these IRS kinases, including PKC, JNK, ERK, p38 MAPK, and IκB kinase β (IKKβ), could be activated by ROS (Fig. 5). In cultured L6 myotubes, insulin stimulated glucose uptake and glycogen synthesis, and H_2O_2 treatment prevented activation of both processes. Inhibition of glucose transport and not glycogen synthesis by H_2O_2 was found to be dependent on activation of the p38 MAPK pathway and not JNK, ERK1/2, or Akt (7). These findings provide good evidence that particular sets of ROS-sensing IRS kinases are required for the insulin resistance induced by ROS in a tissue/organ-dependent manner.

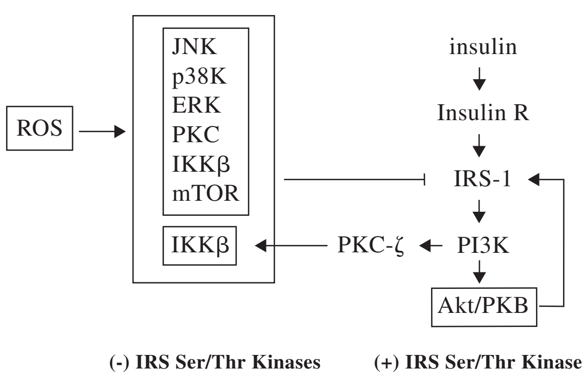

FIG. 5. ROS activate several IRS kinases in the vasculature that inhibit insulin signaling and, therefore, contribute to insulin resistance. mTOR, mammalian target of rapamycin.

INHIBITION OF INSULIN SIGNALING BY ROS IN THE VASCULATURE

Despite the increasing evidence that a relationship between insulin resistance and ROS exists in the vasculature, the precise mechanism of their interaction is still unclear. In a recent study by our research group, we examined whether ROS, such as H_2O_2, could inhibit Akt activation induced by insulin in cultured VSMCs. We found that H_2O_2 clearly inhibits insulin-induced Akt activation, as well as IR binding and IR autophosphorylation in VSMCs. We further showed that the mechanism of the Akt inhibition by H_2O_2 does not involve PKC (20).

In accord with reports by Hansen *et al.* (32), we have also shown that H_2O_2 decreased the autophosphorylation of IRβ. However, this inhibition we demonstrated may be partially due to the decreased receptor binding induced by H_2O_2. Therefore, inhibition of insulin function by H_2O_2 may be regulated at multiple levels proximal to Akt. ROS produced by vascular pathogens such as AngII activate multiple Ser/Thr kinases in ECs and VSMCs (18, 67) that are also implicated as negative IRS kinases. Whether ROS inhibits IRS function by activating IRS kinase(s) in VSMCs and ECs remains to be studied. Therefore, although there is the relative lack of vascular studies regarding insulin and ROS, the above findings together should provide a solid foundation from which to explore this topic focusing on vascular insulin resistance to promote a better understanding of the diabetic/insulin resistance and cardiovascular disease connection.

PERSPECTIVE AND FUTURE DIRECTION

The complex problems associated with insulin resistance, as well as the subsequent increased cardiovascular disease risk, do not simply involve primary insulin targets such as muscle and liver, but rather vascular insulin signaling and functions modulated by multiple factors such as ROS, NO, eNOS, ONOO⁻, and AngII. Insulin function as observed in ECs and VSMCs protects against cardiovascular diseases and insulin resistance that lead to endothelial and vascular dysfunction. This review discussed findings that indicate targeting ROS production may improve both insulin resistance and cardiovascular diseases such as hypertension, restenosis, and atherosclerosis. This has strong support because many of the current treatments for either diabetes/metabolic syndrome or cardiovascular disease appeared to have additional antioxidant properties. Therefore, characterizing the vascular mechanism that ROS utilize to induce vascular insulin resistance should provide important clinical relevance and lead to the elimination of many of the current risk factors for development of insulin/ROS-mediated diseases in the vasculature.

ACKNOWLEDGMENTS

We thank Trinita Fitzgerald, Kunie Eguchi, and Julia Baker for their excellent technical assistance and Dr. Tadashi Inagami for his scientific inputs. This work was supported in part by NIH grant 1 K01 HL76575–01 (G.D.F.), NIH grant HL076770 (S.E.), NIH-NCRR grant 2G12RR03032 (E.D.M), and AHA Grant-In-Aid 0150829B (E.D.M).

ABBREVIATIONS

Akt, protein kinase B; AngII, angiotensin II; BH_4, tetrahydrobiopterin; cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; H_2O_2, hydrogen peroxide; IKKβ, IκB kinase β; iNOS, inducible nitric oxide synthase; IR, insulin receptor; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MKP-1, MAPK phosphatase-1; NFκB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\bullet -}$, superoxide anion; $ONOO^-$, peroxynitrite anion; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; VSMCs, vascular smooth muscle cells.

REFERENCES

1. Andreozzi F, Laratta E, Sciacqua A, Perticone F, and Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ Res* 94: 1211–1218, 2004.
2. Beckman JA, Creager MA, and Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 287: 2570–2581, 2002.
3. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 271: C1424–C1437, 1996.
4. Begum N and Ragolia L. High glucose and insulin inhibit VSMC MKP-1 expression by blocking iNOS via p38 MAPK activation. *Am J Physiol Cell Physiol* 278: C81–C91, 2000.
5. Begum N, Ragolia L, Rienzie J, McCarthy M, and Duddy N. Regulation of mitogen-activated protein kinase phosphatase-1 induction by insulin in vascular smooth muscle cells. Evaluation of the role of the nitric oxide signaling pathway and potential defects in hypertension. *J Biol Chem* 273: 25164–25170, 1998.
6. Berk BC. Redox signals that regulate the vascular response to injury. *Thromb Haemost* 82: 810–817, 1999.
7. Blair AS, Hajduch E, Litherland GJ, and Hundal HS. Regulation of glucose transport and glycogen synthesis in L6 muscle cells during oxidative stress. Evidence for cross-talk between the insulin and SAPK2/p38 mitogen-activated protein kinase signaling pathways. *J Biol Chem* 274: 36293–36299, 1999.
8. Brown EJ and Schreiber SL. A signaling pathway to translational control. *Cell* 86: 517–520, 1996.
9. Cruzado M, Risler N, Castro C, Ortiz A, and Ruttler ME. Proliferative effect of insulin on cultured smooth muscle cells from rat mesenteric resistance vessels. *Am J Hypertens* 11: 54–58, 1998.
10. Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, and Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 334: 952–957, 1996.
11. Dimmeler S, Assmus B, Hermann C, Haendeler J, and Zeiher AM. Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. *Circ Res* 83: 334–341, 1998.
12. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605, 1999.
13. Eguchi S, Iwasaki H, Ueno H, Frank GD, Motley ED, Eguchi K, Marumo F, Hirata Y, and Inagami T. Intracellular signaling of angiotensin II-induced p70 S6 kinase phosphorylation at Ser(411) in vascular smooth muscle cells. Possible requirement of epidermal growth factor receptor, Ras, extracellular signal-regulated kinase, and Akt. *J Biol Chem* 274: 36843–36851, 1999.
14. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 52: 1–8, 2003.
15. Finkel T. Redox-dependent signal transduction. *FEBS Lett* 476: 52–54, 2000.
16. Folli F, Kahn CR, Hansen H, Bouchie JL, and Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest* 100: 2158–2169, 1997.
17. Fonseca VA. Management of diabetes mellitus and insulin resistance in patients with cardiovascular disease. *Am J Cardiol* 92: 50J–60J, 2003.
18. Frank GD. Activation of tyrosine kinases by reactive oxygen species in vascular smooth muscle cells: significance and involvement of EGF receptor transactivation by angiotensin II. *Antioxid Redox Signal* 5: 771–780, 2003.
19. Frank GD, Eguchi S, Yamakawa T, Tanaka S, Inagami T, and Motley ED. Involvement of reactive oxygen species in the activation of tyrosine kinase and extracellular signal-regulated kinase by angiotensin II. *Endocrinology* 141: 3120–3126, 2000.
20. Gardner CD, Eguchi S, Reynolds CM, Eguchi K, Frank GD, and Motley E D. Hydrogen peroxide inhibits insulin

signaling in vascular smooth muscle cells. *Exp Biol Med* 228: 836–842, 2003.
21. Giasson E and Meloche S. Role of p70 S6 protein kinase in angiotensin II-induced protein synthesis in vascular smooth muscle cells. *J Biol Chem* 270: 5225–5231, 1995.
22. Ginsberg HN. Insulin resistance and cardiovascular disease. [see comment]. *J Clin Invest* 106: 453–458, 2000.
23. Gockerman A, Prevette T, Jones JI, and Clemmons DR. Insulin-like growth factor (IGF)-binding proteins inhibit the smooth muscle cell migration responses to IGF-I and IGF-II. *Endocrinology* 136: 4168–4173, 1995.
24. Goetze S, Kintscher U, Kawano H, Kawano Y, Wakino S, Fleck E, Hsueh WA, and Law RE. Tumor necrosis factor alpha inhibits insulin-induced mitogenic signaling in vascular smooth muscle cells. *J Biol Chem* 275: 18279–18283, 2000.
25. Goetze S, Blaschke F, Stawowy P, Bruemmer D, Spencer C, Graf K, Grafe M, Law RE, and Fleck E. TNFalpha inhibits insulin's antiapoptotic signaling in vascular smooth muscle cells. *Biochem Biophys Res Commun* 287: 662–670, 2001.
26. Griendling KK and FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation* 108: 2034–2040, 2003.
27. Griendling KK and Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept* 91: 21–27, 2000.
28. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
29. Griendling KK, Sorescu D, and Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 86: 494–501, 2000.
30. Gupta S, Yang S, Cohen RA, Krane RJ, and Saenz De Tejada I. Altered contractility of urinary bladder in diabetic rabbits: relationship to reduced Na^+ pump activity. *Am J Physiol* 271: C2045–C2052, 1996.
31. Haffner SM, Agil A, Mykkanen L, Stern MP, and Jialal I. Plasma oxidizability in subjects with normal glucose tolerance, impaired glucose tolerance, and NIDDM. *Diabetes Care* 18: 646–653, 1995.
32. Hansen LL, Ikeda Y, Olsen GS, Busch AK, and Mosthaf L. Insulin signaling is inhibited by micromolar concentrations of H_2O_2. Evidence for a role of H_2O_2 in tumor necrosis factor alpha-mediated insulin resistance. *J Biol Chem* 274: 25078–25084, 1999.
33. Hattori Y, Hattori S, and Kasai K. Lipopolysaccharide activates Akt in vascular smooth muscle cells resulting in induction of inducible nitric oxide synthase through nuclear factor-kappa B activation. *Eur J Pharmacol* 481: 153–158, 2003.
34. Heitzer T, Schlinzig T, Krohn K, Meinertz T, and Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 104: 2673–2678, 2001.
35. Hermann C, Assmus B, Urbich C, Zeiher AM, and Dimmeler S. Insulin-mediated stimulation of protein kinase Akt: a potent survival signaling cascade for endothelial cells. *Arterioscler Thromb Vasc Biol* 20: 402–409, 2000.
36. Hixon ML, Muro-Cacho C, Wagner MW, Obejero-Paz C, Millie E, Fujio Y, Kureishi Y, Hassold T, Walsh K, and Gualberto A. Akt1/PKB upregulation leads to vascular smooth muscle cell hypertrophy and polyploidization. *J Clin Invest* 106: 1011–1020, 1011.
37. Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, and Bergman R. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 93: 1809–1817, 1996.
38. Hsu YH, Chen JJ, Chang NC, Chen CH, Liu JC, Chen TH, Jeng CJ, Chao HH, and Cheng TH. Role of reactive oxygen species-sensitive extracellular signal-regulated kinase pathway in angiotensin II-induced endothelin-1 gene expression in vascular endothelial cells. *J Vasc Res* 41: 64–74, 2004.
39. Hsueh WA and Law RE. Insulin signaling in the arterial wall. *Am J Cardiol* 84: 21J–24J, 1999.
40. Hu Y, Dietrich H, Metzler B, Wick G, and Xu Q. Hyperexpression and activation of extracellular signal-regulated kinases (ERK1/2) in atherosclerotic lesions of cholesterol-fed rabbits. *Arterioscler Thromb Vasc Biol* 20: 18–26, 2000.
41. Izumi Y, Kim S, Namba M, Yasumoto H, Miyazaki H, Hoshiga M, Kaneda Y, Morishita R, Zhan Y, and Iwao H. Gene transfer of dominant-negative mutants of extracellular signal-regulated kinase and c-Jun NH_2-terminal kinase prevents neointimal formation in balloon-injured rat artery. *Circ Res* 88: 1120–1126, 2001.
42. Jacob A, Molkentin JD, Smolenski A, Lohmann SM, and Begum N. Insulin inhibits PDGF-directed VSMC migration via NO/cGMP increase of MKP-1 and its inactivation of MAPKs. *Am J Physiol Cell Physiol* 283: C704–C713, 2002.
43. Jialal I, Crettaz M, Hachiya HL, Kahn CR, Moses AC, Buzney SM, and King GL. Characterization of the receptors for insulin and the insulin-like growth factors on micro- and macrovascular tissues. *Endocrinology* 117: 1222–1229, 1985.
44. Jung F. Haendeler J, Goebel C, Zeiher AM, and Dimmeler S. Growth factor-induced phosphoinositide 3-OH kinase/Akt phosphorylation in smooth muscle cells: induction of cell proliferation and inhibition of cell death. *Cardiovasc Res* 48: 148–157, 2000.
45. Junquero DC, Schini VB, Scott-Burden T, and Vanhoutte PM. Enhanced production of nitric oxide in aortae from spontaneously hypertensive rats by interleukin-1 beta. *Am J Hypertens* 6: 602–610, 1993.
46. Kahn AM, Husid A, Allen JC, Seidel CL, and Song T. Insulin acutely inhibits cultured vascular smooth muscle cell contraction by a nitric oxide synthase-dependent pathway. *Hypertension* 30: 928–933, 1997.
47. Kahn AM, Allen JC, Seidel CL, Lichtenberg DS, Song T, and Zhang S. Insulin increases NO-stimulated guanylate cyclase activity in cultured VSMC while raising redox potential. *Am J Physiol Endocrinol Metab* 278: E627–E633, 2000.
48. Kahn AM, Allen JC, Seidel CL, and Zhang S. Insulin inhibits migration of vascular smooth muscle cells with inducible nitric oxide synthase. *Hypertension* 35: 303–306, 2000.

49. Kahn AM, Allen JC, and Zhang S. Insulin increases NADH/NAD+ redox state, which stimulates guanylate cyclase in vascular smooth muscle. *Am J Hypertens* 15: 273–279, 2002.
50. Kamata H and Hirata H. Redox regulation of cellular signalling. *Cell Signal* 11: 1–14, 1999.
51. Katusic ZS. Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol* 281: H981–H986, 2001.
52. Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF, and Hamilton CA. Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. *Hypertension* 33: 1353–1358, 1999.
53. Knebel A, Rahmsdorf HJ, Ullrich A, and Herrlich P. Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. *EMBO J* 15: 5314–5325, 1996.
54. Konishi H, Tanaka M, Takemura Y, Matsuzaki H, Ono Y, Kikkawa U, and Nishizuka Y. Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. *Proc Natl Acad Sci U S A* 94: 11233–11237, 1997.
55. Konishi H, Yamauchi E, Taniguchi H, Yamamoto T, Matsuzaki H, Takemura Y, Ohmae K, Kikkawa U, and Nishizuka Y. Phosphorylation sites of protein kinase C delta in H_2O_2-treated cells and its activation by tyrosine kinase in vitro. *Proc Natl Acad Sci U S A* 98: 6587–6592, 2001.
56. Kuboki K, Jiang ZY, Takahara N, Ha S, Igarashi M, Yamauchi T, Feener E, Herbert T, Rhodes CJ, and King GL. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. *Circulation* 101: 676–681, 2000.
57. Kunsch C and Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res* 85: 753–766, 1999.
58. Li D, Sweeney G, Wang Q, and Klip A. Participation of PI3K and atypical PKC in Na^+-K^+-pump stimulation by IGF-I in VSMC. *Am J Physiol* 276: H2109–H2116, 1999.
59. McFarlane SI, Kumar A, and Sowers JR. Mechanisms by which angiotensin-converting enzyme inhibitors prevent diabetes and cardiovascular disease. *Am J Cardiol* 91: 30H–37H, 2003.
60. Miller FJ Jr, Gutterman DD, Rios CD, Heistad DD, and Davidson BL. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. *Circ Res* 82: 1298–1305, 1998.
61. Montagnani M, Chen H, Barr VA, and Quon MJ. Insulin stimulated activation of eNOS is independent of Ca^{2+} but requires phosphorylation by Akt at Ser1179. *J Biol Chem* 276: 30392–30398, 2001.
62. Montagnani M, Ravichandran LV, Chen H, Esposito DL, and Quon MJ. Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1 are required for insulin-stimulated production of nitric oxide in endothelial cells. *Mol Endocrinol* 16: 1931–1942, 2002.
63. Motley ED, Kabir SM, Eguchi K, Hicks AL, Gardner CD, Reynolds CM, Frank GD, and Eguchi S. Protein kinase C inhibits insulin-induced Akt activation in vascular smooth muscle cells. *Cell Mol Biol (Noisy-le-grand)* 47: 1059–1062, 2001.
64. Motley ED, Kabir SM, Gardner CD, Eguchi K, Frank GD, Kuroki T, Ohba M, Yamakawa T, and Eguchi S. Lysophosphatidylcholine inhibits insulin-induced Akt activation through protein kinase C-alpha in vascular smooth muscle cells. *Hypertension* 39: 508–512, 2002.
65. Motley ED, Eguchi K, Gardner C, Hicks AL, Reynolds CM, Frank GD, Mifune M, Ohba M, and Eguchi S. Insulin-induced Akt activation is inhibited by angiotensin II in the vasculature through protein kinase C-alpha. *Hypertension* 41: 775–780, 2003.
66. Nakao J, Ito H, Kanayasu T, and Murota S. Stimulatory effect of insulin on aortic smooth muscle cell migration induced by 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid and its modulation by elevated extracellular glucose levels. *Diabetes* 34: 185–191, 1985.
67. Ohtsu H, Frank GD, Utsunomiya H, and Eguchi S. Redox-dependent protein kinase regulation by angiotensin II: mechanistic insight and its pathophysiology. *Antioxid Redox Signal* 2005 (in press).
68. Pandolfi A, Grilli A, Cilli C, Patruno A, Giaccari A, Di Silvestre S, De Lutiis MA, Pellegrini G, Capani F, Consoli A, and Felaco M. Phenotype modulation in cultures of vascular smooth muscle cells from diabetic rats: association with increased nitric oxide synthase expression and superoxide anion generation. *J Cell Physiol* 196: 378–385, 2003.
69. Paolisso G and Giugliano D. Oxidative stress and insulin action: is there a relationship? *Diabetologia* 39: 357–363, 1996.
70. Pullen N and Thomas G. The modular phosphorylation and activation of p70s6k. *FEBS Lett* 410: 78–82, 1997.
71. Reaven GM and Chen YD. Insulin resistance, its consequences, and coronary heart disease. Must we choose one culprit? *Circulation* 93: 1780–1783, 1996.
72. Resnick HE, Shorr RI, Kuller L, Franse L, and Harris TB. Prevalence and clinical implications of American Diabetes Association-defined diabetes and other categories of glucose dysregulation in older adults: the health, aging and body composition study. *J Clin Epidemiol* 54: 869–876, 2001.
73. Romashkova JA and Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* 401: 86–90, 1999.
74. Rudich A, Kozlovsky N, Potashnik R, and Bashan N. Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes. *Am J Physiol* 272: E935–E940, 1997.
75. Rudich A, Tirosh A, Potashnik R, Hemi R, Kanet H, and Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes* 47: 1562–1569, 1998.
76. Saltiel AR. Diverse signaling pathways in the cellular actions of insulin. *Am J Physiol* 270: E375–E385, 1996.
77. Schini-Kerth VB and Vanhoutte PM. Nitric oxide synthases in vascular cells. *Exp Physiol* 80: 885–905, 1995.
78. Scott-Burden T, Elizondo E, Ge T, Boulanger CM, and Vanhoutte PM. Simultaneous activation of adenylyl cyclase and protein kinase C induces production of nitric

oxide by vascular smooth muscle cells. *Mol Pharmacol* 46: 274–282, 1994.
79. Sowers JR. Insulin and insulin-like growth factor in normal and pathological cardiovascular physiology. *Hypertension* 29: 691–699, 1997.
80. Sowers JR. Insulin resistance and hypertension. *Am J Physiol Heart Circ Physiol* 286: H1597–H1602, 2004.
81. Sowers JR, Epstein M, and Frohlich ED. Diabetes, hypertension, and cardiovascular diseases. *Hypertension* 37: 1053–1059, 2001.
82. Stabile E, Zhou YF, Saji M, Castagna M, Shou M, Kinnaird TD, Baffour R, Ringel MD, Epstein SE, and Fuchs S. Akt controls vascular smooth muscle cell proliferation in vitro and in vivo by delaying G1/S exit. *Circ Res* 93: 1059–1065, 2003.
83. Stout RW. Insulin as a mitogenic factor: role in the pathogenesis of cardiovascular disease. *Am J Med* 90: 62S–65S, 1991.
84. Takagi Y, Kashiwagi A, Tanaka Y, Maegawa H, and Shigeta Y. Insulin-specific activation of S6 kinase and its desensitization in cultured rat vascular smooth muscle cells. *Atherosclerosis* 113: 19–27, 1995.
85. Tiefenbacher CP. Tetrahydrobiopterin: a critical cofactor for eNOS and a strategy in the treatment of endothelial dysfunction? *Am J Physiol Heart Circ Physiol* 280: H2484–H2488, 2001.
86. Tirosh A, Potashnik R, Bashan N, and Rudich A. Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. *J Biol Chem* 274: 10595–10602, 1999.
87. Tirupattur PR, Ram JL, Standley PR, and Sowers JR. Regulation of Na^+,K^+-ATPase gene expression by insulin in vascular smooth muscle cells. *Am J Hypertens* 6: 626–629, 1993.
88. Trovati M, Massucco P, Mattiello L, Costamagna C, Aldieri E, Cavalot F, Anfossi G, Bosia A, and Ghigo D. Human vascular smooth muscle cells express a constitutive nitric oxide synthase that insulin rapidly activates, thus increasing guanosine 3′:5′-cyclic monophosphate and adenosine 3′:5′-cyclic monophosphate concentrations. *Diabetologia* 42: 831–839, 1999.
89. Tsai EC, Hirsch IB, Brunzell JD, and Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes* 43: 1010–1014, 1994.
90. Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, and Griendling KK. Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 274: 22699–22704, 1999.
91. Wang CC, Gurevich I, and Draznin B. Insulin affects vascular smooth muscle cell phenotype and migration via distinct signaling pathways. *Diabetes* 52: 2562–2569, 2003.
92. Witztum JL. The role of oxidized LDL in atherosclerosis. *Adv Exp Med Biol* 285: 353–365, 1991.
93. Xi XP, Graf K, Goetze S, Hsueh WA, and Law RE. Inhibition of MAP kinase blocks insulin-mediated DNA synthesis and transcriptional activation of c-fos by Elk-1 in vascular smooth muscle cells. *FEBS Lett* 417: 283–286, 1997.
94. Yenush L and White MF. The IRS-signalling system during insulin and cytokine action. *Bioessays* 19: 491–500, 1997.
95. Yenush L, Zanella C, Uchida T, Bernal D, and White MF. The pleckstrin homology and phosphotyrosine binding domains of insulin receptor substrate 1 mediate inhibition of apoptosis by insulin. *Mol Cell Biol* 18: 6784–6794, 1998.
96. Zeng G and Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 98: 894–898, 1999.
97. Zeng G, Nystrom FH, Ravichandran LV, Cong L, Kirby M, Mostowski H, and 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* 101: 1539–1545, 2000.
98. Zhang S, Yang Y, Kone BC, Allen JC, and Kahn AM. Insulin-stimulated cyclic guanosine monophosphate inhibits vascular smooth muscle cell migration by inhibiting Ca/calmodulin-dependent protein kinase II. *Circulation* 107: 1539–1544, 2003.
99. Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. *Trends Cell Biol* 11: 437–441, 2001.

Address reprint requests to:
Evangeline D. Motley, Ph.D.
Department of Physiology
Meharry Medical College
Nashville, TN 37208

E-mail: emotley@mmc.edu

Received for publication September 25, 2004; accepted February 2, 2005.

This article has been cited by:

1. Xin Wang , Hao Wu , Hongli Chen , Rui Liu , Jiangzheng Liu , Tao Zhang , Weihua Yu , Chunxu Hai . 2012. Does Insulin Bolster Antioxidant Defenses via the Extracellular Signal–Regulated Kinases-Protein Kinase B-Nuclear Factor Erythroid 2 p45-Related Factor 2 Pathway?. *Antioxidants & Redox Signaling* **16**:10, 1061-1070. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
2. Manuel Ros Pérez, Gema Medina-Gómez. 2011. Obesity, adipogenesis and insulin resistance. *Endocrinología y Nutrición (English Edition)* . [CrossRef]
3. Barbara Mlinar, Janja Marc. 2011. Review: New insights into adipose tissue dysfunction in insulin resistance. *Clinical Chemistry and Laboratory Medicine* ---. [CrossRef]
4. Manuel Ros Pérez, Gema Medina-Gómez. 2011. Obesidad, adipogénesis y resistencia a la insulina. *Endocrinología y Nutrición* . [CrossRef]
5. Justin L. Rains, Sushil K. Jain. 2011. Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine* **50**:5, 567-575. [CrossRef]
6. Eric P. Davidson, Lawrence J. Coppey, Nigel A. Calcutt, Christine L. Oltman, Mark A. Yorek. 2010. Diet-induced obesity in Sprague-Dawley rats causes microvascular and neural dysfunction. *Diabetes/Metabolism Research and Reviews* **26**:4, 306-318. [CrossRef]
7. Stéliana Ghibu, Carole Richard, Catherine Vergely, Marianne Zeller, Yves Cottin, Luc Rochette. 2009. Antioxidant Properties of an Endogenous Thiol: Alpha-lipoic Acid, Useful in the Prevention of Cardiovascular Diseases. *Journal of Cardiovascular Pharmacology* **54**:5, 391-398. [CrossRef]
8. M.F. Meyer, D. Lieps, H. Schatz, M. Pfohl. 2008. Impaired flow-mediated vasodilation in type 2 diabetes: Lack of relation to microvascular dysfunction. *Microvascular Research* **76**:1, 61-65. [CrossRef]
9. Britta Diesel, Alexandra KiemerActivation of Cytoprotective Signaling Pathways by Alpha-Lipoic Acid **20080652**, . [CrossRef]
10. Peter Kovacic. 2007. Protein electron transfer (mechanism and reproductive toxicity): Iminium, hydrogen bonding, homoconjugation, amino acid side chains (redox and charged), and cell signaling. *Birth Defects Research Part C: Embryo Today: Reviews* **81**:1, 51-64. [CrossRef]
11. Nick R. Leslie . 2006. The Redox Regulation of PI 3-Kinase–Dependent Signaling. *Antioxidants & Redox Signaling* **8**:9-10, 1765-1774. [Abstract] [Full Text PDF] [Full Text PDF with Links]
12. J PAN, X ZHENG, P YANG, Y QIN, Y RUI, L MA, F ZHOU, H KANG. 2006. Different expressions of Nogo-B1 and Nogo-B2 in mouse heart microvascular endothelial cell dysfunction induced by lysophosphatidylcholine. *Microvascular Research* **72**:1-2, 42-47. [CrossRef]
13. Ashok K. Srivastava . 2005. Redox Regulation of Insulin Action and Signaling. *Antioxidants & Redox Signaling* **7**:7-8, 1011-1013. [Citation] [Full Text PDF] [Full Text PDF with Links]