

Editorial

Atherogenesis and Diabetes: Focus on Insulin Resistance and Hyperinsulinemia

Aterogénesis y diabetes: resistencia a la insulina e hiperinsulinemia

Rosalinda Madonna^{a,b} and Raffaele De Caterina^{a,*}^aIstituto di Cardiologia, Università G. d'Annunzio-Chieti, Chieti, Italy^bTexas Heart Institute and St. Luke's Episcopal Hospital, Houston, TX, United States

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INSULIN RESISTANCE, HYPERINSULINEMIA AND VASCULAR DISEASE: DEFINING THE PROBLEM

Macro- and microvascular complications are both main causes of morbidity and mortality in type 1 and type 2 diabetes,¹ but macrovascular complications have an increased incidence even before the onset of type 2 diabetes.² While high blood glucose³ and glucose-induced protein and lipid modifications—the advanced glycation endproducts⁴—can be triggers of both macro- and microvascular disease once diabetes (both type 1 and type 2) has occurred, factors causing macrovascular disease in the setting of the metabolic syndrome and prediabetes have long been debated. Certainly in diabetes, and probably also in the setting of the metabolic syndrome,^{5–8} atherosclerotic vascular and coronary heart disease occur over and above the clustering of other accompanying risk factors, such as hypertriglyceridemia, low levels of high-density lipoproteins and hypertension. Insulin resistance before the onset of diabetes is, by definition, characterized by hyperinsulinemia, and this has long been speculated to be causally linked to vascular disease.^{9–12} In this brief review we will address the biological plausibility and the evidence for hyperinsulinemia to be a causal mechanism in the development of atherosclerosis preceding and following the onset of type 2 diabetes.

SELECTIVE INSULIN RESISTANCE AND COMPENSATORY HYPERINSULINEMIA: PATHOPHYSIOLOGY

Originally, Reaven et al. defined the insulin resistance syndrome as a cluster of cardiovascular risk factors, including glucose intolerance, dyslipidemia, and hypertension, associated with enhanced cardiovascular disease.¹³ They described the metabolic syndrome as a clinical condition characterized by insulin resistance, impaired fasting plasma glucose, obesity, dyslipidemia, and hypertension.¹³ Later, two definitions of the metabolic syndrome have been proposed, one by the National Cholesterol Education Program Adult Treatment Panel III¹⁴ and the other by the World Health Organization.¹⁵ Here, insulin resistance—as measured by the reference method of the hyperinsulinemic euglycemic clamp or by surrogate methods, such as the

frequently sampled intravenous glucose tolerance test, insulin suppression test, or HOMA index—can be demonstrated in up to 76% of subjects,¹⁶ and is accompanied by compensatory hyperinsulinemia.¹⁶ Although molecular mechanisms of insulin resistance are still incompletely understood, abnormalities in insulin signaling have been described.¹⁷ In peripheral tissues, including the skeletal muscle and the liver, in normal conditions insulin initiates its action by binding its specific cell surface receptor, ie, insulin receptor (IR), which is a heterotetrameric protein consisting of 2 extracellular α -subunits and 2 transmembrane β -subunits connected by disulfide bridges. Insulin binding to the extracellular α -subunit induces conformational changes of the IR, which in turn causes the dimerization of adjacent receptors and the activation of the tyrosine kinase domain of the intracellular part of the β -subunit. Once the tyrosine kinase activity of IR is initiated, it promotes the autophosphorylation of the β -subunit itself and the rapid phosphorylation of so-called “docking proteins”, such as IR substrates (IRS)-1, -2, -3, and -4, and several other proteins, including collagen homology proteins (shc) and src homology 2 (SH2), that in turn activate multiple intracellular signaling intermediates (Fig. 1). Therefore, IRS, shc, and SH2 proteins play important regulatory roles in the insulin signaling cascade. In their phosphorylated form, these proteins become points of anchoring for intracellular proteins containing complementary SH2 domains. In particular, the interaction between IRS-1 proteins and phosphatidylinositol (PI) 3-kinase determines the activation of Akt (also known as protein kinase B), which is critical in the mechanism of insulin action on GLUT-4 translocation, glucose transport, and the activation of nitric oxide (NO) synthase (“metabolic signal pathway”). In contrast, nonmetabolic, proliferative, mitogenic, pro-inflammatory effects of insulin are mediated by the activation of Ras (mostly through shc and, to a lesser degree, IRS proteins), Raf, and mitogen-activated protein kinases (MAPK) (“growth signaling pathway”).¹⁸ In insulin-resistant animals and *in vitro* models a reduced activation of insulin signaling via the IRS-1/PI3-kinase pathway can be demonstrated, resulting in diminished glucose uptake, reduced NO synthesis, and reduced glucose utilization in insulin target tissues. The same decrease in glucose transport is sensed at the level of pancreatic beta cells, and induces a compensatory increase in insulin secretion. At the same time, however, the MAPK-mediated insulin pathway remains unaffected.¹⁹ One can easily understand that this selective imbalance of the two signal transduction pathways in such conditions of hyperinsulinemia may lead to an excessive

* Corresponding author: Istituto di Cardiologia, Università G. d'Annunzio-Chieti, C/o Ospedale SS. Annunziata, Via dei Vestini, 66013 Chieti, Italy.

E-mail address: rdcater@unich.it (R. De Caterina).

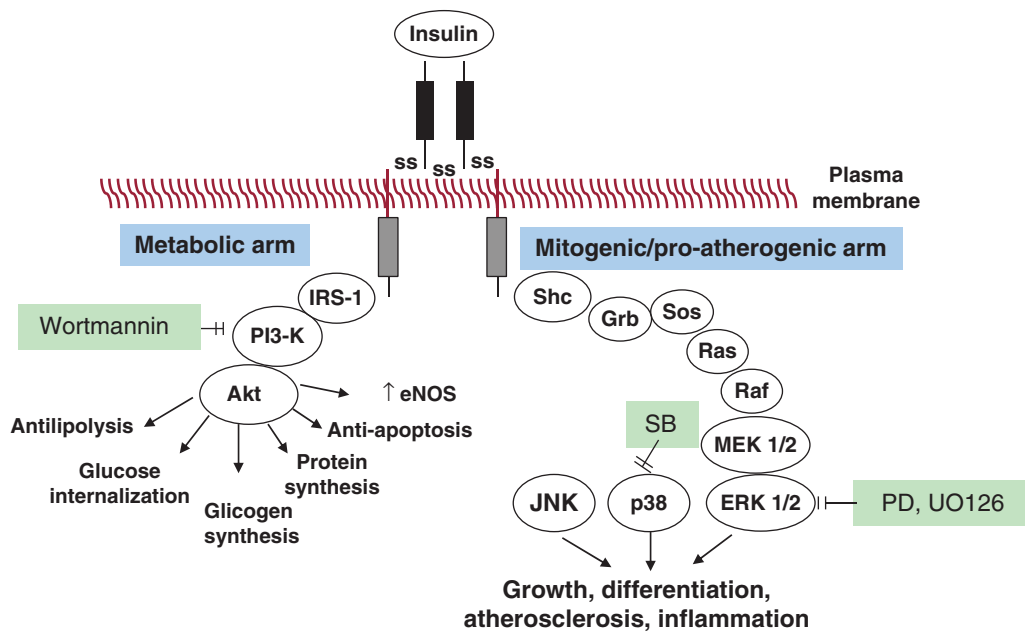


Figure 1. The insulin signaling pathway and its impairment in insulin resistance. Upon binding to its tyrosine-kinase receptor, insulin induces receptor dimerization, and activation of a cascade of phosphorylation events, producing two classes of effects: a) “metabolic” effects, promoting glucose transport, glycogen and protein synthesis, inhibition of lipolysis, protection from apoptosis, and the release of nitric oxide (broadly described as “anti-inflammatory” effects), and b) growth- and differentiation-promoting effects, which lead to promotion of inflammation and atherogenesis (ie, mitogenic, pro-inflammatory insulin signaling). Akt, protein kinase B (PKB); eNOS, endothelial nitric oxide synthase; ERK, extracellular receptor kinase; IRS-1, insulin substrate receptor-1; JNK, c-Jun NH2-1 terminal kinase; MEK, mitogen-activated protein kinase/extracellular receptor kinase; p38, p38 mitogen-activated protein kinase; PD (PD98059) and UO126, extracellular receptor kinase 1/2 inhibitors; PI3-Kinase, phosphatidylinositol(PI)3-kinase; wortmannin, PI3-kinase inhibitor.

proliferative/growth-promoting signal, at the same time allowing the maintenance of normal glucose transport and glucose homeostasis. Compensatory hyperinsulinemia stimulates various proliferative and pro-atherogenic events in vascular smooth muscle and endothelial cells. Such effects include the increased production of plasminogen activator inhibitor type-1 (PAI-1), endothelin, proinflammatory cytokines and the increased surface expression of adhesion molecules.^{19–22}

Insulin has an important role in the maintenance of blood vessel homeostasis through the activation of endothelium-derived NO. Insulin increases endothelial NO production by activating NOS-III (endothelial NOS) by rapid posttranslational mechanisms, which are mediated by the PI3K/Akt signaling pathway.²³ In insulin resistance states the PI3K/Akt pathway is selectively inhibited, and this leads to endothelial dysfunction, with a consequent increase in vascular tone and hypertension, increased interaction between endothelial cells and leukocytes, and a prothrombotic state. This “selective” insulin resistance has been shown in skeletal muscle from obese people and patients with type 2 diabetes,²⁴ and in the vasculature and the myocardium of obese Zucker rats. Here, the normal physiological anti-atherogenic effects of insulin, due largely to its capacity to increase NO production, are turned into pro-atherogenic effects.²⁵

A VICIOUS CIRCLE BETWEEN HYPERINSULINEMIA AND INSULIN RESISTANCE

High plasma concentrations of insulin in conditions of insulin resistance also can trigger a vicious circle that further increases insulin resistance²⁶ through a suppression of the effects mediated by the PI3K/AKT/NO axis, which may unbalance the system through the net promotion of effects related to MAPK activation. Since insulin triggers a series of biological effects through the

binding and activation of its receptor (IR), endowed with tyrosine-kinase activity on specific substrates including IRS -1 and -2,²⁷ mice with a targeted deletion of IRS-1 and IRS-2 genes have a phenotype of insulin resistance.²⁸

Animal models of hyperinsulinemia, such as the *ob/ob* mice and the obese Zucker rats, have low levels of IRS-1 and IRS-2 proteins in the liver.^{29,30} These models are characterized by insulin resistance and a reduced function of the IR/IRS-1/PI3K/AKT axis in the liver and the skeletal muscle. It has been shown that brief *in vitro* incubations of myoblasts with high concentrations of insulin determine a PI3K-mediated reduction of IRS-1 protein expression and a desensitization of insulin signal transduction mechanisms.⁹ Finally, a prolonged exposure of cultured myoblasts to high concentrations of insulin is associated with a reduction of the IR/IRS-1/PI3K/AKT axis activity.³¹ We have demonstrated that prolonged exposure of human umbilical vein endothelial cells to high insulin levels induces a downregulation of the PI3K(AKT/eNOS) axis, paralleled by increased expression of vascular cell adhesion molecule 1 (VCAM-1).³² However, the molecular mechanisms through which hyperinsulinemia begets or worsens insulin resistance are still largely unknown.

HYPERINSULINEMIA AND VASCULAR DISEASE: EVIDENCE FROM THE BENCH

Animal experiments^{33,34} and several *in vitro* studies provided evidence for the biological plausibility of the hypothesis according to which high concentrations of insulin are pro-atherogenic. A link between coronary artery disease and high insulin concentrations was first proposed in the late 1960's¹⁰ and subsequently confirmed (for a review, see Reddy et al.³⁵). *In vitro*, insulin has been shown to stimulate the proliferation and migration of arterial smooth muscle cells in tissue culture preparations²¹ and to induce

monocyte adhesion by increasing the expression of VCAM-1 in endothelial cells.^{22,36,37} VCAM-1 is probably the adhesion molecule most relevant to the development of atherosclerosis.³⁸ Such increased expression in the presence of insulin occurs in a system where insulin may still increase NO bioavailability, which would normally inhibit endothelial activation and atherogenesis.³⁹ Therefore these findings mean that a net effect of high insulin concentrations on endothelial cells is mostly a pro-inflammatory phenotype. We have also shown that these effects can be potentiated by the PI-3-kinase inhibitor wortmannin,²² leading us to postulate that they may be further amplified in conditions of insulin resistance mimicked by wortmannin. Because insulin's ability to induce endothelial activation (for which VCAM-1 expression is both a marker and a mediator) is a plausible explanation for macrovascular disease accompanying hyperinsulinemic conditions, we examined potential molecular mechanisms involved in this specific pattern of endothelial activation. Human umbilical vein endothelial cells were incubated with insulin (0–24 h) ± inhibitors of signaling pathways potentially involved. Incubation of endothelial cells with inhibitors of ERK1/2 failed to affect insulin-induced VCAM-1 expression. Conversely, the p38 MAPK inhibitors SB203580 and SB202190, the protein kinase C (PKC)-β isoform inhibitor LY379196, and—partially—the c-Jun NH₂-terminal kinase inhibitor SP600127, all tested at concentrations around their IC₅₀ for inhibition of substrate phosphorylation, decreased insulin effect on VCAM-1. Gene silencing of p38 MAPK by small interfering RNAs, which suppressed the expression of p38 MAPK, suppressed insulin-stimulated VCAM-1 expression.^{22,36,37} Treatment with insulin also led to activation of NF-κB.^{22,36}

In animals, long-term treatment with insulin has been shown to induce arterial lesions which are rich in lipids and to stimulate wall thickening.¹⁰ The mechanisms responsible for these lesions are increased cholesterol synthesis in the adipose tissue, an imbalanced ratio between receptors for low density lipoproteins and high density lipoproteins (with an increase of the former and a reduction of the latter), and increased low density lipoproteins binding to arterial smooth muscle cells.¹⁰ Insulin is also a growth factor able to promote angiogenesis and smooth muscle cell proliferation by activating the same pathways that are activated by IGFs.⁴⁰ These insulin effects appear to be involved in the retinal neovascularization, playing therefore a key role in the pathophysiology of diabetic microangiopathy and—potentially—atherosclerotic plaque destabilization.^{41–43}

Among the other potential mechanisms by which high levels of insulin favor atherosclerosis, endothelial dysfunction,⁴⁴ and inhibition of macrophage apoptosis are probably also relevant.⁴⁵ Endothelial dysfunction precedes and predicts macrovascular events. In healthy human subjects, the infusion of insulin, attaining pathophysiologically relevant insulin concentrations (>120 pmol/L), can induce severe endothelial dysfunction in large arteries.⁴⁴ The responsible mechanisms likely involve increased intracellular oxidative stress.⁴⁶ *In vitro* studies have shown that insulin stimulates the production of endothelin, the activity of the sympathetic system, and sodium retention.⁴⁷ Furthermore, insulin facilitates smooth muscle cell migration and proliferation, increases the production of extracellular matrix, and induces a pro-coagulant status,⁴⁸ thus also possibly contributing to post-angioplasty restenosis, more frequently observed in diabetic compared with nondiabetic patients.⁴⁹

HYPERINSULINEMIA AND CARDIOVASCULAR DISEASE: EVIDENCE FROM THE BEDSIDE

Despite the strong pathophysiology and experimental evidence for proatherogenic effects of hyperinsulinemia secondary to

insulin resistance, patients with type 2 diabetes very often undergo insulin administration in order to normalize hyperglycemia, free fatty acids levels, and glycated hemoglobin. This treatment often implies insulin administration at very high doses (up to 100 or even 625 U/day),⁵⁰ which causes the appearance of untoward effects, such as weight increase, inhibition of residual endogenous insulin secretion,⁵¹ and the overexpression of the MAPK pathway.¹⁹ However, because of the favorable effects of insulin on blood glucose and on high glucose-mediated deleterious effects of vascular function, the evidence for net deleterious effects of high doses of insulin in diabetes cannot be clear-cut. The DAI (Diabetes and Informatics Study Group, Italian Association of Diabetologists and Italian National Institute of Health) Study,⁵² a multicenter cohort study on the prevalence and incidence of cardiovascular events (myocardial infarction, cerebral thromboembolism, and peripheral amputations) in type 2 diabetic patients, showed that, compared with oral antidiabetic treatment (such as metformin, not entailing increased insulin secretion), insulin treatment was associated with a higher number of cardiovascular events in men and women with type 2 diabetes. In patients with type 2 diabetes, insulin therapy has been shown to independently increase the risk of foot ulcers,⁵³ hypertension,⁵⁴ and high ADP-dependent platelet aggregation.⁵⁵ In the Framingham Heart Study, diabetic patients treated with insulin showed the highest incidence of morbidity and mortality for cardiovascular disease.⁵⁶ In the First National Health and Nutrition Examination Survey, among 7381 observed patients, those with diabetes treated with insulin showed increased risk of all-cause death and death attributable to cardiovascular disease.⁵⁷ In the Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type II Diabetes, patients under intensive insulin therapy showed a 32% incidence of cardiovascular events compared with 21% in patients under standard insulin therapy.⁵⁸ In the Atherosclerotic Risk in Communities study, patients on treatment with sulfonylureas (which also leads to increased insulin concentrations) had a relative risk for cardiovascular disease of 1.82, while patients on insulin therapy had a relative risk of 2.64.⁵⁹ The Kumamoto study, where patients on insulin treatment did not show an increased risk of macrovascular disease, did not contribute substantially to addressing this question because the patients were hypoinsulinemic and nonobese.⁶⁰ A recent study has shown that the mean amplitude of glycemic excursions from continuous glucose monitoring data correlated positively and independently with urinary 8-iso-prostaglandin F_{2α} excretion, a marker of oxidative stress, in patients with poorly controlled diabetes on oral hypoglycemic agents.⁶¹ The authors did not find such associations in insulin-treated type 1 and type 2 diabetic patients, suggesting that insulin treatment itself inhibits oxidative stress in these patients. Yet, insulin effects on cellular homeostasis could also depend on insulin concentrations, since supra-physiological doses of insulin have been shown to induce generation of reactive oxygen species *in vitro*.⁶² Overall, exogenous insulin produces favorable (reduction of hyperglycemia) and adverse (promotion of atherogenesis) effects.⁶³ This is a warning for a less extensive use of insulin in type 2 diabetes. In patients with blood glucose levels >300 mg/dL, an initial insulin administration can decrease glucotoxicity^{50,64,65}; after that, a reduction of insulin resistance by weight reduction, an increase in physical exercise, and the use of insulin sensitizers, such as metformin or the glitazones, would probably be a more rational choice to prevent cardiovascular complications in patients with type 2 diabetes. Of note, 5 large randomized trials of intensive glucose control vs standard therapy in type 2 diabetes have demonstrated no reduction in total or cardiovascular mortality^{58,66–69}; in contrast, such reduction has been found in the EDIC Study⁷⁰ in type 1 diabetes, where insulin resistance is not the primary problem and

insulin treatment replaces a primary failure of insulin production by the pancreatic beta cells.

CONCLUSIONS

Pathophysiological insulin concentrations increase the production of endothelin, proinflammatory cytokines, endothelial leukocyte adhesion molecules, and PAI-1, exerting overall vascular pro-inflammatory effects. Results of *in vitro* and *in vivo* studies point to a pathogenic role of pathophysiological and pharmacological insulin concentrations in vascular disease. Further research on the use of specific inhibitors of the MAPK and PKC pathways, as novel pharmacological agents addressing the pro-atherogenic insulin signaling, are warranted.

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CONFLICTS OF INTEREST

None declared.

REFERENCES

- Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA*. 2002;287:2570–81.
- Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA*. 1990;263:2893–8.
- Cosentino F, Eto M, De Paolis P, Van der Loo B, Bachschmid M, Ullrich V, et al. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoïd profile in human endothelial cells: role of protein kinase C and reactive oxygen species. *Circulation*. 2003;107:1017–23.
- Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc Res*. 2004;63:582–92.
- Vanuzzo D, Pilotto L, Miolo R, Pirelli S. [Cardiovascular risk and cardiometabolic risk: an epidemiological evaluation]. *G Ital Cardiol (Rome)*. 2008;9:S6–17.
- Gupta AK, Poulter NR. The concept of the metabolic syndrome is it dead yet? *J Am Coll Cardiol*. 2010;56:1355–6.
- Mente A, Yusuf S, Islam S, McQueen MJ, Tanomsup S, Onen CL, et al. Metabolic syndrome and risk of acute myocardial infarction: a case-control study of 26,903 subjects from 52 countries. *J Am Coll Cardiol*. 2010;55:2390–8.
- Tenenbaum A, Fisman EZ. "The metabolic syndrome... is dead": these reports are an exaggeration. *Cardiovasc Diabetol*. 2011;10:11.
- Pyörälä K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care*. 1979;2:131–41.
- Stout RW. Insulin and atheroma. 20-yr perspective. *Diabetes Care*. 1990;13:631–54.
- Muntoni S, Draznin B. Effects of chronic hyperinsulinemia in insulin-resistant patients. *Curr Diab Rep*. 2008;8:233–8.
- Mitsuhashi T, Hibi K, Kosuge M, Morita S, Komura N, Kusama I, et al. Relation between hyperinsulinemia and nonculprit plaque characteristics in nondiabetic patients with acute coronary syndromes. *JACC Cardiovasc Imaging*. 2011;4:392–401.
- Reaven GM, Chang H, Ho H, Jeng CY, Hoffman BB. Lowering of plasma glucose in diabetic rats by antilipolytic agents. *Am J Physiol*. 1988;254:E23–30.
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–97.
- World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Geneva: WHO; 1999.
- Cheal KL, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Ford ES. Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. *Diabetes*. 2004;53:1195–200.
- Russell JC, Proctor SD. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc Pathol*. 2006;15:318–30.
- Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*. 2006;7:85–96.
- Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, et al. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem*. 2002;277:1794–9.
- Ridray S. Hyperinsulinemia and smooth muscle cell proliferation. *Int J Obes Relat Metab Disord*. 1995;19 Suppl 1:S39–51.
- Golovchenko I, Goalstone ML, Watson P, Brownlee M, Draznin B. Hyperinsulinemia enhances transcriptional activity of nuclear factor-kappaB induced by angiotensin II, hyperglycemia, and advanced glycosylation end products in vascular smooth muscle cells. *Circ Res*. 2000;87:746–52.
- Madonna R, Pandolfi A, Massaro M, Consoli A, De Caterina R. Insulin enhances vascular cell adhesion molecule-1 expression in human cultured endothelial cells through a pro-atherogenic pathway mediated by p38 mitogen-activated protein-kinase. *Diabetologia*. 2004;47:532–6.
- Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca²⁺ but requires phosphorylation by Akt at Ser(1179). *J Biol Chem*. 2001;276:30392–8.
- Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest*. 2000;105:311–20.
- Wang CC, Goalstone ML, Draznin B. Molecular mechanisms of insulin resistance that impact cardiovascular biology. *Diabetes*. 2004;53:2735–40.
- Combettes-Souverein M, Issad T. Molecular basis of insulin action. *Diabetes Metab*. 1998;24:477–89.
- Cheatham B, Kahn CR. Insulin action and the insulin signaling network. *Endocr Rev*. 1995;16:117–42.
- Araki E, Lipes MA, Patti ME, Bruning JC, Haag 3rd B, Johnson RS, et al. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature*. 1994;372:186–90.
- Saad MJ, Araki E, Miralpeix M, Rothenberg PL, White MF, Kahn CR. Regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance. *J Clin Invest*. 1992;90:1839–49.
- Anai M, Funaki M, Ogihara T, Terasaki J, Inukai K, Katagiri H, et al. Altered expression levels and impaired steps in the pathway to phosphatidylinositol 3-kinase activation via insulin receptor substrates 1 and 2 in Zucker fatty rats. *Diabetes*. 1998;47:13–23.
- Bertacca A, Ciccarone A, Cecchetti P, Vianello B, Laurenza I, Maffei M, et al. Continually high insulin levels impair Akt phosphorylation and glucose transport in human myoblasts. *Metabolism*. 2005;54:1687–93.
- Madonna R, De Caterina R. Prolonged exposure to high insulin impairs the endothelial PI3-kinase/Akt/nitric oxide signalling. *Thromb Haemost*. 2009;101:345–50.
- Duff GL, Mc MG. The effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit. *J Exp Med*. 1949;89:611–30.
- Cruz Jr AB, Amatuzio DS, Grande F, Hay LJ. Effect of intra-arterial insulin on tissue cholesterol and fatty acids in alloxan-diabetic dogs. *Circ Res*. 1961;9:39–43.
- Reddy KJ, Singh M, Bangit JR, Batsell RR. The role of insulin resistance in the pathogenesis of atherosclerotic cardiovascular disease: an updated review. *J Cardiovasc Med (Hagerstown)*. 2010;11:633–47.
- Madonna R, Massaro M, Pandolfi A, Consoli A, De Caterina R. The prominent role of p38 mitogen-activated protein kinase in insulin-mediated enhancement of VCAM-1 expression in endothelial cells. *Int J Immunopathol Pharmacol*. 2007;20:539–55.
- Madonna R, Massaro M, De Caterina R. Insulin potentiates cytokine-induced VCAM-1 expression in human endothelial cells. *Biochim Biophys Acta*. 2008;1782:511–6.
- Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest*. 2001;107:1255–62.
- De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone Jr MA, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest*. 1995;96:60–8.
- Ruiz-Torres A, Lozano R, Melón J, Carraro R. On how insulin may influence ageing and become atherogenic throughout the insulin-like growth factor-1 receptor pathway: in vitro studies with human vascular smooth muscle cells. *Gerontology*. 2005;51:225–30.
- Zunker P, Schick A, Buschmann HC, Georgiadis D, Nabavi DG, Edelmann M, et al. Hyperinsulinism and cerebral microangiopathy. *Stroke*. 1996;27:219–23.
- Inukai T, Matsutomo R, Tayama K, Aso Y, Takemura Y. Relation between the serum level of C-peptide and risk factors for coronary heart disease and diabetic microangiopathy in patients with type-2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 1999;107:40–5.
- Sugimoto K, Baba M, Suda T, Yasujima M, Yagihashi S. Peripheral neuropathy and microangiopathy in rats with insulinoma: association with chronic hyperinsulinemia. *Diabetes Metab Res Rev*. 2003;19:392–400.
- Arcao G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, et al. Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation*. 2002;105:576–82.

45. Iida KT, Suzuki H, Sone H, Shimano H, Toyoshima H, Yatoh S, et al. Insulin inhibits apoptosis of macrophage cell line, THP-1 cells, via phosphatidylinositol-3-kinase-dependent pathway. *Arterioscler Thromb Vasc Biol.* 2002;22:380–6.
46. Cai XJ, Lister CA, Buckingham RE, Pickavance L, Wilding J, Arch JR, et al. Down-regulation of orexin gene expression by severe obesity in the rats: studies in Zucker fatty and Zucker diabetic fatty rats and effects of rosiglitazone. *Brain Res Mol Brain Res.* 2000;77:131–7.
47. Sarafidis PA, Bakris GL. Review: Insulin and endothelin: an interplay contributing to hypertension development? *J Clin Endocrinol Metab.* 2007;92:379–85.
48. Aronson D, Bloomgarden Z, Rayfield EJ. Potential mechanisms promoting restenosis in diabetic patients. *J Am Coll Cardiol.* 1996;27:528–35.
49. Radke PW, Friesse K, Buhr A, Nagel B, Harland LC, Kaiser A, et al. Comparison of coronary restenosis rates in matched patients with versus without diabetes mellitus. *Am J Cardiol.* 2006;98:1218–22.
50. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. *Ann Intern Med.* 1999;131:281–303.
51. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. *Diabetes Care.* 1992;15:318–68.
52. DAI study group. The DAI prospective study on macrovascular complications in patients with type 2 diabetes. Characteristics of the study population. *Ann Ist Super Sanita.* 2001;37:289–96.
53. Faglia E, Favales F, Quarantiello A. Are insulin-treated type 2 subjects at higher risk for foot ulcers? *Diabetes Care.* 1999;22:1379–80.
54. Bruno G, Merletti F, Boffetta P, Cavallo-Perin P, Barger G, Gallone G, et al. Impact of glycaemic control, hypertension and insulin treatment on general and cause-specific mortality: an Italian population-based cohort of type II (non-insulin-dependent) diabetes mellitus. *Diabetologia.* 1999;42:297–301.
55. Angiolillo DJ, Bernardo E, Ramírez C, Costa MA, Sabaté M, Jimenez-Quevedo P, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. *J Am Coll Cardiol.* 2006;48:298–304.
56. Garcia MJ, McNamara PM, Gordon T, Kannel WB. Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes.* 1974;23:105–11.
57. Kleinman JC, Donahue RP, Harris MI, Finucane FF, Madans JH, Brock DB. Mortality among diabetics in a national sample. *Am J Epidemiol.* 1988;128:389–401.
58. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med.* 2009;360:129–39.
59. Saito I, Folsom AR, Brancati FL, Duncan BB, Chambless LE, McGovern PG. Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Intern Med.* 2000;133:81–91.
60. Shichiri M, Kishikawa H, Ohkubo Y, Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care.* 2000;23 Suppl 2:B21–9.
61. Monnier L, Colette C, Mas E, Michel F, Cristol JP, Boegner C, et al. Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. *Diabetologia.* 2009;53:562–71.
62. Ceolotto G, Bevilacqua M, Papparella I, Baritono E, Franco L, Corvaja C, et al. Insulin generates free radicals by an NAD(P)H, phosphatidylinositol 3'-kinase-dependent mechanism in human skin fibroblasts ex vivo. *Diabetes.* 2004;53:1344–51.
63. BARI 2D Study Group, Frye RL, August P, Brooks MM, Hardison RM, Kelsey SF, et al. A randomized trial of therapies for type 2 diabetes and coronary artery disease. *New Engl J Med.* 2009;360:2503–15.
64. Madonna R, De Caterina R. Cellular and molecular mechanisms of vascular injury in diabetes—part I: pathways of vascular disease in diabetes. *Vascul Pharmacol.* 2011;54:68–74.
65. Madonna R, De Caterina R. Cellular and molecular mechanisms of vascular injury in diabetes—part II: cellular mechanisms and therapeutic targets. *Vascul Pharmacol.* 2011;54:75–9.
66. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352:837–53.
67. Charbonnel B, Dormandy J, Erdmann E, Massi-Benedetti M, Skene A. The prospective pioglitazone clinical trial in macrovascular events (PROactive): can pioglitazone reduce cardiovascular events in diabetes? Study design and baseline characteristics of 5238 patients. *Diabetes Care.* 2004;27:1647–53.
68. Ray KK, Seshasai SR, Wijesuriya S, Sivakumaran R, Netherton S, Preiss D, et al. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. *Lancet.* 2009;373:1765–72.
69. Skyler JS, Bergenstal R, Bonow RO, Buse J, Deedwania P, Gale EA, et al. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. *Circulation.* 2009;119:351–7.
70. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med.* 2005;353:2643–53.