The Insulin-Like Growth Factor System in Vascular Smooth Muscle: Interaction With Insulin and Growth Factors

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Vascular smooth muscle cells (SMCs) occur throughout the vascular tree and have important physiological functions. They are also involved in pathological processes such as development and progression of atherosclerotic lesions, restenosis following angioplasty, and in hypertension. This review is focused on the role of the insulin-like growth factor (IGF) system in proliferation, migration, and hypertrophy of vascular SMCs and its interaction with insulin and other growth factors. The IGF-I receptor is highly expressed in SMCs in intact arteries and in cultured SMCs and is activated by binding of IGF-I to the two α-subunits. Insulin and IGF-II from the circulation can interact with the IGF-I receptor at higher concentrations. Insulin receptors are few or absent in SMCs and circulating insulin concentrations in vivo are probably too low for a direct action of insulin on the IGF-I receptor in SMCs. Receptor activation initiates a number of signal transduction pathways. Increased phosphatidylinositol turnover and calcium mobilization correlates with actin filament reorganization and stimulation of directed migration of the SMC in a gradient of IGF-I. The effects of IGF-I receptor activation on signal transduction pathways (eg, the MAP kinase cascade) implicated in DNA synthesis and proliferation are weak and this correlates with the meager mitogenic activityof IGF-I in SMC. Several components of the IGF-system in SMC are regulated by growth factors such as platelet-derived growth factor (PDGF)-BB and basic fibroblast growth factor (bFGF). IGF-I is downregulated by decreased metabolism (eg, diabetes and fasting) and in these states levels of IGF-I mRNA and IGFBP-2 and -4 mRNA are decreased. On the contrary, SMC hypertrophy is associated with increased levels of IGF-I, IGFBP-2, and IGFBP-4 mRNA. In conclusion, the IGF-system in SMCs is likely to play an important role in SMC migration and the response of the SMCs to metabolic deprivation, and hypertrophy.

VASCULAR SMOOTH MUSCLE CELLS (SMCs) occur throughout the vascular tree, and are the only cell type present in the media of mammalian arteries. In the young growing organism, the vascular SMC proliferates, migrates, and synthesizes extracellular matrix components such as collagen, elastin, fibronectin, and proteoglycans.[1,2] This state of the SMC is referred to as the "synthetic phenotype." As the vessel matures, the vascular SMC enters a quiescent state. The quiescent SMC (the "contractile phenotype") is filled with myofilaments and takes part in regulation of blood pressure and flow. Many pathological conditions are associated with conversion of the quiescent SMC to a more synthetic phenotype that is able to proliferate, migrate, and secrete proteins such as extracellular matrix components in the adult vessel wall. This process has been shown to occur in development and progression of atherosclerotic lesions[3,4] and in restenosis following angioplasty.[5,6] Less well studied are the changes in SMCs that occur during hypertension and hypertrophy.[7] The phenotypic state of the vascular SMC is believed to be induced and regulated by locally produced autocrine/paracrine molecules such as growth factors and cytokines, circulating hormones, and hemodynamic factors.[1,2,3,7] SMCs in the synthetic phenotype can respond to a number of growth-regulatory molecules, growth factors, and chemoattractants, such as platelet-derived growth factor (PDGF) isoforms, basic fibroblast growth factor (bFGF), interleukin-1, transforming growth factor-β, and insulin-like growth factor-I (IGF-I).

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0026-0495/95/4410-4010$03.00/0

This review is focused on the role of the IGF system in proliferation, migration, and hypertrophy of vascular SMCs and its interaction with insulin and other growth factors.

COMPONENTS OF THE IGF SYSTEM IN VASCULAR SMOOTH MUSCLE

Receptors

Specific receptors for IGF-I are abundant in plasma membranes from intact bovine arteries,[8] in cultured rat[9] and bovine[10] aortic SMCs, as well as in human vascular SMCs.[11] Human SMCs from newborn aorta express approximately 15,000 high-affinity IGF-I–binding sites per cell.[12] The K_d for IGF-I interaction with its receptor is in the range of 0.1 nmol/L in vascular SMCs.[9,10,13] The molecular size of the vascular SMC IGF-I receptor α-subunit (135 to 145 kd) has no apparent difference from that of the α-subunit expressed in other tissues.[13-15] The $\alpha_2\beta_2$IGF-I receptor dimer binds only one IGF-I molecule with high affinity, but a second IGF-I molecule can bind to the receptor dimer with a lower affinity, a phenomenom referred to as negative cooperativity (for review, see De Meyts[16]). High-affinity binding of IGF-I to the IGF-I receptor involves cross-linking of two distinct areas of the two α-subunits brought together by one IGF-I molecule in human SMCs and other cell types.[17] This process is similar but not identical to the interaction of insulin with the insulin receptor.[16]

Local Production

IGF-I mRNA is expressed in quiescent vascular SMCs in vivo and in cultured vascular SMCs in vitro.[18-22] Secretion of IGF-I protein has been shown in cell cultures of vascular SMCs.[23,24] Size distributions of SMC IGF-I mRNA transcripts are similar to those in other tissues,[25] with a relative expression of IGF-Ia and IGF-Ib mRNA of 1:10.[26]

Compared with the expression of IGF-I mRNA, expression of IGF-II mRNA is considerably lower in SMCs such

as rat bladder SMCs and rat aortic SMCs, and is under the limit of detection in our solution hybridization assay (H.J. Arnqvist, unpublished, January 1995).

An interesting aspect is that other cell types such as macrophages and monocytes, which are present at high numbers in the vascular wall during formation and progression of atherosclerotic lesions, secrete IGF-I, which may interact with the surrounding SMCs.[27,28] Furthermore, IGF-I is released from the α-granules of platelets following degranulation.[29] Thus, SMCs express IGF-I receptors and the IGF-I gene and produce IGF-I both in the quiescent vascular wall and in cultured vascular SMCs. During pathological conditions, other cell types may contribute to IGF-I interaction with SMCs in a paracrine manner.

Binding Proteins

Six binding proteins for IGFs (IGFBPs) have been characterized.[30] Affinity of the binding proteins for IGFs is on the same order of magnitude (nanomolar range) as the affinity of the receptors.[31] The two major forms of IGFBPs produced and secreted by SMCs are IGFBP-2 and IGFBP-4. Thus, mRNA for IGFBP-2 and IGFBP-4 can be detected in rat aortic SMCs, in intact-rat aorta, and in rat urinary bladder SMCs.[32,33] IGFBP-2 and IGFBP-4 are secreted in the culture medium by porcine vascular SMCs.[34] There is evidence that rat aortic SMCs also can express IGFBP-3.[35]

The function of IGFBPs is still largely unknown. IGFBP-1 has been found to enhance the effect of IGF-I on DNA synthesis under certain conditions in vascular SMCs,[36] an effect that depends on serine phosphorylation of IGFBP-1 and might involve an increased binding of IGF-I to its receptor in the presence of IGFBP-1[37] (for review, see Clemmons[38]). The two SMC-derived IGFBPs do not seem to potently alter the action of IGF-I in vascular SMCs. A high concentration (500 ng/mL) of IGFBP-4 has a slight inhibitory (15% decrease) effect on IGF-I–induced DNA synthesis in vascular SMCs, whereas exogenously added IGFBP-2 is without effects.[39] Some reports show that IGFBPs can exert effects independently of IGF-I. For example, IGFBP-1 has recently been shown to enhance cell migration via interaction with $\alpha_5\beta_1$-integrins.[40] Other effects of IGFBPs may include protection of IGF-I from degradation, or localization of IGF-I at its site of action.[38]

IGF-I IS A WEAK VASCULAR SMOOTH MUSCLE GROWTH FACTOR IN VITRO AND IN VIVO

DNA Synthesis and Proliferation In Vitro

IGF-I stimulates DNA synthesis in vascular SMCs of rat, bovine, porcine, or human origin.[9-12,23] Nanomolar or lower concentrations are required to produce half-maximal stimulation of DNA synthesis. Stimulatory effects of IGF-II and insulin on SMC DNA synthesis can be demonstrated, but the potency of these factors is 10- to 1,000-fold lower than IGF-I, suggesting that the effects are mediated mainly through the IGF-I receptor.[9,10]

Following addition of IGF-I to human newborn aortic SMCs, DNA synthesis in the major population of the responding cells peaks at 14 hours,[12] which indicates that the effect is due to a direct effect of IGF-I receptor stimulation and is not a secondary effect due to secretion of other mitogens. Interestingly, in these cells, the time required for maximal stimulation of DNA synthesis seems to be shorter in a population of SMCs stimulated with IGF-I, as compared with a population stimulated with PDGF-BB.[12] This suggests that the mechanisms of IGF-I–induced DNA synthesis differ from those of PDGF-induced DNA synthesis, or that different populations of cells respond to IGF-I and PDGF-BB. It should be noted that the maximal DNA synthesis induced by IGF-I is in general considerably lower than the maximal DNA synthesis induced by PDGF or serum.[12,13]

The meager stimulation of DNA synthesis by IGF-I seems too weak to produce a measurable and consistent increase in cell number, as has been reported in human aortic SMCs[12] and myometrial SMCs.[41] Accordingly, PDGF-BB increases the cell number of human aortic SMCs 3 days after addition, whereas IGF-I does not.[12] The cell number increase following IGF-I stimulation of rat aortic SMCs is in the range of 10% to 20% as compared with controls, whereas PDGF-BB produces a 60% increase in cell number during the same conditions.[13]

Taken together, these observations show that IGF-I is a weak growth factor when added in the absence of other growth factors to SMCs from various origins.

Lack of Effects on the Mitogen-Activated Protein Kinase Cascade

Many growth factor tyrosine kinase receptors activate a signal transduction pathway that includes recruitment of growth factor receptor–bound protein-2 (GRB-2) and the *ras*-GTP exchange molecule SOS through a *src*-homology 2 (SH2) domain in GRB-2 that interacts with the activated, phosphorylated receptor. These events further lead to conversion of inactive *ras*-GDP to active *ras*-GTP, activation of *raf,* mitogen-activated protein (MAP) kinase kinase, MAP kinase, and transcription factors, and eventually DNA synthesis and proliferation on differentiation.[42] The IGF-I receptor does not seem to bind GRB-2 directly, but receptor activation leads to phosphorylation of insulin receptor substrate (IRS)-1 or -2, which binds various SH2-containing proteins.[43] Thus, the IGF-I receptor may activate the MAP kinase cascade, at least partly, through phosphorylation of IRS, which subsequently binds GRB-2 and activates SOS, *ras,* and downstream enzymes.[43] However, although PDGF evokes a marked activation of MAP kinase kinase and MAP kinase (Erk1 and Erk2) in human SMCs, IGF-I is unable to induce activation of the MAP kinase cascade in these cells.[12] The meager ability of IGF-I to activate the MAP kinase cascade in SMCs is probably not due to the lower number of IGF-I receptors per cell as compared with the number of PDGF receptors per cell, but to a different signaling capacity of IGF-I as compared with PDGF. IRS isoforms expressed in SMCs have not yet been identified, and it is possible that the levels of these signal transduction molecules are low in SMCs, making the subsequent activation of the MAP kinase cascade weak. However, activation of the MAP kinase cascade has been

demonstrated to be 100- to 1,000-fold more efficiently coupled to PDGF receptor activation as compared with IGF-I receptor activation in cells expressing IRS-1,[44] suggesting that the signaling capacity of the PDGF receptor and the IGF-I receptor is truly different in this respect.

DNA Synthesis in Normal and Diabetic Rat Aorta

So far, there are few data on the in vivo effects of IGF-I in SMCs. A commonly used in vivo model of SMC proliferation and migration is balloon injury, in which balloon catheterization of rat aorta or carotid artery injures the endothelium and the underlying SMCs and induces proliferation and migration of medial SMCs, followed by formation of neointima and intimal proliferation.[45,46]

After balloon catheterization of rat aorta, DNA synthesis in the media peaks at 2 days after the injury.[47] This procedure is accompanied by an increased IGF-I receptor gene expression that peaks at 24 hours after injury and increased IGF-I gene expression that peaks at 48 hours after injury.[47] Treatment of rats with exogenous IGF-I (35 nmol/d) following balloon catheterization does not lead to increased DNA synthesis in the media, and doses of 150 nmol/d are required to increase DNA synthesis in medial SMCs (Y. Chen et al, unpublished results). Metabolic deprivation of SMCs in the artery, induced by diabetes on fasting, is associated with decreased IGF-I gene expression,[18] as well as decreased levels of IGFBP-2 and IGFBP-4 mRNA.[33] Levels of IGF-I receptor mRNA do not change during these conditions.[48] Furthermore, balloon catheterization of arteries in diabetic or fasted rats results in significantly lower DNA synthesis as compared with that observed in normal rats.[47,49] Treatment of diabetic rats with 35 nmol/d exogenous insulin decreases blood glucose, increases plasma levels of IGF-I, and significantly increases DNA synthesis in medial SMCs following balloon catheterization.[47] However, treatment of diabetic rats with exogenous IGF-I increases SMC medial DNA synthesis to the same extent as an equimolar dose of insulin without improving the diabetic state.[47] These results suggest that IGF-I has a direct effect on SMC DNA synthesis, whereas insulin may increase DNA synthesis indirectly through increased levels of circulating IGF-I.

Thus, diabetic vascular SMCs are more sensitive to IGF-I than nondiabetic vascular SMCs. This may indicate that IGF-I increases DNA synthesis more efficiently when the SMC is in a metabolically deprived state.

IGF-I INDUCES DIRECTED MIGRATION OF VASCULAR SMOOTH MUSCLE

Migration and Chemotaxis Mediated Through the IGF-I Receptor

Directed migration of vascular SMCs is a key event in formation and progression of atherosclerotic lesions and in restenosis following balloon catheterization. PDGF has long been known to stimulate directed migration and chemotaxis of vascular SMCs,[50] and PDGF-BB is the strongest known chemoattractant for SMCs.

In contrast to its weak mitogenic effects, IGF-I acts as a potent chemoattractant for cultured human SMCs of newborn or adult origin.[12,51] In fact, IGF-I is typically able to induce 50% of the maximal migratory effect observed with PDGF-BB in a Boyden-chamber assay. This is significant, since the PDGF receptor outnumbers the IGF-I receptor by approximately sixfold. The concentration of IGF-I required to produce half-maximal stimulation of migration ranges from 0.08 to 0.2 nmol/L. The effect of IGF-I on migration and chemotaxis is mediated through the IGF-I receptor, with no apparent contribution from the IGF-II receptor or insulin receptors. The migratory effect of IGF-I can be inhibited by an IGF-I receptor–blocking antibody (αIR-3). However, IGF-II and insulin can induce migration through interaction with the IGF-I receptor when present in higher concentrations.[12] Thus, IGF-I is a potent chemoattractant for vascular SMCs, and the effects are comparable to the effects of PDGF-BB. Induction of migration of SMCs can be considered a potentially important biological function of IGF-I in the vascular wall.

Induction of Migration Correlates With Stimulation of Calcium Mobilization and Phosphatidylinositol Turnover

Phospholipase C (PLC) catalyzes hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). Generation of IP_3 leads to mobilization of calcium from intracellular stores, whereas DAG activates different isoenzymes of protein kinase C (PKC). IGF-I is a potent inducer of phosphatidylinositol turnover in human aortic SMCs.[12] Accordingly, an early decrease in PIP_2 lipid mass with a reciprocal increase in DAG lipid mass is observed within 30 seconds following IGF-I stimulation. Consistent with the hydrolysis of PIP_2, IGF-I causes an early dose-dependent increase in intracellular calcium levels in human arterial SMCs. The maximal effects of IGF-I on early PIP_2 hydrolysis, DAG formation, and calcium mobilization are comparable to the maximal effects induced by PDGF-BB.[12] The fact that increased phosphatidylinositol turnover is a common element of signal transduction for chemoattractants in SMCs and that inhibition of PLC activation blocks migration[52] indicates that this signal transduction pathway is involved in migration and chemotaxis. PIP_2 hydrolysis and calcium mobilization induced by IGF-I may result in a localized transient actin filament reorganization (for review, see Stossel[53]). This reorganization of actin filaments is probably required for protrusion of filopodia in the gradient of IGF-I.

INTERACTION BETWEEN THE IGF SYSTEM AND OTHER GROWTH FACTORS

IGF-I Enhances DNA Synthesis Induced by PDGF

Although the mitogenic effect of IGF-I in the absence of other growth factors is weak, the effects of PDGF or bFGF on DNA synthesis can be enhanced by IGF-I in SMCs.[11,13,54-56] Furthermore, the effects of IGF-I and PDGF on induction of c-*myc* are additive in cultured aortic SMCs, suggesting different mechanisms of action of IGF-I and PDGF to induce DNA synthesis and proliferation.

These observations indicate that IGF-I may play a role in

proliferation of SMCs when present with growth factors such as PDGF, and the presence of IGF-I may be required for a maximal proliferative response.

Growth Factor Regulation of IGF-I, IGF-I Receptors, and Binding Proteins

IGF-I gene expression in SMCs is regulated by different growth factors. Results on the effects of growth factors on IGF-I mRNA levels are inconsistent and may reflect different states of the cultured SMCs. PDGF and bFGF have been reported to cause a rapid (within 4 to 6 hours) and sustained (at least 24 hours) decreased IGF-I gene expression and a decreased secretion of IGF-I in rat aortic SMCs.[21,56] The decrease may be mediated through increased levels of intracellular calcium following stimulation with PDGF or bFGF, since an increase of intracellular calcium levels has been found to have a similar effect.[58] Furthermore, protein synthesis seems to be required for the inhibitory effect of PDGF isoforms on IGF-I gene expression.[56] In contrast to these findings, Delafontaine et al[59] reported an increased level of IGF-I mRNA following stimulation of rat aortic SMCs with PDGF isoforms. The discrepancies of these results and other studies may be due to differences in confluency of the SMCs or in the ability of PDGF to induce calcium mobilization and/or protein synthesis. PDGF has also been shown to increase radioimmunoassayable IGF-I in culture media from porcine aortic SMCs.[54]

IGF-I is able to increase levels of IGF-I mRNA in rat aortic SMCs under conditions in which PDGF causes decreased levels of IGF-I mRNA.[21] The effect of IGF-I on IGF-I mRNA is observed 3 hours after addition of IGF-I, and is sustained for up to 24 hours. High concentrations of insulin (10 μmol/L) produce a similar effect on IGF-I mRNA, most likely through interaction with the IGF-I receptor. Growth hormone, a well-known regulator of IGF-I gene expression in other tissues,[60] does not regulate levels of IGF-I mRNA in cultured rat aortic SMCs.[21] Angiotensin II has been found to increase IGF-I mRNA in SMCs.[61] Thus, it appears that mitogenic growth factors, ie, PDGF and bFGF, at least under some conditions, result in decreased levels of IGF-I mRNA, whereas weak growth factors, ie, IGF-I and angiotensin II, result in increased levels of IGF-I mRNA. The biological significance and the cause of the inconsistency of results related to IGF-I gene expression need further study.

The complexity of growth factor regulation of the IGF system in SMCs is further enhanced by the ability of the same growth factors to regulate IGF-I receptors and IGFBPs. Several growth factors modify levels of IGF-I receptors on SMCs, thereby modifying the biological effects of IGF-I. For example, addition of PDGF or FGF to cultured rat aortic SMCs results in an increased number of IGF-I receptors 2 to 3 hours later,[55] in what seems to be a PKC-dependent manner.[62] In other studies, PDGF has been shown to increase promoter activity of the IGF-I receptor gene.[63]

Further, the number of IGF-I receptors per SMC can be increased by angiotensin II, through a mechanism that differs from the mechanism used by PDGF.[62]

Another point of interaction of growth factors with the IGF system in vascular SMCs is regulation of IGFBPs. IGF-I and insulin have been described to increase IGFBP-4 expression (protein and mRNA) in porcine SMCs, and the subsequent binding of IGF-I to IGFBPs results in degradation and appearance of IGFBP-4 proteolytic fragments.[39] Since insulin does not bind to IGFBPs, IGFBP-4 induced by insulin is not degraded. However, no significant effect of IGF-I on levels of IGFBP-4 mRNA were found in another study on rat aortic SMCs.[35] PDGF has been shown to increase IGFBP-4 gene expression and secretion from rat aortic SMCs.[56] Levels of IGFBP-2 do not seem to be regulated to the same extent as IGFBP-4 by growth factors.

THE IGF SYSTEM IN SMOOTH MUSCLE HYPERTROPHY

Metabolic Effects of IGF-I in Smooth Muscle

Hypertrophy is characterized by increased cell size without appreciable increase in cell number. Thus, amino acid uptake and protein synthesis are required for hypertrophy. This is perhaps where IGF-I has its strongest effects as compared with other growth factors. For example, IGF-I is generally a more potent inducer of protein synthesis as compared with DNA synthesis in SMCs. Low (nanomolar) concentrations of IGF-I stimulate amino acid uptake, glucose metabolism, and protein synthesis in intact bovine arteries,[64] and metabolic effects are also induced by IGF-I in cultured vascular SMCs (K.E. Bornfeldt et al, unpublished, January 1995). This suggests that the IGF-I receptor is more efficiently coupled to intracellular signal transduction pathways involved in anabolic effects as compared with those induced by mitogenic growth factors such as PDGF. IGF-I also potently stimulates production of extracellular matrix components in SMCs, such as fibronectin[65] and elastin.[66,67]

IGF-I and IGFBP-2 and IGFBP-4 Are Regulated in Hypertrophy

Hypertrophy of vascular SMCs is associated with changes in the IGF system. In the rat portal vein, expression of IGF-I mRNA and IGF-I protein is increased in hypertrophy induced by partial ligation of the portal vein.[32] Increased levels of IGF-I and IGFBP-4 mRNA are also observed in rat aorta subjected to coarctation.[68,69] In a urinary-bladder model of SMC hypertrophy induced by partial outlet obstruction, the developing hypertrophy is associated with a transient increase in IGF-I mRNA and pronounced, sustained increases of mRNA encoding IGFBP-2 and IGFBP-4.[70] Accordingly, levels of IGFBP-2 protein are increased in hypertrophying bladder (Y. Chen et al, unpublished results, January 1995). The induction of IGF-I mRNA in hypertrophy is approximately sixfold the increases of IGF-I mRNA observed during SMC proliferation induced by balloon catheterization, suggesting that the IGF system plays a more important role in hypertrophy as compared with hyperplasia. IGF-I expression is increased in several other models of hypertrophy, such as during

increased vascular load in rat femoral artery[71] and during hypertrophy of skeletal muscle and heart muscle.[72-74] Regression of hypertrophy after removal of the outlet obstruction of the bladder is associated with decreased levels of mRNA for IGF-I, IGFBP-2, and IGFBP-4, whereas levels of IGF-I receptor mRNA do not change during development and regression of hypertrophy.[70]

Finally, infusion of exogenous IGF-I exaggerates hypertrophy of the kidney in diabetic rats.[75] In summary, the IGF system appears to play an important role in hypertrophying tissues.

INTERACTIONS BETWEEN INSULIN AND THE IGF SYSTEM

Receptor Interaction

Expression of insulin receptors is low or absent in vascular SMCs.[9,10,15] When present in sufficient concentrations, insulin is able to interact with the IGF-I receptor in SMCs. The concentrations of insulin required for IGF-I receptor interaction are 100- to 1,000-fold those of IGF-I in cultured SMCs[9-11,15] and in plasma membranes from arteries.[8] Insulin analogs such as B10 Asp, in which a basic amino acid (histidine) in position B10 has been replaced with an acidic amino acid (aspartic acid), are more potent than native insulin in interacting with the insulin receptor and have a higher mitogenic effect as compared with native insulin.[9,16,76] In rat aortic SMCs, which are devoid of insulin receptors, the insulin analog B10 Asp is more potent than native insulin in interacting with the IGF-I receptor and stimulating DNA synthesis through activation of the IGF-I receptor.[9] This may be explained by the fact that IGF-I has an acidic amino (glutamic acid) acid in the corresponding position, and the single amino acid substitution makes the insulin molecule chemically and biologically more similar to IGF-I. This modification may enhance binding of the B10 Asp insulin analog to the IGF-I receptor in SMCs.

Since such high concentrations of insulin are required for IGF-I receptor interaction, and since SMCs are essentially devoid of insulin receptors, the main effects of insulin on the IGF system in SMCs may be through regulation of circulating IGF-I and IGFBPs.

Insulin Regulation of Circulating IGF-I and IGFBPs

Circulating IGF-I is regulated by growth hormone, insulin, and nutrition.[77,78] In insulin-deficient states such as diabetes, levels of circulating IGF-I are low. The liver is considered the major source of circulating IGF-I, and in the rat expression of IGF-I mRNA in liver is approximately 100-fold higher than in peripheral tissues.[18] However, in man, there is little difference in the tissue level of IGF-I gene expression between liver and peripheral tissues.[79] It is therefore conceivable that the liver is not the main source of circulating IGF-I, and this is also suggested by direct measurements of liver IGF-I production in man.[80] The major part of circulating IGF-I is bound to IGFBP-3, whereas the free biologically active fraction is regulated by IGFBP-1. IGFBP-1 is to a large extent produced in the liver, and is directly and acutely (within hours) regulated by insulin.[77,80] Insulin is thus an important regulator of IGF-I activity. The ratio of portal insulin concentration to peripheral insulin concentration differs between healthy subjects and insulin-treated diabetic patients, and the route of insulin delivery may determine how IGF-I activity is affected by insulin.

Insulin Resistance, Hyperinsulinemia, and Cardiovascular Risk Factors

There is some epidemiological evidence that in nondiabetic and glucose-intolerant individuals, hyperinsulinemia is associated with cardiovascular disease.[81-84] Furthermore, insulin resistance with compensatory hyperinsulinemia is associated with risk factors for cardiovascular disease such as hypertension, dyslipidemia, and decreased fibrinolysis.[85-88] Thus, insulin by itself has been suggested to promote atherosclerosis.[89] However, this association is not strong and consistent,[90] and hyperinsulinemia as a causative factor for atherosclerotic disease has accordingly been questioned.[91,92] Today, no firm evidence on a mechanism for an atherogenic action of insulin exists.[91,93] No consistent direct effect of insulin in concentrations observed in vivo in insulin-resistant states or during insulin treatment has been found in vascular SMCs.[13] Insulin treatment of patients with non–insulin-dependent diabetes mellitus (NIDDM) and secondary treatment failure on oral agents markedly increases peripheral insulin concentrations and at the same time improves both glycemic control and other cardiovascular risk factors such as dyslipidemia and albuminuria.[94-98] Furthermore, insulin treatment of NIDDM patients after an acute myocardial infarction has been shown to reduce mortality.[99] These findings argue against insulin as a cause of cardiovascular morbidity in patients with NIDDM. Increased proinsulin and split-proinsulin concentrations have also been found to correlate with certain cardiovascular risk factors.[100] It is not known if this is a reflection of increased proinsulin release from β cells under stress in patients with varying degrees of insulin resistance, or if it has any pathogenetic meaning. Proinsulin does not seem to affect SMCs directly, except at extremely high concentrations,[13] and no mechanism whereby proinsulin could promote atherosclerosis has been identified.

Endogenous and Exogenous Hyperinsulinemia

In an insulin-resistant patient, the pancreas tries to overcome insulin resistance by increasing insulin production.[85] This increases the supply of insulin to the liver, where a large proportion of it, approximately 50%, is extracted and will also increase peripheral insulin concentrations.[101] Treatment with sulfonylureas also increases insulin supply to the liver (unless it causes blood glucose levels to decrease sufficiently to decrease the glucose stimulus for insulin secretion) and will thereby result in endogenous hyperinsulinemia. In contrast, treatment with subcutaneous insulin in patients with NIDDM primarily results in peripheral hyperinsulinemia, and endogenous insulin secretion is decreased.[102] This means that, for example, fat tissue, vasculature, and liver are exposed to

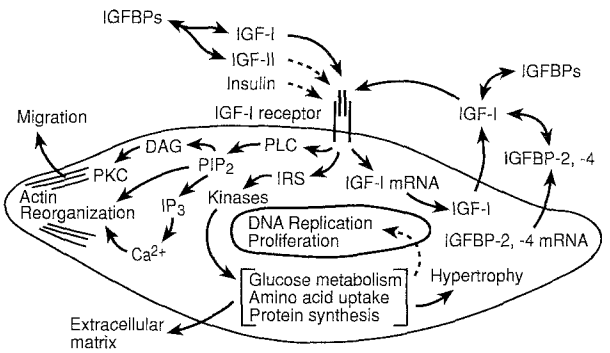
different levels of insulin during endogenous hyperinsulinemia in insulin-resistant states such as NIDDM combined with obesity than in exogenously produced hyperinsulinemia caused by insulin treatment. It is therefore conceivable that endogenous and exogenous hyperinsulinemia may have qualitatively different effects on lipoprotein metabolism and other processes of importance for atherosclerotic disease.

SUMMARY

Our current understanding of some of the actions and biological effects of the IGF system in a SMC is shown schematically in Fig 1. Locally produced IGF-I or IGF-I from the circulation binds to the two α-subunits of the IGF-I receptor, which is highly expressed in SMCs in the intact artery and in culture. Insulin and IGF-II can interact with the IGF-I receptor at high concentrations. Insulin receptors are few or absent in SMCs, and circulating insulin concentrations in vivo are probably too low for a direct action on the IGF-I receptor in SMCs. Receptor activation initiates a number of signal transduction pathways. Increased phosphatidylinositol turnover and calcium mobilization correlates with actin filament reorganization and stimulation of directed migration of the SMC in a gradient of IGF-I. Phosphorylation and activation of various kinases (and IRS-1 or -2) are likely to be important in the increased metabolism (amino acid uptake, protein synthesis, and glucose metabolism) of the SMC. The effects of IGF-I receptor activation on signal transduction pathways (eg, the MAP kinase cascade) associated with DNA synthesis and proliferation are weak, and this correlates with the meager mitogenic activity of IGF-I in SMCs. In contrast to the potent mitogen PDGF, the weaker mitogenic effects of IGF-I may be dependent on the basal metabolic state of the SMC (in culture or in the vascular wall) and may preferentially be due to the ability of IGF-I to potently increase metabolism in SMCs. The increased metabolic state of the SMC following stimulation with IGF-I may thus be responsible for the increased DNA synthesis in cultured cells and in diabetic aortic smooth muscle. Activation of the IGF-I receptor leads to increased levels of IGF-I mRNA, which may contribute to further IGF-I receptor activation through an autocrine loop. Several components of the IGF system in SMCs are regulated by growth factors such as PDGF-BB and bFGF. These include IGF-I expression, IGF-I receptor expression, and IGFBP-4 expression.

The IGF system is downregulated by states characterized by decreased metabolism (eg, diabetes and fasting), and in these states, levels of IGF-I mRNA and IGFBP-2 and -4 mRNA are decreased. In contrast, SMC hypertrophy is associated with increased levels of IGF-I, IGFBP-2, and IGFBP-4 mRNA.

In conclusion, the IGF system in smooth muscle is extensively regulated and is likely to play an important role in SMC migration, response of the SMC to metabolic deprivation, and hypertrophy.



	PDGF-BB	IGF-I	Diabetes/ Fasting	Hypertrophy
IGF-I mRNA	↑/↓	↑	↓	↑
IGF-I	↑/↓	N.A.	↓	↑
IGF-I receptor mRNA	↑	?	—	—
IGF-I receptor	↑	N.A.	—	—
IGFBP-2 mRNA	—	—	↓	↑
IGFBP-2	—	—	?	↑
IGFBP-4 mRNA	↑	↑/—	↓	↑
IGFBP-4	↑	↑/↓	?	?

Fig 1. Components and regulation of the IGF system in SMC. Some of the fundamental mechanisms involved in regulation of the SMC IGF-system are shown in the table. ↑, Increased levels; ↓, decreased levels; —, no significant effect; ?, not determined; N.A., not applicable.

REFERENCES

1. Schwartz SM, Heimark RL, Majesky MW: Developmental mechanisms underlying pathology of arteries. Physiol Rev 70:1177-1209, 1990

2. Campbell GR, Campbell JH: Smooth muscle phenotypic changes in arterial wall homeostasis: Implications for the pathogenesis of atherosclerosis. Exp Mol Pathol 42:139-162, 1985

3. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 362:801-809, 1993

4. Nilsson J: Cytokines and smooth muscle cells in atherosclerosis. Cardiovasc Res 27:1184-1190, 1993

5. Epstein SE, Speir E, Unger EF, et al: The basis of molecular strategies for treating coronary restenosis after angioplasty. J Am Coll Cardiol 23:1278-1288, 1994

6. Ferns GAA, Stewart-Lee AL, Änggård EE: Arterial response to mechanical injury: Balloon catheter de-endothelialization. Atherosclerosis 92:89-104, 1992

7. Pauletteo P, Sarazani R, Rappelli A, et al: Differentiation and growth of vascular smooth muscle cells in experimental hypertension. Am J Hypertens 7:661-674, 1994

8. Bornfeldt KE, Arnqvist HJ, Dahlkvist HH, et al: Characterization of receptors for insulin-like growth factor-I in plasma membranes isolated from bovine mesenteric arteries. Acta Endocrinol (Copenh) 177:428-434, 1988

9. Bornfeldt KE, Gidlöf R, Wastesson Ä, et al: Binding and biological effects of insulin, insulin analogues and insulin-like growth factors in rat aortic smooth muscle cells. Comparison of

maximal growth promoting activities. Diabetologia 34:307-313, 1991

10. King GL, Goodman AD, Buzney S, et al: Receptors and growth promoting effects of insulin and insulin-like growth factor on cells from bovine retinal capillaries and aorta. J Clin Invest 75:1028-1036, 1985

11. Banskota NK, Taub R, Zellner K, et al: Characterization of induction of protooncogene c-*myc* and cellular growth in human vascular smooth muscle cells by insulin and IGF-I. Diabetes 38:123-129, 1989

12. Bornfeldt KE, Raines EW, Nakano T, et al: Insulin-like growth factor-I and platelet derived growth factor-BB induce directed migration of human arterial smooth muscle cells via signaling pathways that are distinct from those of proliferation. J Clin Invest 93:1266-1274, 1994

13. Bornfeldt KE, Arnqvist HJ: Actions of insulin-like growth factor I and insulin in vascular smooth muscle: Receptor interaction and growth promoting effects, in Flyvbjerg A, Ørskov H, Alberti KGMM (eds): Growth Hormone and Insulin-Like Growth Factor I in Human and Experimental Diabetes. Chichester, UK, Wiley. 1993, pp 159-191

14. Jialal I, Crettaz M, Hachiya HL, et al: Characterization of the receptors for insulin and insulin like growth factors on micro- and macrovascular tissues. Endocrinology 117:1222-1229, 1985

15. Lee PDK, Hintz RL, Rosenfeldt RG, et al: Presence of insulin like growth factor receptors and lack of insulin receptors on fetal bovine smooth muscle cells. In Vitro Cell Dev Biol 24:921-926, 1988

16. De Meyts P: The structural basis of insulin and insulin-like growth factor-I receptor binding and negative co-operativity, and its relevance to mitogenic versus metabolic signalling. Diabetologia 37:S135-S148, 1994 (suppl 2)

17. Christoffersen CT, Bornfeldt KE, Rotella CM, et al: Negative cooperativity in the insulin-like growth factor-I receptor and a chimeric IGF-I/insulin receptor. Endocrinology 135:472-475, 1994

18. Bornfeldt KE, Arnqvist HJ, Enberg B, et al: Regulation of insulin-like growth factor-I and growth hormone receptor gene expression by diabetes and nutritional state in rat tissues. J Endocrinol 122:651-656, 1989

19. Sarazani R, Brecher P, Chobanian AV: Growth factor expression in aorta of normotensive and hypertensive rats. J Clin Invest 83:1404-1408, 1989

20. Murphy LJ, Ghahary A, Chakrabarti S: Insulin regulation of IGF-I expression in rat aorta. Diabetes 39:657-662, 1990

21. Bornfeldt KE, Arnqvist HJ, Norstedt G: Regulation of insulin-like growth factor-I gene expression by growth factors in cultured vascular smooth muscle cells. J Endocrinol 125:381-386, 1990

22. Delafontaine P, Bernstein KE, Alexander RW: Insulin-like growth factor I gene expression in vascular cells. Hypertension 17:693-699, 1991

23. Clemmons DR, van Wyk JJ: Evidence for a functional role of endogenously produced somatomedinlike peptides in the regulation of DNA-synthesis in cultured human fibroblasts and porcine smooth muscle cells. J Clin Invest 75:1914-1918, 1985

24. Clemmons DR: Variables controlling the secretion of a somatomedin-like peptide by cultured porcine smooth muscle cells. Circ Res 56:418-426, 1985

25. Hoyt EC, van Wyk JJ, Lund PK: Tissue and development specific regulation of a complex family of rat insulin-like growth factor-I messenger ribonucleic acids. Mol Endocrinol 22:1077-1086, 1988

26. Cercek B, Fishbein MC, Forrester JS, et al: Induction of insulin-like growth factor-I mRNA in rat aorta after balloon denudation. Circ Res 66:1755-1760, 1990

27. Rom WN, Basset P, Fells GA, et al: Alveolar macrophages release an insulin-like growth factor I-type molecule. J Clin Invest 82:1685-1693, 1988

28. Kirstein M, Aston C, Hintz R, et al: Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product–modified proteins. J Clin Invest 90:439-446, 1992

29. Karey KP, Sirbasku DA: Human platelet-derived mitogens. II. Subcellular localization of insulin like growth factors I and II by purification and N-α amino acid analysis. Blood 74:1084-1092, 1989

30. Shimasaki S, Ling N: Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). Prog Growth Factor Res 3:243-266, 1991

31. Sara VR, Hall K: Insulin-like growth factors and their binding proteins. Physiol Rev 70:591-614, 1990

32. Chen Y, Bornfeldt KE, Arner A, et al: Increase in insulin-like growth factor-I in hypertrophying smooth muscle. Am J Physiol 266:E224-E229, 1994

33. Chen Y, Arnqvist HJ: Differential regulation of insulin-like growth factor binding protein-2 and -4 mRNA in muscle tissues and liver by diabetes or fasting. J Endocrinol 143:235-242, 1994

34. McCusker RH, Camacho-Hubner C, Clemmons DR: Identification of the types of insulin-like growth factor–binding proteins that are secreted by muscle cells in vitro. J Biol Chem 264:7795-8000, 1989

35. Kamyar A, Pirola CJ, Wang HM, et al: Expression and insulin-like growth factor–dependent proteolysis of insulin-like growth factor–binding protein-4 are regulated by cell confluence in vascular smooth muscle cells. Circ Res 74:576-585, 1994

36. Elgin RG, Busby WH Jr, Clemmons DR: An insulin-like growth factor (IGF) binding protein enhances the response to IGF-I. Proc Natl Acad Sci USA 84:3254-3258, 1987

37. Busby WH Jr, Klapper DG, Clemmons DR: Purification of a 31,000-dalton insulin-like growth factor binding protein from human amniotic fluid: Isolation of two forms with different biological activities. J Biol Chem 263:14203-14210, 1988

38. Clemmons DR: IGF binding proteins and their functions. Mol Reprod Dev 35:368-375, 1993

39. Cohick WS, Gockerman A, Clemmons DR: Vascular smooth muscle cells synthesize two forms of insulin-like growth factor binding proteins which are regulated differentially by the insulin like growth factors. J Cell Physiol 157:52-60, 1993

40. Jones JI, Gockerman A, Busby WH Jr, et al: Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the α5β1 integrin by means of its Arg-Gly-Asp sequence. Proc Natl Acad Sci USA 90:10553-10557, 1993

41. Tang XM, Rossi MJ, Masterson BJ, et al: Insulin-like growth factor-I (IGF-I), IGF-I receptors, and IGF-I action in endometrial stromal and myometrial smooth muscle cells in vitro. Biol Reprod 50:1113-1122, 1994

42. Davies RJ: The mitogen-activated protein kinase signal transduction pathway. J Biol Chem 268:14553-14556, 1993

43. Myers MG Jr, Sun X-J, White M: The IRS-1 signaling system. TIBS 19:289-293, 1994

44. Mastick CC, Kato H, Robert CT Jr, et al: Insulin and insulin-like growth factor-I receptors similarly stimulate deoxyribonucleic acid synthesis despite differences in cellular protein tyrosine phosphorylation. Endocrinology 135:214-222, 1994

45. Goldberg ID, Stemerman MB, Schnipper LE, et al: Vascular smooth muscle cell kinetics: A new assay for studying patterns of cellular proliferation in vivo. Science 205:920-922, 1979

46. Capron L, Jarnet J, Kazandjian S, et al: Growth promoting effect of diabetes and insulin on arteries. Diabetes 35:973-978, 1986

47. Bornfeldt KE, Arnqvist HJ, Capron L: In vivo proliferation of rat vascular smooth muscle in relation to diabetes mellitus,

insulin-like growth factor I and insulin. Diabetologia 35:104-108, 1992

48. Bornfeldt KE, Skottner A, Arnqvist HJ: In vivo regulation of messenger RNA encoding insulin-like growth factor-I (IGF-I) by diabetes, insulin and IGF-I in rat skeletal muscle, heart muscle and vascular smooth muscle. J Endocrinol 135:203-211, 1992

49. Ridray S, Capron L, Heudes D, et al: Effects of fasting and refeeding on the proliferative response of rat aorta to injury. Am J Physiol 261:H190-H195, 1991

50. Grotendorst GR, Chang T, Seppä HEJ, et al: Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. J Cell Physiol 113:261-266, 1982

51. Grant MB, Wargovich TJ, Ellis EA, et al: Localization of insulin-like growth factor I and inhibition of coronary smooth muscle cell growth by somatostatin analogues in human coronary smooth muscle cells. A potential treatment for restenosis. Circulation 89:1511-1517, 1994

52. Kundra V, Escobedo JA, Kazlauskas A, et al: Regulation of chemotaxis by the platelet-derived growth factor-BB. Nature 367:474-476, 1994

53. Stossel TP: On the crawling on animal cells. Science 260:1086-1094, 1993

54. Clemmons DR: Exposure to platelet-derived growth factor modulates the porcine aortic smooth muscle cell response to somatomedin-C. Endocrinology 117:77-83, 1985

55. Pfeifle B, Boeder H, Ditschuneit H: Interactions of receptors for insulin-like growth factor I, platelet-derived growth factor, and fibroblast growth factor in rat aortic cells. Endocrinology 120:2251-2258, 1987

56. Giannella-Neto D, Kamyar A, Sharifi B, et al: Platelet-derived growth factor isoforms decrease insulin-like growth factor I gene expression in rat vascular smooth muscle cells and selectively stimulate the biosynthesis of insulin-like growth factor binding protein 4. Circ Res 71:646-656, 1992

57. Ko Y, Stiebler H, Nuckenig G, et al: Synergistic action of angiotensin II, insulin-like growth factor-I and transforming growth-factor-β on platelet-derived growth factor-BB, basic fibroblast growth factor, and epidermal growth factor–induced DNA synthesis in vascular smooth muscle cells. Am J Hypertens 6:496-499, 1993

58. Hovis JG, Meyer T, Teasdale RM, et al: Intracellular calcium regulates insulin-like growth factor-I messenger ribonucleic acid levels. Endocrinology 132:1931-1938, 1993

59. Delafontaine P, Bernstein KE, Alexander RW: Insulin-like growth factor-I gene expression in vascular cells. Hypertension 17:693-699, 1991

60. Mathews LS, Norstedt G, Palmiter RD: Regulation of insulin-like growth factor I gene expression by growth hormone. Proc Natl Acad Sci USA 83:9343-9347, 1986

61. Delafontaine P, Lou H: Angiotensin II regulates insulin-like growth factor I gene expression in vascular smooth muscle cells. J Biol Chem 268:16866-16870, 1993

62. Ververis JJ, Ku L, Delafontaine P: Regulation of insulin-like growth factor-I receptors on vascular smooth muscle cells by growth factors and phorbol esters. Circ Res 72:1285-1292, 1993

63. Rubini M, Werner H, Gandini E, et al: Platelet-derived growth factor increases the activity of the promoter of the insulin-like growth factor-I (IGF-I) receptor gene. Exp Cell Res 211:374-379, 1994

64. Bornfeldt K, Arnqvist HJ: Effect of insulin-like growth factor I (IGF-I) on metabolism in bovine mesenteric arteries. Acta Physiol Scand 131:563-568, 1987

65. Tamaroglio TA, Lo CS: Regulation of fibronectin by insulin-like growth factor-I in cultured rat thoracic aortic smooth muscle cells and glomerular mesangial cells. Exp Cell Res 215:338-346, 1994

66. Rich CB, Ewton DZ, Martin BM, et al: IGF-regulation of elastogenesis: Comparison of aortic and lung cells. Am J Physiol 263:L276-L282, 1992

67. Wolfe BL, Rich CB, Goud HD, et al: Insulin-like growth factor-I regulates transcription of the elastin gene. J Biol Chem 268:12418-12426, 1993

68. Fath KA, Alexander RW, Delafontaine P: Abdominal coarctation increases insulin-like growth factor I mRNA levels in rat aorta. Circ Res 72:271-277, 1993

69. Anwar A, Delafontaine P: Hypertension increases insulin-like growth factor binding protein-4 mRNA levels in rat aorta. Hypertension 24:679-685, 1994

70. Chen Y, Bornfeldt KE, Arner A, et al: Different regulation of gene expression encoding insulin-like growth factor-I, insulin-like growth factor–binding protein-2 and -4 during smooth muscle hypertrophy. Growth Regul 5:45-52, 1995

71. Hansson H-A, Jennische E, Skottner A: IGF-I expression in blood vessels varies with vascular load. Acta Physiol Scand 129:165-169, 1987

72. DeVol DL, Rotwein P, Sadow JL, et al: Activation of insulin-like growth factor gene expression during work induced skeletal muscle growth. Am J Physiol 259:E89-E95, 1990

73. Wåhlander H, Isgaard J, Jennische E, et al: Left ventricular insulin-like growth factor I increases in early renal hypertension. Hypertension 19:25-32, 1992

74. Russell-Jones DL, Leach RM, Ward JPT, et al: Insulin-like growth factor-I gene expression is increased in the right ventricular hypertrophy induced by chronic hypoxia in the rat. J Mol Endocrinol 10:99-102, 1993

75. Flyvbjerg A, Bornfeldt KE, Ørskov H, et al: Effect of insulin-like growth factor I infusion on renal hypertrophy in experimental diabetes in rats. Diabetologia 34:715-720, 1991

76. Drejer K, Kruse V, Larsen UD, et al: Receptor binding and tyrosine kinase activation by insulin analogues with extreme affinities studied in human hepatoma HepG2 cells. Diabetes 40:1488-1495, 1991

77. Sara V, Hall K: Insulin-like growth factors and their binding proteins. Physiol Rev 70:591-614, 1990

78. Clemmons DR, Underwood LE: Nutritional regulation of IGF-I and IGF binding proteins. Annu Rev Nutr 11:393-412, 1991

79. Möller C, Arner P, Sonnenfeld T, et al: Quantitative comparison of insulin-like growth factor mRNA levels in human and rat tissues analyzed by a solution hybridization assay. J Mol Endocrinol 7:213-222, 1991

80. Brismar K, Fernquist-Forbes E, Wahren J, et al: Effect of insulin on the hepatic secretion of IGFBP-1 and IGF-I in insulin dependent diabetes. J Clin Endocrinol Metab (in press)

81. Pyörälä K: Relationship of glucose intolerance and plasma insulin to the incidence of coronary heart disease: Results from two population studies in Finland. Diabetes Care 2:131-141, 1979

82. Welborn TA, Wearne K: Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care 2:154-160, 1979

83. Fontbonne A, Tchobroutsky G, Eschwege E, et al: Coronary heart disease mortality risk: Plasma insulin level is a more sensitive marker than hypertension or abnormal glucose tolerance in overweight males: The Paris Prospective Study. Int J Obes 12:557-565, 1988

84. Rönnemaa T, Laakso M, Pyörälä K, et al: High fasting plasma insulin is an indicator of coronary heart disease in non–insulin-dependent diabetic patients and nondiabetic subjects. Arterioscler Thromb 11:80-90, 1991

85. Pyörälä K, Laakso M, Uusitupa M: Diabetes and atheroscle-

rosis: An epidermiologic view. Diabetes Metab Rev 3:463-524, 1987

86. Standl E, Janka HU: High serum insulin concentrations in relation to other cardiovascular risk factors in macrovascular disease of type 2 diabetes. Horm Metab Res 15:46-51, 1985 (suppl)

87. Bierman E: Atherogenesis in diabetes. Arterioscler Thromb 12:647-656, 1992

88. Jarrett RJ: Risk factors for coronary heart disease in diabetes mellitus. Diabetes 41:1-3, 1992 (suppl 2)

89. Stout RW: Insulin and atheroma—20-yr perspective. Diabetes Care 13:631-654, 1990

90. Welin L, Eriksson H, Larsson B, et al: Hyperinsulinaemia is not a major coronary risk factor in elderly men. The study of men born in 1913. Diabetologia 35:766-770, 1992

91. Jarrett RJ: In defence of insulin: A critique of syndrome X. Lancet 340:469-471, 1992

92. Liu QZ, Knowler WC, Nelson RG, et al: Insulin treatment, endogenous insulin concentration, and ECG abnormalities in diabetic Pima Indians: Cross-sectional and prospective analyses. Diabetes 41:1141-1150, 1992

93. Lindström T, Arnqvist HJ, von Schenck HH: Effect of conventional and intensified insulin therapy on free-insulin profiles and glycemic control in NIDDM. Diabetes Care 15:27-34, 1992

94. Lindström T, Arnqvist HJ, Olsson AG: Effect of different insulin regimens on plasma lipoprotein and apolipoprotein concentrations in patients with non–insulin-dependent diabetes mellitus. Atherosclerosis 81:137-144, 1990

95. Taskinen M-R, Kuusi T, Helve E, et al: Insulin therapy induces antiatherogenic changes of serum lipoproteins in noninsulin-dependent diabetes. Arteriosclerosis 8:168-177, 1988

96. Lindström T, Olsson AG, von Schenck H, et al: Insulin treatment improves microalbuminuria and other cardiovascular risk factors in patients with type 2 diabetes mellitus. J Int Med 235:253-261, 1994

97. Lindström T, Eriksson P, Olsson AG, et al: Long-term improvement of glycemic control by insulin treatment in NIDDM patients with secondary failure. Diabetes Care 17:719-721, 1994

98. Jain SK, Nagi DK, Slavin BM, et al: Insulin therapy in type 2 diabetic subjects suppresses plasminogen activator inhibitor (PAI-1) activity and proinsulin-like molecules independently of glycaemic control. Diabetic Med 10:27-32, 1993

99. Malmberg K: Myocardial infarction and diabetes mellitus. Doctoral thesis, University of Stockholm, Stockholm, Sweden, 1994

100. Nagi DK, Hendra TJ, Ryle AJ, et al: The relationships of concentrations of insulin, intact proinsulin and 32-33 split proinsulin with cardiovascular risk factors in type 2 (non–insulin-dependent) diabetic subjects. Diabetologia 33:532-537, 1990

101. Polonsky KS, Rubenstein AH: C-peptide as a measure of the secretion and hepatic extraction of insulin–Pitfalls and limitations. Diabetes 33:486-494, 1984

102. Lindström T, Arnqvist HJ, Ludvigsson J, et al: C-peptide profiles in patients with non–insulin-dependent diabetes mellitus before and during insulin treatment. Acta Endocrinol (Copenh) 126:477-483, 1992