

## **Review**

# **Regulation of smooth muscle cell accumulation in diabetes-accelerated atherosclerosis**

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**Summary.** Diabetes leads to accelerated formation/progression of lesions of atherosclerosis. Cardiovascular disease thus develops earlier in people with type 1 or type 2 diabetes compared to people without diabetes, and cardiovascular (macrovascular) disease is the major cause of death in adults with diabetes. The molecular and cellular mechanisms leading to diabetes-accelerated atherosclerosis are not well understood. The arterial smooth muscle cell (SMC), one of the three or four principal cell types in atherosclerosis, has been extensively studied over the years. Proliferation and accumulation of SMCs are believed to play important roles in the progression of macrophage-rich lesions to fibroatheromas. Further progression of these atheromas into complicated vulnerable lesions that are likely to cause the acute clinical symptoms of atherosclerosis (myocardial infarction and stroke) may involve cell death and loss of SMCs from the fibrous cap of the lesion.

Recent animal studies have shown that diabetes causes a marked increase in SMC accumulation and proliferation in atheromas. Hyperglycemia, advanced glycation end-products, insulin and lipid abnormalities associated with the diabetic environment have been suggested to increase SMC accumulation. Indeed, it is becoming increasingly clear that macrovascular disease associated with diabetes is a multifactorial disease. We review the factors and mechanisms that may regulate SMC proliferation and accumulation in different stages of lesion progression in diabetes. We propose that lipid abnormalities associated with diabetes can act in combination with growth factors present in the diabetic environment to increase SMC accumulation and accelerate lesion progression.

**Key words:** Diabetes, Glucose, Growth factors, Macrovascular complications, Non-esterified fatty acids

## **Atherosclerosis and subsequent cardiovascular disease are accelerated by diabetes**

Diabetes is associated with both microvascular and macrovascular complications. While microvascular disease can lead to blindness, renal failure and neuropathies, macrovascular disease leads to stroke, myocardial infarction and amputation of limbs. Macrovascular disease caused by atherosclerosis is more common and occurs at an earlier age in people with type 1 and type 2 diabetes than in the general population (Ruderman and Haudenschild, 1984). It is estimated that 75-80% of adult diabetic patients die from complications of atherosclerosis. Furthermore, although women have a lower susceptibility to cardiovascular disease than men, women with diabetes lose this gender-related protection. Together, there is a 2- to 10-fold increased risk of macrovascular disease in people with diabetes, depending on gender and type of diabetes. The incidence of diabetes is now increasing by epidemic proportions world-wide (Zimmet et al., 2001), and diabetes-accelerated macrovascular disease is predicted to become a major health care issue in the near future. These reports stress the need for studies of the mechanisms responsible for the increased atherosclerosis and subsequent cardiovascular disease in diabetes.

Micro- and macrovascular complications of diabetes appear to be regulated, in part, by distinct mechanisms. The microvascular complications are believed to be due to hyperglycemia-induced damage of microvascular endothelial cells and neuronal cells, leading to increased microvascular permeability and other deleterious events (Brownlee, 2001). Large clinical studies support an important role for hyperglycemia in microvascular complications of diabetes (DCCT, 1993; UKPDS, 1998). Although hyperglycemia may play a role in macrovascular disease, hyperglycemia alone is not sufficient since normalization of glucose levels does not significantly improve macrovascular disease in people with type 1 or type 2 diabetes (DCCT, 1993; UKPDS, 1998).

The cellular and molecular mechanisms responsible for diabetes-accelerated atherosclerosis are not well

understood. Early studies on lesions from humans with and without diabetes showed that the morphology and cellular composition of the lesions are similar in the diabetic and non-diabetic environment (Strandness et al., 1964; Ferrier, 1967). The main difference between people with and without diabetes is the accelerated formation and/or progression of these lesions in diabetes. Clearly, an effort is needed to elucidate what cell types in the lesion are directly affected by the diabetic environment and what cellular responses occur as a consequence of these primary events.

### **The role of the smooth muscle cell in progression of atherosclerotic lesions**

It is generally believed that accumulation of lipid-loaded macrophages in the arterial wall initiates formation of lesions of atherosclerosis. In humans, accumulation of lipid-loaded macrophages is often seen in areas with intimal thickening (smooth muscle cell mass) that has formed in the subendothelial space due to adaptations to local mechanical forces (Stary et al., 1995). In many small animal models, infiltration of monocytes and subsequent activation and differentiation of these cells into lipid-loaded macrophages is seen in areas without preexisting intimal thickening (Ross, 1993). These initial events are followed by an increased accumulation of lipid-loaded macrophages and extracellular lipid, which leads to formation of a lipid core and a lesion, defined by the American Heart Association as an atheroma (Stary et al., 1995). The next step in the sequence of progression of the lesion is increased accumulation of smooth muscle cells (SMCs) in the intima and formation of a fibroatheroma. Finally, the lesion is destabilized, possibly by thinning of the SMC-rich fibrous cap and/or increased macrophage death, events which can lead to plaque rupture, thrombosis, and the acute clinical manifestations of atherosclerosis (Ross, 1993; Glass and Witztum, 2001). The intimal SMC is thought to play important roles in the two latter stages of lesion progression.

### ***The role of the SMC in transition of atheromas to fibroatheromas***

The accumulation of SMCs in atheromas in non-diabetic animal models is generally believed to be due to increased migration of SMCs from the underlying media of the artery into the intima, accompanied by increased proliferation and possibly decreased death/apoptosis. However, when atheromas are formed in areas with a preexisting mass of intimal SMCs, migration may be a minor contributor to the subsequent SMC accumulation. Since no markers of SMC migration are known, the only evidence for SMC migration is the appearance of SMCs in areas that were previously devoid of SMCs. Proliferation of SMCs, on the other hand, can be measured by expression of molecules required for cell cycle progression (such as proliferating cell nuclear

antigen; PCNA) or incorporation of thymidine analogs (such as BrdU) into synthesized DNA. SMC proliferation appears to play a significant role in formation of fibroatheromas, although proliferation rates are much lower *in vivo* than *in vitro* (Gordon and Rekhter, 1997). A variety of molecules have been shown to stimulate proliferation of SMCs. Among the most potent growth factors for SMCs in culture are platelet-derived growth factor B chain homodimer (PDGF-BB) and fibroblast growth factor-2 (FGF-2). Others are relatively weak when added alone, but enhance the effects of stronger mitogens. Insulin-like growth factor I (IGF-I) belongs to the latter group of growth factors (Bornfeldt et al., 1994). SMC proliferation is also regulated by other factors; components of the extracellular matrix, and  $O_2$  tension, to name a few (for review see Berk, 2001). Although these factors stimulate proliferation of cultured SMCs, one should bear in mind that their *in vivo* and *ex vivo* effects may differ. For example, PDGF appears to act as a weak SMC mitogen *in vivo* (Ferns et al., 1991; Jawien et al., 1992).

Advanced lesions with a thick fibrous cap of SMCs are less vulnerable and less likely to rupture and cause clinical symptoms. Thus, it is not clear if SMC accumulation should be viewed as “good” or “bad”, and it is quite likely that “good” or “bad” depends on the stage of the lesion (see below and Schwartz et al., 2000). What is clearer is that accumulation of SMCs in the neointima is an integral part of lesion progression that likely converts a reversible fatty streak into a non-reversible fibroatheroma.

### ***The role of the SMC in progression of fibroatheromas to vulnerable lesions***

Much research has recently focused on the processes that drive formation of vulnerable lesions, which are likely to rupture and cause acute clinical symptoms. A thin fibrous cap and a low number of SMCs versus macrophages are often seen in ruptured lesions. Several studies have shown an increased SMC death/apoptosis in these advanced lesions (Geng and Libby, 1995; Kockx and Herman, 2000; McCarthy and Bennett, 2000), but macrophages also account for a significant part of cell death in such lesions (Tabas, 2000). Loss of SMCs may lead to plaque instability because most of the interstitial collagen fibers, which are important for the strength of the fibrous cap, are produced by SMCs (Kockx and Herman, 2000; Rekhter et al., 2000). Furthermore, increased SMC apoptosis in the arterial wall has recently been shown to induce secretion of pro-inflammatory cytokines and to increase invasion of monocytes, which would contribute to a decreased ratio of SMCs vs macrophages (Schaub et al., 2000). Conversely, it has been shown that treatments that prevent or lead to regression of cardiovascular disease cause an increased SMC involvement, a reduced macrophage involvement (Kockx et al., 1998; Rong et al., 2001), and an increased collagen content in lesions (Aikawa et al., 1998).

However, despite the correlation between loss of SMCs in the fibrous cap and plaque rupture, there is not yet any direct evidence that SMC death indeed causes plaque rupture.

### **What factors contribute to the increased SMC accumulation in atheromas in diabetes?**

We have recently shown, using a new porcine model of diabetes-accelerated atherosclerosis, that diabetes results in a marked accumulation and proliferation (measured as expression of PCNA) of SMCs in fibroatheromas (Suzuki et al., 2001). Several factors associated with diabetes have been proposed to stimulate SMC proliferation. The best-studied factors include glucose, advanced glycation end-products (AGEs), insulin and non-esterified fatty acids. Below we discuss the different factors that may contribute to SMC proliferation in diabetes.

#### **1. High glucose levels**

A large number of studies have examined the effects of high glucose levels on the proliferative capacity of isolated arterial SMCs from various species. The results are contradictory. Whereas several studies have demonstrated that high glucose levels can stimulate SMC proliferation (e.g. Natarajan et al., 1992; Yasunari et al., 1995; Begum and Ragolia, 2000; Watson et al., 2001), other studies have found no stimulatory effect (Sakakibara et al., 1993; Xia et al., 1995; Williams et al., 1997; Suzuki et al., 2001; Indolfi et al., 2001), and yet other studies have shown an inhibitory effect of high glucose levels on SMCs (Peiro et al., 2001). Interpretation of these results is complicated by the facts that SMCs from species that do not develop diabetes-accelerated atherosclerosis (e.g. rats and rabbits) are often used, and that different incubation times and cell culture conditions are used.

To investigate the direct effect of high glucose levels on proliferation of SMCs in a relevant model, we have used a new porcine model of diabetes-accelerated atherosclerosis, in which SMC accumulation and proliferation (PCNA-positive SMCs) are increased in lesions of atherosclerosis (Suzuki et al., 2001). Analysis of the effect of high glucose on SMCs isolated from these animals revealed that high glucose levels (15-50 mM) do not induce proliferation in the presence or absence of growth factors despite an increased rate of the citric acid cycle under high glucose conditions (Suzuki et al., 2001).

It is possible that the contradictory effects of high glucose on proliferation of isolated SMCs can be explained, in part, by indirect effects of high glucose levels. For example, high glucose has been shown to induce expression of growth factors and their receptors in SMCs, including vascular endothelial growth factor; VEGF (Natarajan et al., 1997; Williams et al., 1997), transforming growth factor- $\beta$  (TGF- $\beta$ ), FGF-2 (McClain

et al., 1992) and the PDGF- $\beta$  receptor (Inaba et al., 1996). Thus, under certain conditions, high glucose levels may stimulate proliferation by increasing the autocrine actions of growth factors. This phenomenon, as discussed above, is not consistently observed.

Interestingly, SMCs metabolize glucose largely through glycolysis under aerobic conditions, a process that has been referred to as "aerobic glycolysis" (Morrison et al., 1976; Morrison et al., 1978; Paul et al., 1979; Suzuki et al., 2001). We have recently shown that glucose consumption in isolated proliferating human SMCs is high ( $\sim 0.6$  pmoles glucose/h/cell) compared to that of many other cell types (Renard and Bornfeldt, 2001). The high rate of glucose consumption in SMCs is similar to that of malignant cells that are known to exhibit a  $\sim 10$ -fold increase in glucose uptake and high glycolysis under aerobic conditions (Skøyum et al., 1997; Dang and Semenza, 1999). Thus, in this respect, normal human SMCs in culture resemble tumor cells. It is possible that the increased energy required in cells with a high proliferative capacity is supplied primarily by glycolysis rather than by oxidative glucose breakdown. In this context, it has been suggested that glycolysis, although highly unfavorable for the cell in terms of ATP production, may serve as a protective strategy to minimize oxidative stress (Brand and Hermfisse, 1997). Since many of the effects of high glucose have been attributed to an increased oxidative stress, it is possible that SMCs, with their high rate of glycolysis, are largely protected against the oxidative stress induced by high glucose conditions in other cell types, such as endothelial cells (Nishikawa et al., 2000; Brownlee, 2001). Furthermore, the rate of glycolysis in SMCs may be suppressed by an intact endothelium (Morrison et al., 1976), and endothelial damage may therefore contribute to this process.

No *in vivo* studies have investigated whether hyperglycemia may directly stimulate SMC proliferation and accumulation in atheromas. However, some studies have determined cell proliferation in response to balloon catheter injury in diabetic animals. Although the cellular and molecular mechanisms of SMC proliferation in balloon injury models may be quite different from those mediating SMC proliferation and accumulation in atherosclerosis, these studies nevertheless give us important information. For example, a recent study compared the proliferative response of medial and intimal cells (the proliferating cell type(s) was not identified) of the carotid artery after balloon injury in rat models of type 1 and type 2 diabetes (Park et al., 2001). The results showed an increased cell proliferation in the model of type 2 diabetes (obese Zucker rats) but not in the model of type 1 diabetes (streptozotocin-treated rats), indicating that lipids or other factors associated with type 2 diabetes, but not hyperglycemia, may stimulate arterial proliferation (Park et al., 2001). Other studies have shown that balloon injury of arteries of type 1 diabetic rats or rabbits results in decreased DNA synthesis and BrdU incorporation in SMCs compared to

non-diabetic controls, despite the presence of marked hyperglycemia (Bornfeldt et al., 1992; Schiller and McNamara, 1999; Dahlfors et al., 2000). The lack of direct effects of hyperglycemia on SMC proliferation *in vivo* is in agreement with studies that show no increase in neointimal thickness in balloon injured arteries of streptozotocin-diabetic rats 2 weeks after injury (Aoki et al., 2001). Similar studies have been performed in humans subjected to percutaneous transluminal coronary angioplasty (PTCA). PTCA is often associated with an increased mortality of patients with diabetes compared to patients without diabetes. It is uncertain whether this increased mortality is associated with increased SMC accumulation/proliferation. It has been suggested that it is due to elastic recoil of the artery rather than increased cell proliferation (Moreno et al., 1999). Nevertheless, improved glycemic control does not appear to alter the long term outcome of angioplasty in people with diabetes (Hasdai et al., 2001).

Together, the lack of consistent growth-stimulatory effects of high glucose levels in isolated SMCs or arterial tissue and the lack of effects of hyperglycemia on SMC proliferation *in vivo* following balloon injury argue that hyperglycemia does not directly stimulate SMC proliferation and accumulation in diabetes.

## 2. Advanced glycation end-products (AGEs)

Another possibility is that hyperglycemia, through increased formation of AGEs, could contribute to SMC accumulation and proliferation. AGEs can be formed extracellularly and intracellularly as a result of nonenzymatic glycation of proteins under hyperglycemic conditions, and levels of AGEs are elevated in diabetes (Schmidt and Stern, 2001; Vlassara and Palace, 2002). Some AGEs may also be formed as a result of peroxidation of polyunsaturated fatty acids in triglycerides and phospholipids. When derived from lipids, these compounds should be termed advanced lipoxidation end-products (ALEs); or, if their origin is uncertain, AGE/ALEs (Baynes and Thorpe, 2000). AGEs may affect cells by at least three different mechanisms. First, AGE-modification of long-lived extracellular proteins, such as collagen, leads to formation of cross-links and thus to a more rigid scaffold surrounding the SMCs. This modification of the extracellular matrix may affect SMC proliferation (Iino et al., 1996).

Second, extracellular AGEs bind to a number of cellular proteins; the components of the AGE-receptor complex p60, p90 and galectin-3 (Li et al., 1996), receptor for AGEs (RAGE; Neeper et al., 1992), CD36 (Ohgami et al., 2001) and the macrophage scavenger receptor types I and II (Takata et al., 1988; Araki et al., 1995). To date, the only well-defined AGE receptor with signaling capacities is RAGE, which appears to mediate intracellular signal transduction mainly through an increased intracellular oxidative stress (Lander et al., 1997; Wautier et al., 2001). RAGE recognizes and can be activated by a large number of interesting ligands (for

review see Schmidt and Stern, 2001). These include AGE and ALE adducts of proteins (e.g. N<sup>ε</sup>-(carboxymethyl)lysine [CML]), amphoterin, amyloid peptides, S100 polypeptides, and transthyretin. It has been shown that blocking the interaction of RAGE with its ligands decreases atherosclerosis and expression of adhesion molecules in blood vessels of diabetic mice (Park et al., 1998; Kislinger et al., 2001). At present, it is unknown if the effects of RAGE blockade on atherogenesis are due to inhibition of AGE signaling or signaling induced by one or several other RAGE ligands. The S100 polypeptides are especially interesting candidates because these peptides have proinflammatory actions and are present in lesions of atherosclerosis (Bobryshev et al., 1999; Wendt et al., 2002).

Several studies have investigated the effects of extracellular AGEs on SMC proliferation. As with high glucose, the effects of AGEs on SMC proliferation are unconvincing. Furthermore, since different protocols are used to generate AGEs, the studies are difficult to compare. The extent of modification of the used protein is likely to be of importance for the biological effects of AGEs. AGEs have been found to increase SMC proliferation (Mizutani et al., 2000; Hattori et al., 2002) or have no effect (Iino et al., 1996; Sakata et al., 2000; Renard et al., 2001). One study has demonstrated a biphasic effect of AGEs; a growth-stimulatory effect was seen at 1-10  $\mu\text{g/ml}$  and an inhibitory effect at  $>20 \mu\text{g/ml}$  (Satoh et al., 1997). Our studies on human SMCs have shown no growth-stimulatory effects of AGE-modified albumin or CML-modified albumin over a wide range of concentrations (10 - 1000  $\mu\text{g/ml}$ ) and extents of modification, despite expression of RAGE in these cells (Renard et al., 2001). Furthermore, no growth-stimulatory effect was observed when the RAGE ligands S100 and  $\beta$ -amyloid peptide were used (Renard and Bornfeldt, unpublished observations). A recent *in vivo* study shows that although blockade of RAGE leads to a reduced neointimal formation after balloon injury of the rat carotid artery, proliferation of SMCs was not significantly suppressed (Zhou et al., 2001).

Finally, AGEs can be formed intracellularly from glucose-derived dicarbonyl precursors under high glucose conditions, a process that is faster than the extracellular modification of proteins by glucose (for review see Brownlee, 2001). Intracellular proteins modified by AGEs may have altered function, but the effects of intracellular AGE formation on SMC function are unknown.

Together with the lack of effects of hyperglycemia in the *in vivo* studies discussed above (in which AGE formation is also enhanced), these findings suggest that AGEs are not sufficient to explain the increased SMC proliferation and accumulation seen in fibroatheromas in diabetes.

## 3. Triglycerides and non-esterified fatty acids

Diabetes is associated with elevated levels of plasma triglycerides due to lack of proper insulin signaling in

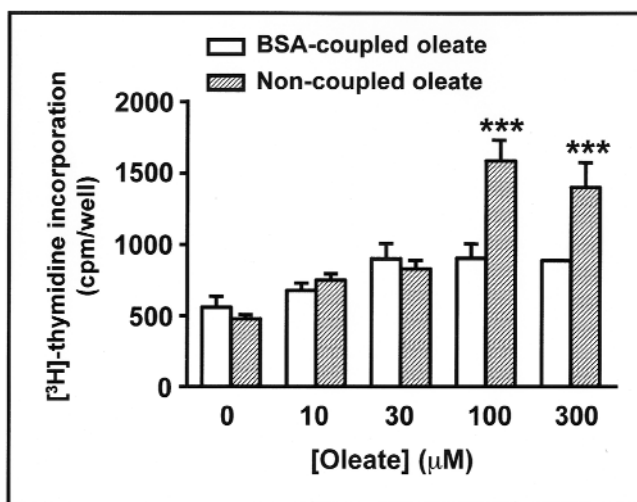
insulin-sensitive tissues (Bianchi et al., 1995; Taskinen, 2001). Hypertriglyceridemia shows a strong correlation with cardiovascular disease in humans (Faergeman, 2000) and is a risk factor for atherosclerosis in diabetes (Semenkovich and Heinecke, 1997). Several animal models of combined atherosclerosis and diabetes also show elevated levels of triglycerides (Dixon et al., 1999; Keren et al., 2000; Gerrity et al., 2001). Although there is a strong correlation between elevated triglyceride levels and macrovascular disease in diabetes, it is not clear to what extent elevated plasma levels of triglycerides contribute to diabetes-accelerated atherosclerosis.

However, evidence that elevated triglycerides in combination with enhanced hydrolysis into non-esterified fatty acids may play an important role in stimulating proliferation and accumulation of lesion SMCs is accumulating. Levels of non-esterified fatty acids within the lesion are likely regulated to a great extent by the activity of lipases, e.g. lipoprotein lipase and secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) present in the lesion. Lipoprotein lipase is the rate-limiting enzyme for hydrolysis of lipoprotein triglycerides (for review see Brunzell, 1995; Mead et al., 1999). Lipoprotein lipase is secreted primarily from muscle and adipose tissue and is then bound to the vascular endothelium via cell surface proteoglycans. In lesions of atherosclerosis, it is also synthesized by macrophages (O'Brien et al., 1992). Interestingly, lipoprotein lipase-deficiency in macrophages has recently been shown to lead to reduced atherosclerosis (Babaev et al., 2000; Clee et al., 2000; Van Eck et al., 2000; Pentikäinen et al., 2002). A role for sPLA<sub>2</sub> activity in atherogenesis has also recently been confirmed in mice overexpressing sPLA<sub>2</sub> (Ivandic et al., 1999). Furthermore, lipoprotein lipase expression has been shown to be increased in macrophages from people with type 2 diabetes (Sartippour and Renier, 2000), and in isolated macrophages exposed to high glucose levels (Sartippour et al., 1998) or to non-esterified fatty acids (Michaud and Renier, 2001).

The most common fatty acids in human plasma are the long-chained saturated palmitate (16:0), and stearate (18:0), the monounsaturated oleate (18:1), the diunsaturated linoleate (18:2) and polyunsaturated arachidonate (20:4). Most of these fatty acids are bound in triglyceride-rich particles in circulation (mainly very low-density lipoproteins; VLDL) and plasma concentrations can vary widely ( $\mu\text{M}$  to mM range). In addition to the elevated levels of circulating triglycerides, diabetes often results in increased levels of circulating non-esterified fatty acids (Erkelend, 1998), which could enter the arterial wall.

A number of studies have examined direct effects of non-esterified fatty acids on proliferation of cultured SMCs from various species. The conditions used in these studies vary, as there are several different approaches to study the effects of non-esterified fatty acids on cells. One approach is to expose the cells to fatty acids in the absence of a carrier protein or without pre-coupling to a carrier protein. In this case, the

effective fatty acid concentration is likely to be similar to the concentration added to the cells, and the concentration of fatty acid in the cellular membrane fraction may even be an order of magnitude higher since most of the fatty acid will bind to the membrane due to its low aqueous solubility (Hamilton and Kamp, 1999). The other approach is to couple the fatty acid to a carrier protein (often fatty acid-free bovine serum albumin; BSA). This method is used because non-esterified fatty acids are complexed to albumin or other carrier proteins in plasma (Hamilton and Kamp, 1999). The number of fatty acid-binding sites on human albumin and BSA has been estimated to  $\sim$  three. If a ratio of fatty acid:BSA of  $\leq$  3:1 is used, the unbound concentration of fatty acid in a water phase is  $<50$  nM for oleate (Richieri et al., 1993). Under these conditions, low amounts of fatty acids enter the cell through passive diffusion or transporters (Hamilton and Kamp, 1999). Thus, the "same" extracellular concentration of any given non-esterified fatty acid can cause dramatically different concentrations in the cellular membranes depending on if it is coupled to a carrier protein or not (Hamilton and



**Fig. 1.** The growth-promoting effect of oleate is modulated by carrier protein-coupling. Porcine SMCs were isolated from the thoracic aorta by an explant method (Suzuki et al., 2001). The cells (50,000 cells/well) were plated in 24-well trays and quiescence was induced by a 2-day incubation in the presence of 1% human plasma-derived serum. The cells were stimulated with or without oleate, at indicated concentrations, that had been coupled to 80  $\mu\text{M}$  bovine serum albumin (BSA) for 1 h at 37  $^{\circ}\text{C}$  before addition to the cells, or with the same concentrations of oleate and BSA without prior coupling. The cells were then incubated for 20 h with the indicated concentrations of oleate/BSA and newly synthesized DNA was labeled with 1  $\mu\text{Ci}/\text{ml}$  [ $^3\text{H}$ ]-thymidine for an additional 3 h. DNA synthesis was measured as trichloroacetic acid-insoluble radioactivity. Basal [ $^3\text{H}$ ]-thymidine incorporation was in the range of 2000 cpm/mg protein. The experiment was repeated four times with similar results. The values are shown as means  $\pm$  SEM of triplicate samples from a representative experiment. Differences between groups were analyzed by two-way analysis of variance (ANOVA). Specific comparison between points was determined by post-hoc comparison using a Bonferroni test for multiple comparisons (Graph Pad Software, San Diego, CA). Levels of significance are denoted by  $p < 0.001$  (\*\*\*).

Kamp, 1999). This is demonstrated by the differences in growth-promoting activities of oleate when added to SMCs before or after coupling BSA. Oleate added to porcine aortic SMCs at concentrations of 100-300  $\mu$ M without prior coupling to BSA (1.3:1 - 4:1 molar ratio fatty acid:BSA) results in a significant increase in DNA synthesis, whereas no mitogenic effect is seen when the same concentrations of oleate are coupled to BSA prior to addition to the SMCs (Fig. 1).

Keeping these methodological considerations in mind, it has been shown that oleate and linoleate exert mitogenic effects on arterial SMCs from various species, whereas stearate, palmitate and arachidonate do not induce proliferation (Lu et al., 1996; Askari et al., 2001). Micromolar concentrations of oleate and linoleate can induce proliferation of isolated SMCs in the absence of other growth factors when added without prior coupling to a carrier protein. Under these conditions, the molecular mechanisms of oleate-induced proliferation have been shown to be mediated by protein kinase C; PKC (Lu et al., 1996), mitogen-activated protein kinase; MAPK/ERK (Lu et al., 1998a) and increased formation of reactive oxygen species (Lu et al., 1998b). The molecular mechanisms of linoleate-induced SMC proliferation under these conditions have been attributed to the conversion of linoleate to the bioactive metabolites hydroperoxyoctadecadienoic acids (HPODEs) and monohydroxyoctadecadienoic acids (HODEs) by lipoxygenases. 13-HPODE has been shown to stimulate proliferation in rat SMCs (Rao et al., 1995), although its mitogenic effects appear to be species-dependent, as porcine SMCs do not proliferate in response to 13-HPODE (Natarajan et al., 2001). Linoleate has also been shown to lead to activation of the transcription factor peroxisome proliferator-activated receptor (PPAR) $\alpha$  and PPAR $\gamma$  (for review see Vamecq and Latruffe, 1999), although there is no evidence that linoleate exerts its mitogenic effects through PPARs in SMCs.

Oleate and linoleate also potentiate the mitogenic effects of more "classic" growth factors, such as angiotensin II (Lu et al., 1996, 1998a,b), endothelin-1 (Kwok et al., 2000) and IGF-I (Askari et al., 2002). This potentiation occurs when the non-esterified fatty acids are added coupled to BSA at a ratio below 3:1 (Askari et al., 2002). Under these conditions, oleate and linoleate alone have little growth-promoting activity. Similarly, under these conditions oleate does not activate the MAPK/ERK pathway (Lu et al., 2000), but does stimulate de novo formation of diacylglycerol (DAG) without a detectable activation of PKC isoforms (Lu et al., 2000; Yu et al., 2001). Oleate-mediated stimulation of DAG levels may be due to the presence of an oleate-dependent phospholipase D, PLD (Kasai et al., 1998) that may be identical to the recently cloned isoform PLD2 (Kim et al., 1999). PLD acts on phospholipids to generate phosphatidic acid that, in turn, is converted to DAG. Oleate has also been shown to inhibit growth-factor-induced DAG kinase  $\alpha$  activation in SMCs (Du et

al., 2001) and since DAG kinase phosphorylates DAG to form phosphatidic acid, this process also leads to increased DAG levels. Furthermore, we have recently shown that inhibition of PLD activity prevents oleate- and linoleate-induced potentiation of the mitogenic effects of IGF-I, and that a DAG kinase inhibitor (R59022) enhances IGF-I-stimulated DNA synthesis in SMCs, similar to the effects of oleate and linoleate (Askari et al., 2002). These findings suggest that PLD and increased intracellular levels of DAG may mediate the growth-promoting effects of low concentrations of oleate and linoleate. However, fatty acids that do not induce SMC proliferation also increase DAG formation. Thus, palmitate and stearate bound to albumin at a physiological ratio increase DAG concentrations in SMCs (Lu et al., 2000; Yu et al., 2001). This raises the interesting possibility that the fatty acid chain composition of DAG is important for its ability to promote SMC proliferation.

Although there is not yet evidence that oleate and linoleate can stimulate SMC proliferation in atheromas, a recent study supports a role for oleate in atherogenesis, as a diet rich in oleate increased atherosclerosis compared to a diet rich in saturated fatty acids (Merkel et al., 2001). In summary, non-esterified fatty acids within the lesion may be important in driving SMC proliferation and accumulation by enhancing the effects of growth factors in atheromas in diabetes.

#### 4. Insulin

Insulin has been suggested to promote atherosclerosis. This is based on the correlation between hyperinsulinemia and cardiovascular disease (Reaven, 1988) and the ability of high concentrations of insulin to promote SMC proliferation (Stout, 1996). However, there is no evidence that insulin directly promotes atherosclerosis. Instead, clinical studies show that insulin therapy is beneficial or has no adverse effect on macrovascular disease and its risk factors (DCCT, 1993; Lindström et al., 1994; Kornowski et al., 1998; Lehto et al., 2000). Furthermore, the growth-promoting effects of insulin on isolated SMCs are meager, and are seen only at high unphysiological concentrations of insulin due to a cross-activation of the IGF-I receptor (Bornfeldt et al., 1991, 1994; Avena et al., 1999). Several *in vivo* studies also support the lack of direct effects of insulin on SMC proliferation after arterial injury (Bornfeldt et al., 1992; Ridray et al., 1992). It is therefore likely that hyperinsulinemia is a marker of insulin resistance and associated lipid abnormalities, and that insulin is unable to directly stimulate SMC proliferation or accumulation in atherosclerotic lesions *in vivo*.

#### 5. Hypertension and the renin-angiotensin system

Type 2 diabetes is often associated with increases in blood pressure, and it has been suggested a large part of

macrovascular complications in people with type 2 diabetes may be attributed to hypertension (Sowers et al., 2001). In people with type 1 diabetes, macrovascular disease is often not seen until microalbuminuria, systolic hypertension (Pinkney et al., 1995) and elevated triglyceride levels (Bianchi et al., 1995) are present. On the other hand, a number of factors, in addition to hypertension, contribute to cardiovascular disease in people with and without diabetes (Sowers et al., 2001). The direct contribution of hypertension to SMC accumulation in diabetes is unknown.

Interestingly, it has recently been shown that infusion of angiotensin II in mice leads to increased atherosclerosis (Daugherty et al., 2000). This effect appears to be independent of changes in blood pressure, and may be due to stimulation of macrophages rather than SMCs (Daugherty et al., 2000). These findings are in line with recent clinical studies that show reduced cardiovascular disease in people with diabetes treated with an ACE inhibitor (HOPE Study Investigators, 2000; see below). Thus, the renin-angiotensin system contributes to macrovascular disease in diabetes through a mechanism(s) that appears to be independent of blood pressure.

## 6. Paracrine factors

It is likely that diabetes-stimulated SMC accumulation and proliferation occur secondary to increased macrophage infiltration into the arterial wall. A variety of growth-regulatory molecules with the ability to stimulate SMCs in a paracrine fashion are released from macrophages. It is thus possible that the increased SMC proliferation seen in fibroatheromas from diabetic animals is due to an increased secretion of growth factors from macrophages (or other cell types) in the lesion, and that macrophages are directly affected by the diabetic environment. For example, AGEs can induce increased expression of at least two SMC growth factors, PDGF (Kirstein et al., 1990) and IGF-I (Kirstein et al., 1992) in monocytes. We have recently shown increased levels of IGF-I immunoreactivity in macrophages in lesions of atherosclerosis from diabetic pigs fed a cholesterol-rich diet compared to non-diabetic animals (Askari et al., 2002).

Perhaps even more interesting is the possibility that the mitogenic actions of growth factors released by macrophages may be enhanced by factors in the diabetic environment, such as non-esterified fatty acids, as discussed above. This is unquestionably an interesting area that requires further investigation.

## What factors may contribute to increased SMC death in vulnerable lesions of atherosclerosis in diabetes?

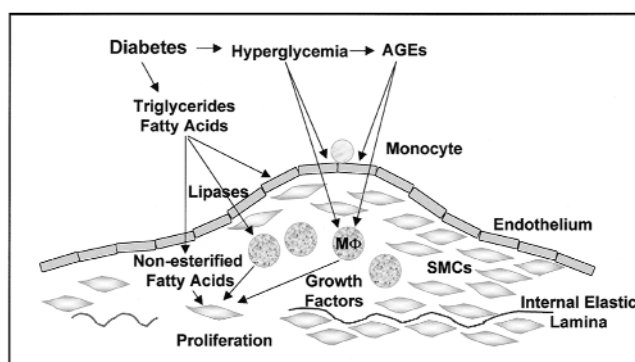
Because death from macrovascular disease is increased in people with diabetes, it is likely that factors in the diabetic environment affect the vulnerability of lesions of atherosclerosis. Loss of SMCs in lesions of

atherosclerosis might contribute to this process, although this hypothesis is supported, so far, only by correlative studies. Some studies suggest that diabetes may be associated with increased SMC death; high glucose levels can increase apoptosis in isolated human SMCs (Peiro et al., 2001) and in rat aorta *in vivo* (Chu et al., 1997). On the other hand, other studies have found that high glucose levels protect against apoptosis in isolated SMCs (Hall et al., 2000). Non-esterified fatty acids may also contribute to death of isolated SMCs under certain conditions (Gouni-Berthold et al., 2001).

In summary, no studies to date have addressed possible direct effects of diabetes on vulnerable lesions, nor has the role of the SMC in these lesions been studied. With the generation of animal models that develop severe vulnerable lesions of atherosclerosis (Rosenfeld et al., 2000; Gerrity et al., 2001), such studies are now feasible.

## What can we learn from intervention trials?

Several major clinical studies have investigated the effects of improved blood glucose, lipid abnormalities and hypertension on macrovascular disease associated



**Fig. 2.** Diabetes-associated increases in non-esterified fatty acids may contribute to SMC proliferation and accumulation in fibroatheromas by synergizing with growth factors present in the atheroma. Diabetes leads to hyperglycemia and increased formation of advanced glycation end-products (AGEs). While hyperglycemia and AGEs may play important roles in affecting the endothelium and/or macrophages, for example by promoting monocyte adherence to the endothelium, macrophage (MΦ) accumulation and secretion of growth factors from macrophages, they do not appear sufficient to directly promote proliferation and accumulation of arterial smooth muscle cells (SMCs). Levels of triglycerides and non-esterified fatty acids are associated with macrovascular complications in both type 1 and type 2 diabetes. Elevated levels of triglycerides and fatty acids in combination with the presence of lipases (e.g. lipoprotein lipase and secretory phospholipase A<sub>2</sub>) in the lesion are likely to lead to increased levels of non-esterified fatty acids within the lesion. Fatty acids have a number of effects on endothelial cells, macrophages and SMCs. In SMCs, the common fatty acids oleate (18:1) and linoleate (18:2) enhance the growth-promoting effects of several growth factors, such as angiotensin II, endothelin-1 and IGF-I. We propose that non-esterified fatty acids synergize with growth factors present in the diabetic environment and that they play an important role in mediating proliferation and accumulation of SMCs in lesions of atherosclerosis in diabetes.

with diabetes. However, one should bear in mind that these studies tell us little about the cellular processes in lesions of atherosclerosis, or the role of the SMC in these lesions. Nevertheless, these studies give us important clues as to which factors contribute to lesion progression in humans with diabetes.

Clinical studies have shown that macrovascular disease of diabetes is a multifactorial disease. Interestingly, improved blood glucose control has been shown to not significantly improve macrovascular complications, whereas microvascular complications are clearly reduced (DCCT, 1993; UKPDS, 1998). Other studies show that, when present, hypertension and elevated lipid levels appear to contribute to a similar extent to macrovascular disease in people with diabetes as in people without diabetes (Huang et al., 2001; Robins, 1991; Steiner, 2001; Yki-Järvinen, 2001).

The effect of hypertension and the renin-angiotensin system on macrovascular disease in people with type 1 and type 2 diabetes was recently studied in the large Heart Outcomes Prevention Evaluation (HOPE) trial. The results from this study show that ramipril, an angiotensin-converting-enzyme (ACE) inhibitor, caused a significant reduction in death from cardiovascular disease in people with diabetes. However, the benefit was not greater in people with diabetes than in people with other risk factors, and did not appear to be due to reduction of blood pressure (HOPE Study Investigators, 2000).

Thus, the renin-angiotensin system, hypertension and lipid abnormalities all contribute significantly to cardiovascular events both in people with and without diabetes.

## Conclusions

Diabetes-accelerated atherosclerosis is a multifactorial disease. One possible reason for this may be that lesion *initiation* and different stages of lesion *progression* are stimulated by different factors in the diabetic environment. We propose that non-esterified fatty acids that enhance the mitogenic effects of growth factors present in the lesion, rather than hyperglycemia or AGEs, contribute to the increased SMC proliferation and accumulation in fibroatheromas in diabetes (Fig. 2).

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## References

Aikawa M., Rabkin E., Okada Y., Voglic S.J., Clinton S.K., Brinckerhoff C.E., Sukhova G.K. and Libby P. (1998). Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen

content of rabbit atheroma: a potential mechanism of lesion stabilization. *Circulation* 97, 2433-2444.

- Aoki M., Morishita R., Hayashi S., Jo N., Matsumoto K., Nakamura T., Kaneda Y. and Ogihara T. (2001). Inhibition of neointimal formation after balloon injury by cilostazol, accompanied by improvement of endothelial dysfunction and induction of hepatocyte growth factor in rat diabetes model. *Diabetologia* 44, 1034-1042.
- Araki N., Higashi T., Mori T., Shibayama R., Kawabe Y., Kodama T., Takahashi K., Shichiri M. and Horiuchi S. (1995). Macrophage scavenger receptor mediates the endocytic uptake and degradation of advanced glycation end products of the Maillard reaction. *Eur. J. Biochem.* 230, 408-415.
- Askari B., Gerrity R.G., Kramer F. and Bornfeldt K.E. (2001). Free fatty acids potentiate the mitogenic effects of insulin-like growth factor-I in arterial smooth muscle cells. *Diabetes* 50, 1393 (Abstr).
- Askari B., Carroll M.A., Capparelli M., Kramer F., Gerrity R.G. and Bornfeldt K.E. (2002). Oleate and linoleate enhance the growth-promoting effects of IGF-I through a phospholipase D-dependent pathway arterial smooth muscle cells. *J. Biol. Chem.* (in press).
- Avena R., Mitchell M.E., Carmody B., Arora S., Neville R.F. and Sidaway A.N. (1999). Insulin-like growth factor-1 receptors mediate intragenicular vascular smooth muscle cell proliferation in response to glucose and insulin not by insulin receptors. *Am. J. Surg.* 178, 156-161.
- Babaev V.R., Patel M.B., Semenkovich C.F., Fazio S. and Linton M.F. (2000). Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in low density lipoprotein receptor-deficient mice. *J. Biol. Chem.* 275, 26293-26299.
- Baynes J.W. and Thorpe S.R. (2000). Glycoxidation and lipoxidation in atherogenesis. *Free. Radic. Biol. Med.* 28, 1708-1716.
- Begum N. and Ragolia L. (2000). High glucose and insulin inhibit VSMC MKP-1 expression by blocking iNOS via p38 MAPK activation. *Am J Physiol. Cell. Physiol.* 278, C81-C91.
- Berk B.C. (2001). Vascular smooth muscle growth: autocrine growth mechanisms. *Physiol. Rev.* 81, 999-1030.
- Bianchi S., Bigazzi R., Quinones Galvan A., Muscelli E., Baldari G., Pecori N., Ciociaro D., Ferrannini E. and Natali A. (1995). Insulin resistance in microalbuminuric hypertension. Sites and mechanisms. *Hypertension* 26, 789-795.
- Bobryshev Y.V., Babaev V.R., Iwasa S., Lord R.S. and Watanabe T. (1999). Atherosclerotic lesions of apolipoprotein E deficient mice contain cells expressing S100 protein. *Atherosclerosis* 143, 451-454.
- Bornfeldt K.E., Gidlöf R.A., Wasteson Å., Lake M., Skottner A. and Arnqvist H.J. (1991). 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.
- Bornfeldt K.E., Arnqvist H.J. and Capron L. (1992). *In vivo* proliferation of rat vascular smooth muscle in relation to diabetes mellitus insulin-like growth factor I and insulin. *Diabetologia* 35, 104-108.
- Bornfeldt K.E., Raines E.W., Nakano T., Graves L.M., Krebs E.G. and Ross R. (1994). 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.
- Brand K.A. and Hermfisse U. (1997). Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *FASEB J.* 11, 388-395.

## *Diabetes and smooth muscle accumulation*

- Brownlee M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813-820.
- Brunzell J.D. (1995). Lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. In: The metabolic and molecular basis of inherited disease. 7th ed. Vol. 2. Scriver C.R., Beaudet A.L., Sly W.S. and Valle D. (eds). McGraw Hill, Inc. New York. pp. 1913-1932.
- Chu Y., Faraci F.M., Ooboshi H. and Heistad D.D. (1997). Increase in TUNEL positive cells in aorta from diabetic rats. *Endothelium* 5, 241-250.
- Clee S.M., Bissada N., Miao F., Miao L., Marais A.D., Henderson H.E., Steures P., McManus J., McManus B., LeBoeuf R.C., Kastelein J.J. and Hayden M.R. (2000). Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis. *J. Lipid Res.* 41, 521-531.
- Dang C.V. and Semenza G.L. (1999). Oncogenic alterations of metabolism. *TIBS* 24, 68-72.
- Dahlfors G., Chen Y., Gustafsson B. and Arnqvist H.J. (2000). Inhibitory effect of diabetes on proliferation of vascular smooth muscle after balloon injury in rat aorta. *Int. J. Exp. Diabetes Res.* 1, 101-109.
- Daugherty A., Manning M.W. and Cassis L.A. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J. Clin. Invest.* 105, 1605-1612.
- DCCT; The Diabetes Control and Complications Trial Research Group. (1993). The effects of intensive treatment of diabetes on the development and progression of long-term complications of insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329, 977-986.
- Dixon J.L., Stoops J.D., Parker J.L., Laughlin M.H., Weisman G.A. and Sturek M. (1999). Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. *Arterioscler. Thromb. Vasc. Biol.* 19, 2981-2992.
- Du X., Jiang Y., Qian W., Lu X. and Walsh J.P. (2001). Fatty acids inhibit growth-factor-induced diacylglycerol kinase  $\alpha$  activation in vascular smooth-muscle cells. *Biochem. J.* 357, 275-282.
- Erkelend D.W. (1998). Diabetic dyslipidaemia. *Eur. Heart J.* 19, H27-H30.
- Faergeman O. (2000). Hypertriglyceridemia and the fibrate trials. *Curr. Opin. Lipidol.* 11, 609-614.
- Ferns G.A., Raines E.W., Sprugel K.H., Motani A.S., Reidy M.A. and Ross R. (1991). Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. *Science* 253, 1129-1132.
- Ferrier T.M. (1967). Comparative study of arterial disease in amputated lower limbs from diabetics and non-diabetics (with special reference to feet arteries). *Med. J. Aust.* 1, 5-11.
- Geng Y.J. and Libby P. (1995). Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1  $\beta$ -converting enzyme. *Am. J. Pathol.* 147, 251-266.
- Gerrity R.G., Natarajan R., Nadler J.L. and Kimsey T. (2001). Diabetes-induced accelerated atherosclerosis in swine. *Diabetes* 50, 1654-1665.
- Glass C.K. and Witztum J.L. (2001). Atherosclerosis. the road ahead. *Cell* 104, 503-516.
- Gordon D. and Reikhter M.D. (1997). The growth of human atherosclerosis: Cell proliferation and collagen synthesis. *Cardiovasc. Pathol.* 6, 103-116.
- Gouni-Berthold I., Berthold H.K., Seul C., Ko Y., Vetter H. and Sachinidis A. (2001). Effects of authentic and VLDL hydrolysis-derived fatty acids on vascular smooth muscle cell growth. *Br. J. Pharmacol.* 132, 1725-1734.
- Hall J.L., Matter C.M., Wang X. and Gibbons G.H. (2000). Hyperglycemia inhibits vascular smooth muscle cell apoptosis through a protein kinase C-dependent pathway. *Circ. Res.* 87, 574-580.
- Hamilton J.A. and Kamp F. (1999). How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? *Diabetes* 48, 2255-2269.
- Hasdai D., Rizza R.A., Grill D.E., Scott C.G., Garratt K.N. and Holmes D.R. Jr. (2001). Glycemic control and outcome of diabetic patients after successful percutaneous coronary revascularization. *Am. Heart J.* 141, 117-123.
- Hattori Y., Suzuki M., Hattori S. and Kasai K. (2002). Vascular smooth muscle cell activation by glycated albumin (amadori adducts). *Hypertension* 39, 22-28.
- HOPE study investigators (2000). Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 355, 253-259.
- Huang E.S., Meigs J.B. and Singer D.E. (2001). The effect of interventions to prevent cardiovascular disease in patients with type 2 diabetes mellitus. *Am. J. Med.* 111, 633-642.
- Iino K., Yoshinari M., Yamamoto M., Kaku K., Doi Y., Ichikawa K., Iwase M. and Fujishima M. (1996). Effect of glycated collagen on proliferation of human smooth muscle cells in vitro. *Diabetologia* 39, 800-806.
- Inaba T., Ishibashi S., Gotoda T., Kawamura M., Morino N., Nojima Y., Kawakami M., Yazaki Y. and Yamada N. (1996). Enhanced expression of platelet-derived growth factor- $\beta$  receptor by high glucose. Involvement of platelet-derived growth factor in diabetic angiopathy. *Diabetes* 45, 507-512.
- Indolfi C., Torella D., Cavuto L., Davalli A.M., Coppola C., Esposito G., Carriero M.V., Rapacciuolo A., Di Lorenzo E., Stabile E., Perrino C., Chieffo A., Pardo F. and Chiariello M. (2001). Effects of balloon injury on neointimal hyperplasia in streptozotocin-induced diabetes and in hyperinsulinemic nondiabetic pancreatic islet-transplanted rats. *Circulation* 103, 2980-2986.
- Ivancic B., Castellani L.W., Wang X.P., Qiao J.H., Mehrabian M., Navab M., Fogelman A.M., Grass D.S., Swanson M.E., de Beer M.C., de Beer F. and Lusis A.J. (1999). Role of group II secretory phospholipase A2 in atherosclerosis: 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A2. *Arterioscler. Thromb. Vasc. Biol.* 19, 1284-1290.
- Jawien A., Bowen-Pope D.F., Lindner V., Schwartz S.M. and Clowes A.W. (1992). Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. *J. Clin. Invest.* 89, 507-511.
- Kasai T., Ohguchi K., Nakashima S., Ito Y., Naganawa T., Kondo N. and Nozawa Y. (1998). Increased activity of oleate-dependent type phospholipase D during actinomycin D-induced apoptosis in Jurkat T cells. *J. Immunol.* 161, 6469-6474.
- Keren P., George J., Shaish A., Levkovitz H., Janakovic Z., Afek A., Goldberg I., Kopolovic J., Keren G. and Harats D. (2000). Effect of hyperglycemia and hyperlipidemia on atherosclerosis in LDL receptor-deficient mice: establishment of a combined model and association with heat shock protein 65 immunity. *Diabetes* 49, 1064-1069.
- Kim J.H., Kim Y., Lee S.D., Lopez I., Arnold R.S., Lambeth J.D., Suh P.G. and Ryu S.H. (1999). Selective activation of phospholipase D2 by unsaturated fatty acid. *FEBS Lett.* 454, 42-46.
- Kirstein M., Brett J., Radoff S., Ogawa S., Stern D. and Vlassara H.

- (1990). Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: role in vascular disease of diabetes and aging. *Proc. Natl. Acad. Sci. USA* 87, 9010-9014.
- Kirstein M., Aston C., Hintz R. and Vlassara H. (1992). 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.
- Kislinger T., Tanji N., Wendt T., Qu W., Lu Y., Ferran L.J. Jr, Taguchi A., Olson K., Bucciarelli L., Goova M., Hofmann M.A., Cataldegirmen G., D'Agati V., Pischetsrieder M., Stern D.M. and Schmidt A.M. (2001). Receptor for advanced glycation end products mediates inflammation and enhanced expression of tissue factor in vasculature of diabetic apolipoprotein E-null mice. *Arterioscler. Thromb. Vasc. Biol.* 21, 905-910.
- Kockx M.M., De Meyer G.R., Buysens N., Knaapen M.W., Bult H. and Herman A.G. (1998). Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. *Circ. Res.* 83, 378-387.
- Kockx M.M. and Herman A.G. (2000). Apoptosis in atherosclerosis: beneficial or detrimental? *Cardiovasc. Res.* 45, 736-746.
- Kornowski R., Mintz G.S., Lansky A.J., Hong M.K., Kent K.M., Pichard A.D., Satler L.F., Popma J.J., Bucher T.A. and Leon M.B. (1998). Paradoxical decreases in atherosclerotic plaque mass in insulin-treated diabetic patients. *Am. J. Cardiol.* 81, 1298-1304.
- Kwok C.F., Shih K.C., Hwu C.M. and Ho L.T. (2000). Linoleic acid and oleic acid increase the endothelin-1 binding and action in cultured rat aortic smooth muscle cells. *Metabolism* 49, 1386-1389.
- Lander H.M., Tauras J.M., Ogiste J.S., Hori O., Moss R.A. and Schmidt A.M. (1997). Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J. Biol. Chem.* 272, 17810-17814.
- Lehto S., Rönkämaa T., Pyörälä K. and Laakso M. (2000). Cardiovascular risk factors clustering with endogenous hyperinsulinemia predict death from coronary heart disease in patients with type II diabetes. *Diabetologia* 43, 148-155.
- Li Y.M., Mitsuhashi T., Wojciechowski D., Shimizu N., Li J., Stitt A., He C., Banerjee D. and Vlassara H. (1996). Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. *Proc. Natl. Acad. Sci. USA* 93, 11047-11052.
- Lindström T., Eriksson P., Olsson A.G. and Arnqvist H.J. (1994). Long-term improvement of glycemic control by insulin treatment in NIDDM patients with secondary failure. *Diabetes Care* 17, 719-721.
- Lu G., Morinelli T.A., Meier K.E., Rosenzweig S.A. and Egan B.M. (1996). Oleic acid-induced mitogenic signaling in vascular smooth muscle cells. A role for protein kinase C. *Circ. Res.* 79, 611-618.
- Lu G., Meier K.E., Jaffa A.A., Rosenzweig S.A. and Egan B.M. (1998a). Oleic acid and angiotensin II induce a synergistic mitogenic response in vascular smooth muscle cells. *Hypertension* 31, 978-985.
- Lu G., Greene E.L., Nagai T. and Egan B.M. (1998b). Reactive oxygen species are critical in the oleic acid-mediated mitogenic signaling pathway in vascular smooth muscle cells. *Hypertension* 32, 1003-1010.
- Lu X., Yang X.Y., Howard R.L. and Walsh J.P. (2000). Fatty acids modulate protein kinase C activation in porcine vascular smooth muscle cells independently of their effect on de novo diacylglycerol synthesis. *Diabetologia* 43, 1136-1144.
- McCarthy N.J. and Bennett M.R. (2000). The regulation of vascular smooth muscle apoptosis. *Cardiovasc. Res.* 45, 747-755.
- McClain D.A., Paterson A.J., Roos M.D., Wei X. and Kudlow J.E. (1992). Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 89, 8150-8154.
- Mead J.R., Cryer A. and Ramji D.P. (1999). Lipoprotein lipase, a key role in atherosclerosis? *FEBS Lett.* 462, 1-6.
- Merkel M., Velez-Carrasco W., Hudgins L.C. and Breslow J.L. (2001). Compared with saturated fatty acids, dietary monounsaturated fatty acids and carbohydrates increase atherosclerosis and VLDL cholesterol levels in LDL receptor-deficient, but not apolipoprotein E-deficient, mice. *Proc. Natl. Acad. Sci. USA* 98, 13294-13299.
- Michaud S.E. and Renier G. (2001). Direct regulatory effect of fatty acids on macrophage lipoprotein lipase: potential role of PPARs. *Diabetes* 50, 660-666.
- Mizutani K., Ikeda K. and Yamori Y. (2000). Resveratrol inhibits AGEs-induced proliferation and collagen synthesis activity in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.* 274, 61-67.
- Moreno P.R., Fallon J.T., Murcia A.M., Leon M.N., Simosa H., Fuster V. and Palacios I.F. (1999). Tissue characteristics of restenosis after percutaneous transluminal coronary angioplasty in diabetic patients. *J. Am. Coll. Cardiol.* 34, 1045-1049.
- Morrison A.D., Berwick L., Orci L. and Winegrad A.I. (1976). Morphology and metabolism of an aortic intima-media preparation in which an intact endothelium is preserved. *J. Clin. Invest.* 57, 650-660.
- Morrison E.S., Frick J. and Kroms M. (1978). The effects of O<sub>2</sub> concentration and albumin on respiration and aerobic glycolysis in rabbit aortic intima-media. *Biochem. Med.* 20, 279-283.
- Natarajan R., Gonzales N., Xu L. and Nadler J.L. (1992). Vascular smooth muscle cells exhibit increased growth in response to elevated glucose. *Biochem. Biophys. Res. Commun.* 187, 552-560.
- Natarajan R., Bai W., Lanting L., Gonzales N. and Nadler J. (1997). Effects of high glucose on vascular endothelial growth factor expression in vascular smooth muscle cells. *Am. J. Physiol.* 273, H2224-H2231.
- Natarajan R., Reddy M.A., Malik K.U., Fatima S. and Khan B.V. (2001). Signaling mechanisms of nuclear factor- $\kappa$ B-mediated activation of inflammatory genes by 13-hydroperoxyoctadecadienoic acid in cultured vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 21, 1408-1413.
- Nepper M., Schmidt A.M., Brett J., Yan S.D., Wang F., Pan Y.C., Elliston K., Stern D. and Shaw A. (1992). Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J. Biol. Chem.* 267, 14998-145004.
- Nishikawa T., Edelstein D., Du X.L., Yamagishi S., Matsumura T., Kaneda Y., Yorek M.A., Beebe D., Oates P.J., Hammes H.P., Giardino I. and Brownlee M. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404, 787-790.
- O'Brien K.D., Gordon D., Deeb S., Ferguson M. and Chait A. (1992). Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* 89, 1544-1550.
- Ohgami N., Nagai R., Ikemoto M., Arai H., Kuniyasu A., Horiuchi S. and Nakayama H. (2001). CD36, a member of the class b scavenger receptor family, as a receptor for advanced glycation end products.

## *Diabetes and smooth muscle accumulation*

- J. Biol. Chem. 276, 3195-3202.
- Park L., Raman K.G., Lee K.J., Lu Y., Ferran L.J. Jr, Chow W.S., Stern D. and Schmidt A.M. (1998). Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nature Med.* 4, 1025-1031.
- Park S.H., Marso S.P., Zhou Z., Foroudi F., Topol E.J. and Lincoff A.M. (2001). Neointimal hyperplasia after arterial injury is increased in a rat model of non-insulin-dependent diabetes mellitus. *Circulation* 104, 815-819.
- Paul R.J., Bauer M. and Pease W. (1979). Vascular smooth muscle: aerobic glycolysis linked to sodium and potassium transport processes. *Science* 206, 1414-1416.
- Peiro C., Lafuente N., Matesanz N., Cercas E., Llergo J.L., Vallejo S., Rodriguez-Manas L. and Sanchez-Ferrer C.F. (2001). High glucose induces cell death of cultured human aortic smooth muscle cells through the formation of hydrogen peroxide. *Br. J. Pharmacol.* 133, 967-974.
- Pentikäinen M.O., Oksjoki R., Öörni K. and Kovanen P.T. (2002). Lipoprotein lipase in the arterial wall: linking LDL to the arterial extracellular matrix and much more. *Arterioscler. Thromb. Vasc. Biol.* 22, 211-217.
- Pinkney J.H., Foyle W.J., Denver A.E., Mohamed-Ali V., McKinlay S. and Yudkin J.S. (1995). The relationship of urinary albumin excretion rate to ambulatory blood pressure and erythrocyte sodium-lithium countertransport in NIDDM. *Diabetologia* 38, 356-362.
- Rao G.N., Alexander R.W. and Runge M.S. (1995). Linoleic acid and its metabolites, hydroperoxyoctadecadienoic acids, stimulate c-Fos, c-Jun, and c-Myc mRNA expression, mitogen-activated protein kinase activation, and growth in rat aortic smooth muscle cells. *J. Clin. Invest.* 96, 842-847.
- Reaven G.M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37, 1595-1607.
- Rekhter M.D., Hicks G.W., Brammer D.W., Hallak H., Kindt E., Chen J., Rosebury W.S., Anderson M.K., Kuipers P.J. and Ryan M.J. (2000). Hypercholesterolemia causes mechanical weakening of rabbit atheroma: local collagen loss as a prerequisite of plaque rupture. *Circ. Res.* 86, 101-108.
- Renard C.B. and Bornfeldt K.E. (2001). Human arterial smooth muscle cells rapidly deplete cell culture media of glucose. *Diabetologia* 44, 1067-1068.
- Renard C.B., Suzuki L.A. and Bornfeldt K.E. (2001). Human arterial smooth muscle cells express RAGE but CML-modified proteins do not induce their proliferation. *Diabetes* 50, 666 (Abstr).
- Richieri G.V., Anel A. and Kleinfeld A.M. (1993). Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* 32, 7574-7580.
- Ridray S., Ktorza A., Picon L. and Capron L. (1992). *In vivo* effect of insulin on the acute proliferative response of the rat aorta to injury. *Arterioscler. Thromb.* 12, 633-638.
- Robins S.J. (2001). Targeting low high-density lipoprotein cholesterol for therapy: lessons from the Veterans Affairs High-density Lipoprotein Intervention Trial. *Am. J. Cardiol.* 88, 12 Suppl 1, 19-23.
- Rong J.X., Li J., Reis E.D., Choudhury R.P., Dansky H.M., Elmaleh V.I., Fallon J.T., Breslow J.L. and Fisher E.A. (2001). Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing macrophage and increasing smooth muscle cell content. *Circulation* 104, 2447-2452.
- Rosenfeld M.E., Polinsky P., Virmani R., Kauser K., Rubanyi G. and Schwartz S.M. (2000). Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. *Arterioscler. Thromb. Vasc. Biol.* 20, 2587-2592.
- Ross R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801-809.
- Ruderman N.B. and Haudenschild C. (1984). Diabetes as an atherogenic factor. *Prog. Cardiovasc. Dis.* 26, 373-412.
- Sakakibara F., Hotta N., Koh N. and Sakamoto N. (1993). Effects of high glucose concentrations and epalrestat on sorbitol and myo-inositol metabolism in cultured rabbit aortic smooth muscle cells. *Diabetes* 42, 1594-1600.
- Sakata N., Meng J. and Takebayashi S. (2000). Effects of advanced glycation end products on the proliferation and fibronectin production of smooth muscle cells. *J. Atheroscler. Thromb.* 7, 169-176.
- Sartippour M.R., Lambert A., Laframboise M., St-Jacques P. and Renier G. (1998). Stimulatory effect of glucose on macrophage lipoprotein lipase expression and production. *Diabetes* 47, 431-438.
- Sartippour M.R. and Renier G. (2000). Upregulation of macrophage lipoprotein lipase in patients with type 2 diabetes: role of peripheral factors. *Diabetes* 49, 597-602.
- Satoh H., Togo M., Hara M., Miyata T., Han K., Maekawa H., Ohno M., Hashimoto Y., Kurokawa K. and Watanabe T. (1997). Advanced glycation endproducts stimulate mitogen-activated protein kinase and proliferation in rabbit vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 239, 111-115.
- Schaub F.J., Han D.K., Liles W.C., Adams L.D., Coats S.A., Ramachandran R.K., Seifert R.A., Schwartz S.M. and Bowen-Pope D.F. (2000). Fas/FADD-mediated activation of a specific program of inflammatory gene expression in vascular smooth muscle cells. *Nature Med.* 6, 790-796.
- Schiller N.K. and McNamara D.B. (1999). Balloon catheter vascular injury of the alloxan-induced diabetic rabbit: the role of insulin-like growth factor-1. *Mol. Cell. Biochem.* 202, 159-167.
- Schmidt A.M. and Stern D.M. (2001). Receptor for AGE (RAGE) is a gene within the major histocompatibility class III region: implications for host response mechanisms in homeostasis and chronic disease. *Front. Biosci.* 6, D1151-D1160.
- Schwartz S.M., Virmani R. and Rosenfeld M.E. (2000). The good smooth muscle cells in atherosclerosis. *Curr. Atheroscler. Rep.* 2, 422-429.
- Semenkovich C.F. and Heinecke J.W. (1997). The mystery of diabetes and atherosclerosis: time for a new plot. *Diabetes* 46, 327-334.
- Skøyum R., Eide K., Berg K. and Rofstad E.K. (1997). Energy metabolism in human melanoma cells under hypoxic and acidic conditions in vitro. *Br. J. Cancer* 76, 421-426.
- Sowers J.R., Epstein M. and Frohlich E.D. (2001). Diabetes, hypertension, and cardiovascular disease: an update. *Hypertension* 37, 1053-1059.
- Stary H.C., Chandler A.B., Dinsmore R.E., Fuster V., Glagov S., Insull W. Jr., Rosenfeld M.E., Schwartz C.J., Wagner W.D. and Wissler R.W. (1995). A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 92, 1355-1374.
- Steiner G. (2001). Treating lipid abnormalities in patients with type 2 diabetes mellitus. *Am. J. Cardiol.* 88, 12 Suppl 1, 37-40.
- Stout R.W. (1996). Hyperinsulinemia and atherosclerosis. *Diabetes* 45 Suppl 3, S45-S46.

- Strandness D.E. Jr, Priest R.E. and Gibbons G.E. (1964). Combined clinical and pathological study of diabetic and nondiabetic peripheral artery disease. *Diabetes* 13, 366-372.
- Suzuki L.A., Poot M., Gerrity R.G. and Bornfeldt K.E. (2001). Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. *Diabetes* 50, 851-860.
- Tabas I. (2000). Cholesterol and phospholipid metabolism in macrophages. *Biochim. Biophys. Acta.* 1529, 164-174.
- Takata K., Horiuchi S., Araki N., Shiga M., Saitoh M. and Morino Y. (1988). Endocytic uptake of nonenzymatically glycosylated proteins is mediated by a scavenger receptor for aldehyde-modified proteins. *J. Biol. Chem.* 263, 14819-14825.
- Taskinen M.R. (2001). Pathogenesis of dyslipidemia in type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 109 (Suppl 2) S180-S188.
- UKPDS Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS33). *Lancet* 352, 837-853.
- Van Eck M., Zimmermann R., Groot P.H., Zechner R. and Van Berkel T.J. (2000). Role of macrophage-derived lipoprotein lipase in lipoprotein metabolism and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 20, E53-E62.
- Vamecq J. and Latruffe N. (1999). Medical significance of peroxisome proliferator-activated receptors. *Lancet* 354, 141-148.
- Vlassara H. and Palace M.R. (2002). Diabetes and advanced glycation endproducts. *J. Intern. Med.* 251, 87-101.
- Watson P.A., Nesterova A., Burant C.F., Klemm D.J. and Reusch J.E. (2001). Diabetes-related changes in cAMP response element-binding protein content enhance smooth muscle cell proliferation and migration. *J. Biol. Chem.* 276, 46142-46150.
- Wautier M.P., Chappey O., Corda S., Stern D.M., Schmidt A.M. and Wautier J.L. (2001). Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am. J. Physiol. Endocrinol. Metab.* 280, E685-E694.
- Wendt T., Bucciarelli L., Qu W., Lu Y., Yan S.F., Stern D.M. and Schmidt A.M. (2002). Receptor for advanced glycation endproducts (RAGE) and vascular inflammation: insights into the pathogenesis of macrovascular complications in diabetes. *Curr. Atheroscler. Rep.* 4, 228-237.
- Williams B., Gallacher B., Patel H. and Orme C. (1997). Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. *Diabetes* 46, 1497-1503.
- Xia P., Kramer R.M. and King G.L. (1995). Identification of the mechanism for the inhibition of Na<sup>+</sup>,K<sup>+</sup>-adenosine triphosphatase by hyperglycemia involving activation of protein kinase C and cytosolic phospholipase A2. *J. Clin. Invest.* 96, 733-740.
- Yasunari K., Kohno M., Yokokawa K., Horio T. and Yoshikawa J. (1995). Aldose reductase inhibitor prevents hyperproliferation and hypertrophy of cultured rat vascular smooth muscle cells induced by high glucose. *Arterioscler. Thromb. Vasc. Biol.* 15, 2207-2212.
- Yki-Järvinen H. (2001). Management of type 2 diabetes mellitus and cardiovascular risk. *Drugs* 60, 975-983.
- Yu H.Y., Inoguchi T., Kakimoto M., Nakashima N., Imamura M., Hashimoto T., Umeda F. and Nawata H. (2001). Saturated non-esterified fatty acids stimulate de novo diacylglycerol synthesis and protein kinase C activity in cultured aortic smooth muscle cells. *Diabetologia* 44, 614-620.
- Zimmet P., Alberti K.G. and Shaw J. (2001). Global and societal implications of the diabetes epidemic. *Nature* 414, 782-787.
- Zhou Z.M., Marso S.P., Schmidt A.M., Stern D.M., Qu W., Forudi F., Wang K., Lincoff A.M. and Topol E.J. (2001). Blockade of receptor for advanced glycation end-products (RAGE) suppresses neointimal formation in diabetic rat carotid injury model. *Circulation* 104 (suppl. 2) 1202 (Abstr).

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