Oxidative Medicine and Cellular Longevity 3:5, 290-303; September/October 2010; © 2010 Landes Bioscience

Pericytopathy

Oxidative stress and impaired cellular longevity in the pancreas and skeletal muscle in metabolic syndrome and type 2 diabetes

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Key words: adult mesenchymal stem cells, antipericyte autoantibodies, apoptosis, beta cells, islet amyloid, islet fibrosis, rarefaction, reactive oxygen species

The pericyte's role has been extensively studied in retinal tissues of diabetic retinopathy; however, little is known regarding its role in such tissues as the pancreas and skeletal muscle. This supportive microvascular mural cell plays an important and novel role in cellular and extracellular matrix remodeling in the pancreas and skeletal muscle of young rodent models representing the metabolic syndrome and type 2 diabetes mellitus (T2DM). Transmission electron microscopy can be used to evaluate these tissues from young rodent models of insulin resistance and T2DM, including the transgenic Ren2 rat, db/db obese insulin resistant—T2DM mouse, and human islet amyloid polypeptide (HIP) rat model of T2DM. With this method, the earliest pancreatic remodeling change was widening of the islet exocrine interface and pericyte hypercellularity, followed by pericyte differentiation into islet and pancreatic stellate cells with early fibrosis involving the islet exocrine interface and interlobular interstitium. In skeletal muscle there was a unique endothelial capillary connectivity via elongated longitudinal pericyte processes in addition to pericyte to pericyte and pericyte to myocyte cell-cell connections allowing for paracrine communication. Initial pericyte activation due to moderate oxidative stress signaling may be followed by hyperplasia, migration and differentiation into adult mesenchymal cells. Continued robust oxidative stress may induce pericyte apoptosis and impaired cellular longevity. Circulating antipericyte autoantibodies have recently been characterized, and may provide a screening method to detect those patients who are developing pericyte loss and are at greater risk for the development of complications of T2DM due to pericytopathy and rarefaction. Once detected, these patients may be offered more aggressive treatment strategies such as early pharmacotherapy in addition to lifestyle changes targeted to maintaining pericyte integrity. In conclusion, we have provided a review of current knowledge regarding the pericyte and novel ultrastructural findings regarding its role in metabolic syndrome and T2DM.

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Submitted: 09/10/10; Revised: 09/16/10; Accepted: 09/16/10
Previously published online:
www.landesbioscience.com/journals/oximed/article/13653
DOI: 10.4161/oxim.3.5.13653

Introduction

Unmitigated oxidative stress as occurs due to the current obesity epidemic with an excess of nutrients and lack of physical exercise resulting in an excess of free fatty acids, triglycerides and glucose associated with the metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) may lead to premature apoptosis and diminished cellular longevity, accelerated aging and accumulation of toxic effects on cellular structure and function.[1] Many cells are vulnerable to this condition; however, one such cell that seems highly vulnerable to the effects of excess reactive oxygen species (ROS) and subject to early vascular injury in this scenario is the microvascular pericyte.

The term pericyte immediately conjures up findings associated with diabetic retinopathy (DR) for most clinicians and researchers. Light microscopic and early transmission electron microscopy (TEM) studies have suggested that pericyte abnormalities play an important role in the pathogenesis of human DR. One of the earliest retinal morphological changes is a selective loss of pericytes, a finding commonly referred to as "pericyte dropout" and/or degenerative "pericyte ghost cells." Other abnormalities associated with pericyte loss in human DR are acellular capillaries with capillary closure, endothelial basement membrane thickening, neovascularization and increased capillary permeability. These pathological abnormalities are thought to be related to microaneurysms, retinal macular edema and hemorrhage, which result in the leading cause of new blindness in the US.[2-4]

The pericyte was initially described by Rouget in 1873 [5] and Zimmerman named this cell the pericyte in 1923,[6] which was commonly referred to as Zimmerman's cell until Kuwabara et al. in 1961 referred to pericytes as intramural cells.[7] Since the pericyte had been previously identified by electron microscopy in 1956 by Farquhar and Hartmann,[8] Ashton and de Oliveira suggested in 1966 that these intramural cells corresponded to the capillary pericyte and therefore both terms currently remain in use.[9]

In 1970, Addison et al. presented a question that still applies today regarding the role of pericyte degeneration. They felt the loss of intramural pericytes appeared to be of crucial importance in DR and felt one of the many questions that would naturally

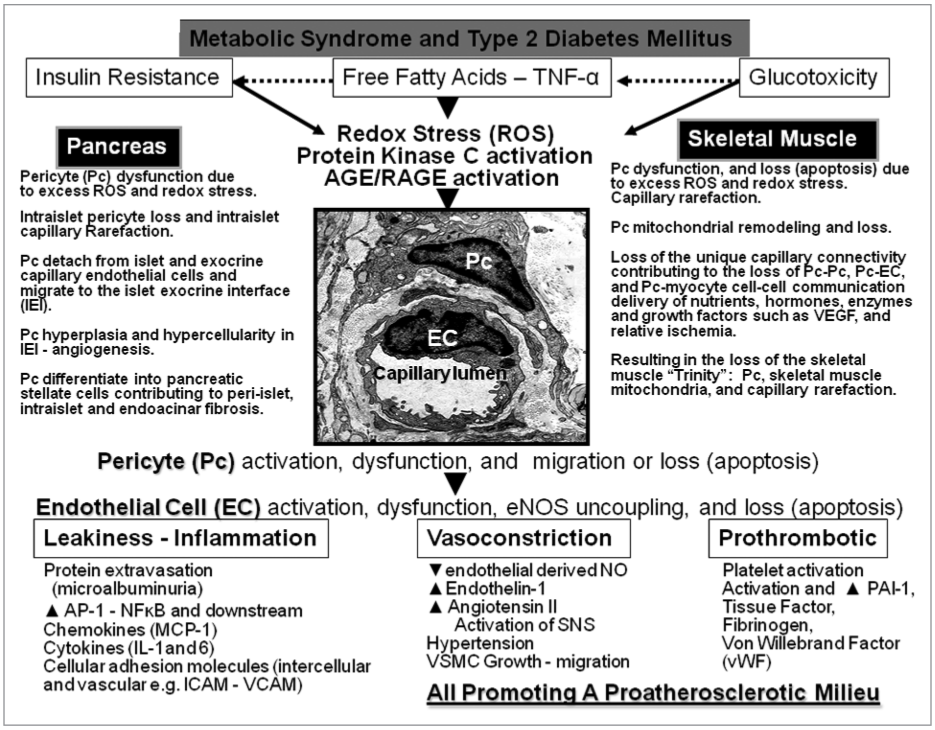

Figure 1. Interactions and pathology of pericytes in the pancreas and skeletal muscle. This image depicts the interaction of insulin resistance, free fatty acids—tumor necrosis factor—alpha (TNFα), glucotoxicity and how they individually and synergistically result in ROS generation, along with multiple other metabolic toxicities to develop the activation of protein kinase C and advanced glycation endproducts (AGE)/receptor for AGE/(RAGE) to result in pericyte (Pc) activation, dysfunction and loss (apoptosis). This is followed by endothelial cell (EC) activation, dysfunction, endothelial nitric oxide synthase (eNOS) uncoupling and loss (apoptosis). Ultrastructural remodeling within these beds result in micro-macrovascular beds developing leakiness, inflammation, vasoconstriction and a prothrombotic milieu. Each of the microvascular beds presented in this review are detailed including the role of the Pc and EC capillary in the pancreatic and skeletal muscle microcirculation in their respective microvascular beds. Additionally, these changes promote a macrovascular proatherosclerotic milieu and thus, these mechanisms contribute to the development of micro-macrovascular disease in metabolic syndrome and type 2 diabetes mellitus.

arise was whether pericytes in other organs in the body would undergo similar degeneration or remodeling changes.[10]

Subsequently, Tilton et al. (1979–1981) were able to demonstrate pericyte degeneration in human skeletal muscle tissue in patients with diabetes utilizing TEM.[11,12]

Many of the early observations regarding retinal pericyte changes in DR have been valuable in understanding the role of the pericyte in the pancreas and skeletal muscle in animal models of the MetS characterized by hypertension (HTN), insulin resistance (IR), oxidative stress (OS), obesity and evolution to T2DM. The following TEM observational findings in pancreatic and skeletal muscle tissue suggest a novel role for this classic microcirculatory mural cell we will refer to as the pericyte. A cartoon outlining and summarizing the interactions and relevant pathophysiology regarding the pericytes role in the pancreas and skeletal muscle is provided (**Fig. 1**).

Vascular Disease in Metabolic Syndrome and Type 2 Diabetes Mellitus

Both micro- and macrovascular disease are increased in metabolic syndrome and T2DM. These diseases are largely triggered by increased redox stress, tissue damage and a response to injury remodeling. These processes initially involve microvessels, which are manifested as microvascular disease and a smoldering more gradual macrovascular disease, which results in accelerated atherosclerotic cardiovascular disease. Macrovascular disease is manifested histologically initially by intimal and medial layers and eventual adventitial remodeling resulting in the vulnerable atherosclerotic plaque prone to rupture and constrictive narrowing. Increased macrovascular disease is strongly associated with redox stress, endothelial dysfunction, atherogenic-diabetic dyslipidemia (increased small low density lipoprotein particles, decreased high density lipoproteins and elevated triglycerides and free fatty acids), hypertension, hypercoagulability (impaired fibrinolysis and platelet hyperaggregability), autonomic neuropathy and eventual glucotoxicity. This ultimately results in ischemia and thrombosis and an associated increased morbidity and mortality. Microvascular disease is manifested histologically by microvascular basement membrane thickening, capillary closure and capillary rarefaction and clinically by impaired endothelial dysfunction and associated endothelial nitric oxide (eNOS) uncoupling. This endothelial dysfunction is widespread in the metabolic syndrome and T2DM and may be clinically detected via flow mediated dilation studies with plethysmography and ultrasound.[13-15]

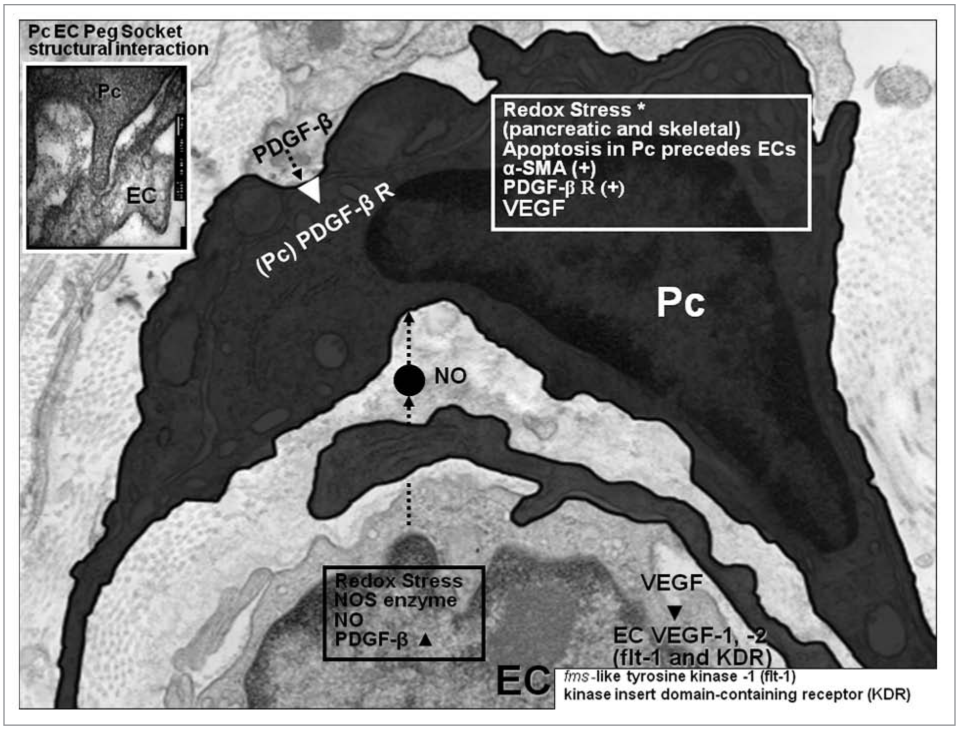

Figure 2. It takes two: the pericyte and endothelial cell for the microcirculation. This image demonstrates a large color-enhanced black pericyte (Pc) located superior and structurally related to its adjacent endothelial cell (EC). This slide not only portrays how the two cells are structurally interacting but also depicts pathways that connect Pcs and ECs in normal states of health and also those involved in disease states from our past studies. These include the necessary roles of redox stress with the Pc, which is believed to be more sensitive to oxidative redox stress (*) as compared to ECs. We have noted that the Pcs undergo apoptotic changes prior to EC loss and capillary rarefaction. We have noted that only the alpha smooth muscle actin is upregulated in the pericyte indicating activation as well as platelet-derived growth factor beta receptor (PDGFβ R). Additionally, only the Pc is capable of synthesizing vascular endothelial cell growth factor (VEGF) and that the EC have only the VEGF Receptors-1–*fms*-like tyrosine kinase-1(flt-1) and VEGFR-2–kinase insert domain-containing receptor (KDR). Importantly, only the ECs are capable of synthesizing PDGFβ and only the Pcs have PDGFβ R. Insert depicts the Pc–EC peg-socket type of ultrastructural cell-cell interaction.

The Pericyte in Microcirculation: Pericyte-Endothelial Interactions

It is difficult to explore the role of the pericyte without discussing its role in the microcirculation and its interactions with capillary endothelial cells. The microcirculation is important for the delivery of nutrients including oxygen, fluids, minerals and hormones as well as removal of toxic metabolic byproducts of metabolism. Additionally the microcirculation is important in maintaining a proper hydrostatic balance in order to sustain proper capillary diffusion mechanics between the microcirculation and tissue interstitium.

The pericyte is a ubiquitous, requisite, mesenchymally derived, pluripotent and postnatally undifferentiated vascular mural cell important for mediating physiological and pathological repair processes. It serves other microcirculation functions including post natal vascular development (angiogenesis), important for maturation and remodeling. The pericyte also provides structural stabilization and a supportive-protective role to capillary endothelial cells. Additionally, changes in pericyte biology are implicated in a variety of microvascular alterations, including wound healing, diabetes, inflammation, HTN, neoplasia and vascular calcification.[16-24] Importantly, pericytes are contractile cells and contribute to the regulation of capillary blood flow, hydrostatic balance and maintenance of proper intracapillary pressure and permeability between the microcirculation and interstitial tissue.[25]

While each of these above interactions between pericytes and endothelial cells are extremely important it is beyond the scope of this review to discuss them in their entirety, especially wound healing and angiogenesis. Therefore, only the primary interactions that apply to what we have found in our ultrastructural studies in the pancreas and skeletal muscle will be emphasized (**Fig. 2**).[16-19,22,25,26]

The pericyte and endothelial cell are interdependent for competent microvessel function; "it takes two": a pericyte and an endothelial cell (**Figs. 2 and 3 and Table 1**).[17,21,26,27]

Additionally, pericytes and vascular smooth muscle cell(s) (VSMC) share a close homology and one may give rise to the other. Emerging evidence also supports the possibility that pericytes may arise from native bone marrow progenitor cells.[28,29] Pericyte coverage of endothelial cells varies in different vascular beds with the highest coverage found in the central nervous system (contributing to the blood brain barrier along with astrocytes) and the retina, where it is almost 1:1 coverage, while in the skeletal muscle the coverage is one pericyte to 100 endothelial cells.[19] In the islet

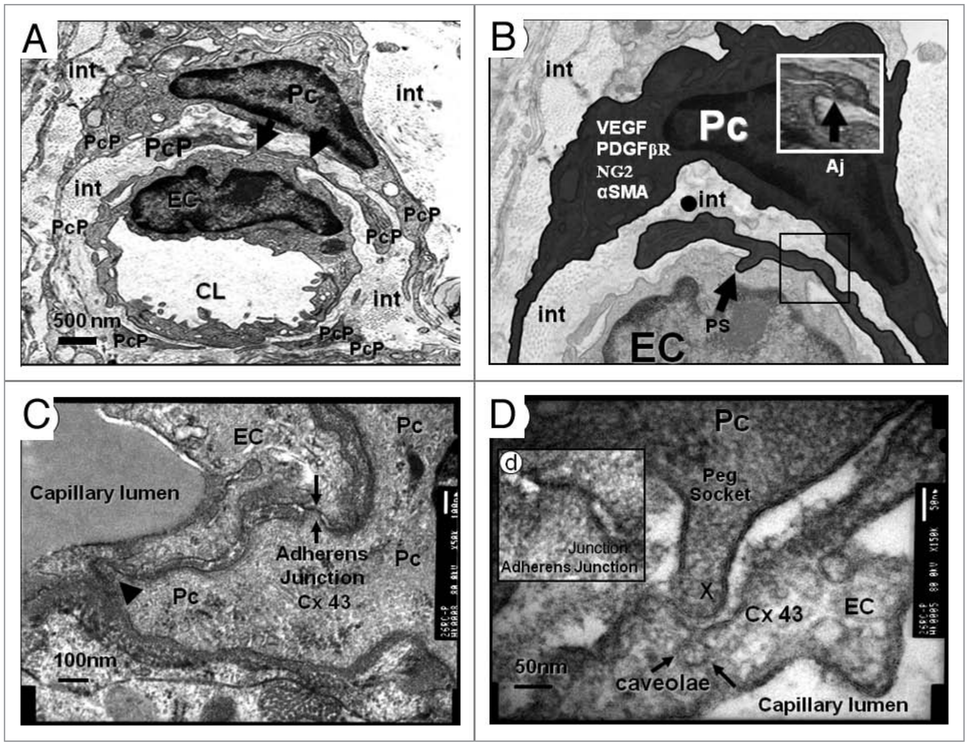

Figure 3. Pericyte-endothelial morphology and connections. (A) demonstrates an intraislet circumferential pericyte (Pc) surrounding an endothelial cell (EC) with its cytoplasmic pericyte processes (PcP). Note that the PcP come in intimate contact with the EC at specific sites termed peg sockets (PS) and adherens junctions (Aj) (arrows). Also note the loose areolar interstitium (int) surrounding these two cells. These contact points are demonstrated in greater detail in (B–D). Magnification x15,000. Bar = 500 nm. (B) is an exploded image of (A) and the Pc has been darkly highlighted to better depict the communication contact structures between the Pc and EC. (C) illustrates both types of endothelial-pericyte communication, cell-cell connections. Note the Peg socket connection (arrowhead) and the adherens junction (arrows) between the Pc and the EC. These structures provide direct communication between these two cells via specific connections containing connexin 43 (Cx 43). Magnification x50,000. Bar = 100 nm. (D) depicts the peg socket connection between the Pc and EC at higher magnification and further demonstrates the presence of a caveolae (arrows), which also provides communication between these two cells. Magnification x150,000. Bar = 50 nm. Insert (d) is an exploded image of the adherens junction in (C). Original magnification x50,000.

we have noted that pericyte coverage is more comparable to the retina, while in the peri-islet–islet exocrine interface region and in the exocrine-acinar portions of the pancreas it appears to be similar to the skeletal muscle vascular bed.

Pericytes seem to be quite vulnerable to multiple metabolic toxicities including ROS and inflammation associated with the MetS, pre-diabetes and overt T2DM. For example, glucotoxicity associated with activation of endogenous aldose reductase enzyme of the polyol pathway with formation of advanced glycation end-products (AGE) and their receptors (RAGE) can promote oxidative stress, inflammation and pericyte injury.[20-22,27,30-32]

Interestingly, pericytes in their native-quiescent stage are known to contain vitamin A-storage droplets (retinoid-lipid vesicles) in their cytoplasm. However, when activated by oxidative stress, downstream cytokines and inflammation, they lose their storage vesicles and undergo proliferation and migration and are capable of differentiating into alpha smooth muscle actin (α-SMA) positive profibrogenic myofibroblast-like pancreatic stellate cells in the pancreatic peri-islet area.[22,33] Although signaling pathways, which activate pericyte differentiation are not fully understood, there exists a central role for ROS, the protein kinase C family and the recently described involvement of extracellular receptor kinase (ERK1/2) and P38 mitogen activated protein kinase (P38 MAPK) signaling pathways.[22,34]

A Unique Capillary Connectivity via Pericytes—"Contineocytes"

The pericyte and its classic circumferential cytoplasmic processes are in close physical contact with capillary endothelial cells. They communicate by intimately sharing their basement membranes through readily identifiable cytoplasmic ultrastructural interdigitations termed peg sockets and cell-cell adherens junctions (**Fig. 3**).[20-22,27] These structural connections allow for paracrine communication and additionally provide physical anchoring attachments allowing their contractile properties to be transmitted to the endothelial cell for capillary constriction, which is necessary for maintaining hydrostatic balance and sustaining proper diffusion mechanics.[35]

In the pancreas (exocrine and islet exocrine interface) and skeletal muscle interstitial matrices, pericytes appear to have very long longitudinal cytoplasmic processes in contrast to the classical circumferential cytoplasmic processes within pancreatic islets. These elongated longitudinal cytoplasmic processes connect at least one or more endothelial capillaries within the endomysial, endoacinar interstitium and interface regions between the islet and exocrine pancreas and skeletal muscle interstitium (**Figs. 4 and 5**). Additionally, there are pericyte-to-pericyte connections (**Fig. 6C and D**), and in skeletal muscle there is a close association of pericytes and myocytes in the endomysium (**Fig. 5D**). This unique longitudinal—connecting phenotype of the pericyte may be referred to as a contineocyte (connecting cell) or interstitial pericyte in contrast to the circumferential capillary mural pericyte (around cell). To our knowledge these TEM images of capillary connectivity within the pancreas and skeletal muscle have not been previously described.

The islet exocrine interface region has been found to be an area where early cellular and extracellular remodeling occurs in young rodent models representative of the MetS [HTN, IR, oxidative stress (OS), obesity] and overt T2DM. Undoubtedly, this diverse structural morphology will be matched by functional diversity as well. Further, this unique capillary connectivity in the pancreas and skeletal muscle may allow for the integration and coordination of neighboring endothelial cell interactions and responses that help regulate microcirculation hemodynamics including the distribution of blood flow within the islet and skeletal muscle endomysium.

Table 1. Pericyte–endothelial cell protein interactions/interdependence and markers

Protein	Pericyte	Endothelial cell
VEGF	+	-
PDGF-β	-	+
PDGF-β(R)	+	-
eNOS	-	+
ET-1	-	+
Basement Membrane (Type IV collagen, laminin, and glycosaminoglycans)	+	+
Markers		
NG2 proteoglycan	+	-
α-SMA	+	-
mAb (3G5)- defined ganglioside cell surface marker	+	-
vWf	-	+
LDL-C R	-	+

VEGF, vascular endothelial growth factor; PDGF-β - PDGF-β(R), platelet derived growth factor beta (receptor); eNOS, endothelial derived nitric oxide synthase; ET-1, endothelin-1; NG2, nerve/glial antigen 2; αSMA, alpha smooth muscle actin; mAb(3G5), monoclonal antibody (3G5)-defined ganglioside antigen; vWf, von Willebrand factor; LDL-C R, low density lipoprotein cholesterol receptor.

Pancreatic Pericyte Story

Pericytopathy (dysfunction—degeneration—apoptosis). At autopsy, human islets demonstrate the co-existence of islet fibrosis and islet amyloid deposition as central features in addition to exocrine pancreatic interstitial fibrosis, adipogenesis, atherosclerosis and acinar loss with atrophy in patients with T2DM (**Fig. 7**).[20-23] Therefore, our group has studied the pancreata (endocrine and exocrine) in young rodent models of MetS and T2DM in order to better understand the early cellular and extracellular matrix changes associated with end-stage islet disease-remodeling (fibrosis and islet amyloid deposition) found in humans.

Definitions. Because multiple young rodent models of IR and T2DM were utilized in gathering the information regarding similar pericyte findings, authors will refer to diseased and control models in the following sections. When indicated, the specific rodent model(s) will be brought to the attention of the reader.

The term diseased model(s) will refer to the rodent models of IR and T2DM. These models will be represented by the male Ren2 transgenic [mRen2]27 model of HTN, OS and IR due to transfection of the Sprague Dawley rat with the mouse renin gene,[19,21] male human islet amyloid polypeptide (HIP) model of T2DM (a Sprague Dawley rat model transfected with the human amylin gene, 2–14 months of age)[25,26] and the male *db/db* mouse model of obesity, IR and T2DM [a genetic, spontaneously developed mouse model (Lepr$^{db/db}$), a leptin receptor-deficient model, 7 weeks of age].[27] The term control model(s) will refer to age-matched male Sprague Dawley control(s) (SDC) and lean C57BL/6 Lepr(db/+) non-diabetic, wild-type (WT) littermate controls (db/dbWT).

The islet exocrine interface (IEI): widening, hypercellularity and early fibrosis. Early remodeling changes within the peri-islet interface region (including fibrosis and islet amyloid deposition) are not fully appreciated with light microscopy in animal models and humans with MetS and T2DM. With the use of TEM, a specific space termed the islet exocrine interface (IEI) has been observed.[20-22] This anatomical region may be the site of the earliest remodeling changes within the pancreas in rodent models of the MetS and T2DM.

In each of the diseased rodent models (Ren2 rat, HIP rat and db/db mouse models) evaluated, TEM demonstrated a widening of the IEI between the endocrine-islet and the exocrine-acinar portion of the pancreas with hypercellularity consisting primarily of pericytes and their elongated longitudinal cytoplasmic processes as compared to control models (**Fig. 8A–C**). These pericytes appear to differentiate into collagen producing cells suggestive of myofibroblasts-like pancreatic stellate cells staining positive for alpha smooth muscle actin and are associated with the early deposition of organized banded fibrillar collagen or fibrosis.[22,24,26] Interestingly, this peri-islet interface also seemed to be an important region for the early mononuclear inflammatory infiltrate (macrophages) found in the db/db models studied and has been recently reported in other rodent models (GK rat, high-fat-fed C57BL/6J mice) and humans with the MetS and T2DM (**Fig. 8D**).[27,34,36] In addition to the uniform finding of IEI widening and pericyte hypercellularity in these three diseased models, they individually had unique morphological cellular and extracellular remodeling.

The non-obese Ren2 model of HTN, IR, OS and early glucose intolerance. The transgenic Ren2 rat model manifests an

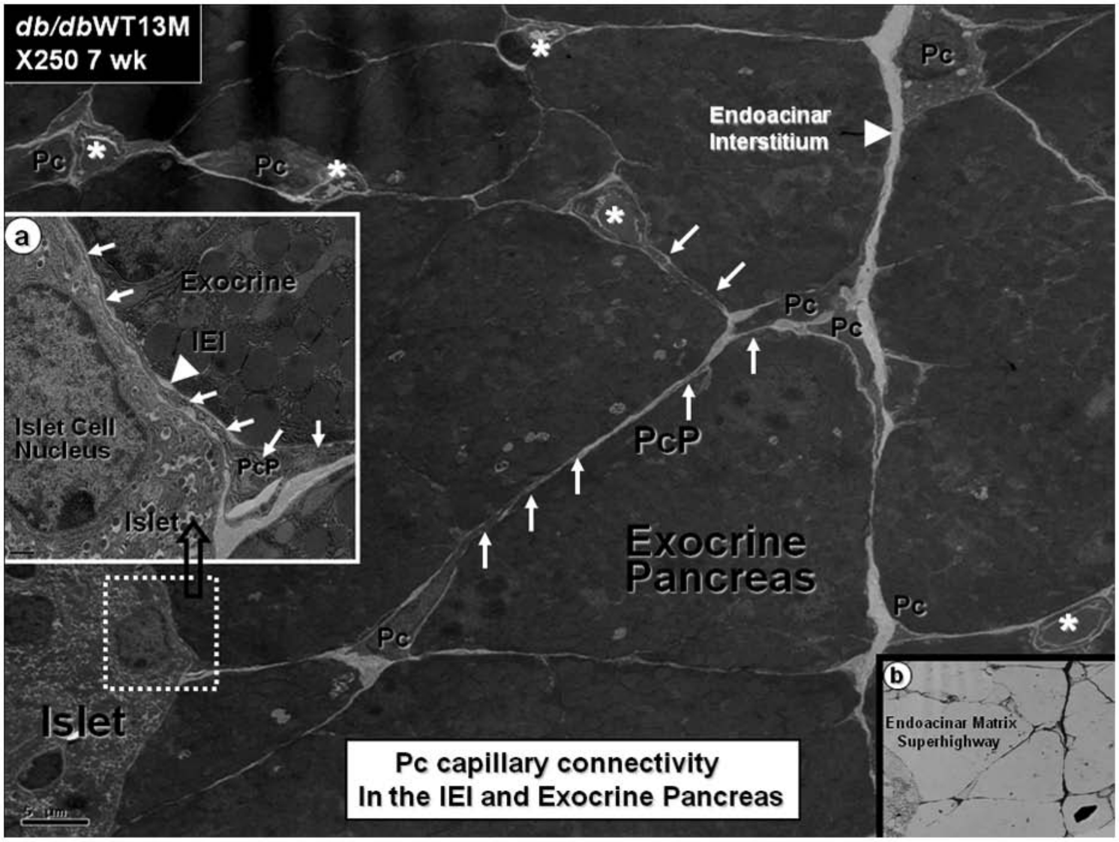

Figure 4. Pericyte capillary connectivity in the islet exocrine interface and exocrine pancreas. This image depicts the pericyte (Pc) connectivity of capillaries (asterisks) via the long pericyte processes (PcP) (arrows) within the endoacinar matrix of the exocrine pancreas and islet exocrine interface (IEI) (arrowhead). The IEI between the islet and the exocrine tissue within the pancreas is continuous as depicted in insert (a). Within exploded insert (a) note the pericyte processes (PcP arrows) traversing the IEI (arrowhead) and the endoacinar matrix demonstrating that these two matrices are continuous within the normal pancreas. This continuous matrix is important as it will later become totally fibrosed and there has been demonstrated a loss of matrix communication due to fibrosis in humans and models with the metabolic syndrome and type 2 diabetes mellitus.[24] Insert (b) is a minimized—inverted image, which demonstrates the IEI and endoacinar matrix in black. This image is reminiscent of a roadmap and that is why we have termed the IEI and endoacinar matrix the "Pancreatic Superhighway." Original magnification X250. Bar = 5 μm. Modified and adapted with permission.[24]

angiotensin II type I receptor and NAD(P)H oxidase-mediated overproduction of ROS in multiple organs including the skeletal muscle and pancreas.[22,24] The Ren2 model is unique, in manifesting a temporal-spatial deposition of collagen with earlier and more robust fibrosis in the exocrine perivascular, periductal and interlobular areas. This is in contrast to the absence of fibrosis in the intra-islet region and early minimal fibrosis found in the peri-islet–IEI region of the pancreas (**Fig. 7D**). These early fibrotic changes in the Ren2 model help to better understand the extensive endoacinar, IEI and intra-islet fibrosis perpetrated by the fibrogenic pancreatic pericyte myofibroblast-like, pancreatic stellate cell observed in the human pancreata at autopsy with MetS and T2DM (**Fig. 7A–C**).

The db/db mouse model of obesity, IR and overt T2DM. In addition to the IEI widening and pericyte hypercellularity, the db/db model manifests hypertrophic islets and depletion of β-cell insulin secretory granules (**Figs. 4, 6C, 8A, 9A and B**).[27] These changes are associated with excessive hyperinsulinemia and IR. Our observational TEM studies did not demonstrate islet microcirculation abnormalities in the young 7-week-old db/db model; however others have noted capillary scarcity, increased diversity of the capillary size, pericapillary fibrosis, pericyte hypertrophy and luminal irregularity in an older 12-week-old db/db model.[37]

The human islet amyloid polypeptide (HIP) rat model of non-obese T2DM. Additional findings of intraislet capillary rarefaction and IEI angiogenesis in the older 8 and 14 month old HIP models reflected a loss of intra-islet pericytes (degeneration—dysfunction—apoptosis) and correlated with increased platelet derived growth factor receptor beta (PDGFR-β) antibody staining and angiogenesis in the IEI (**Figs. 8C and 9C**).[26]

The novel HIP model simulates human pancreatic islet pathology as this model develops the deposition of islet amyloid similar to human patients with MetS and T2DM (**Fig. 10**).

Skeletal Muscle Pericyte Story

Skeletomyopathy–pericytopathy. Skeletal muscle comprises 40–50% of body mass in humans and is the primary tissue responsible for peripheral insulin stimulated glucose uptake and systemic glucose homeostasis. Thus, skeletal muscle IR

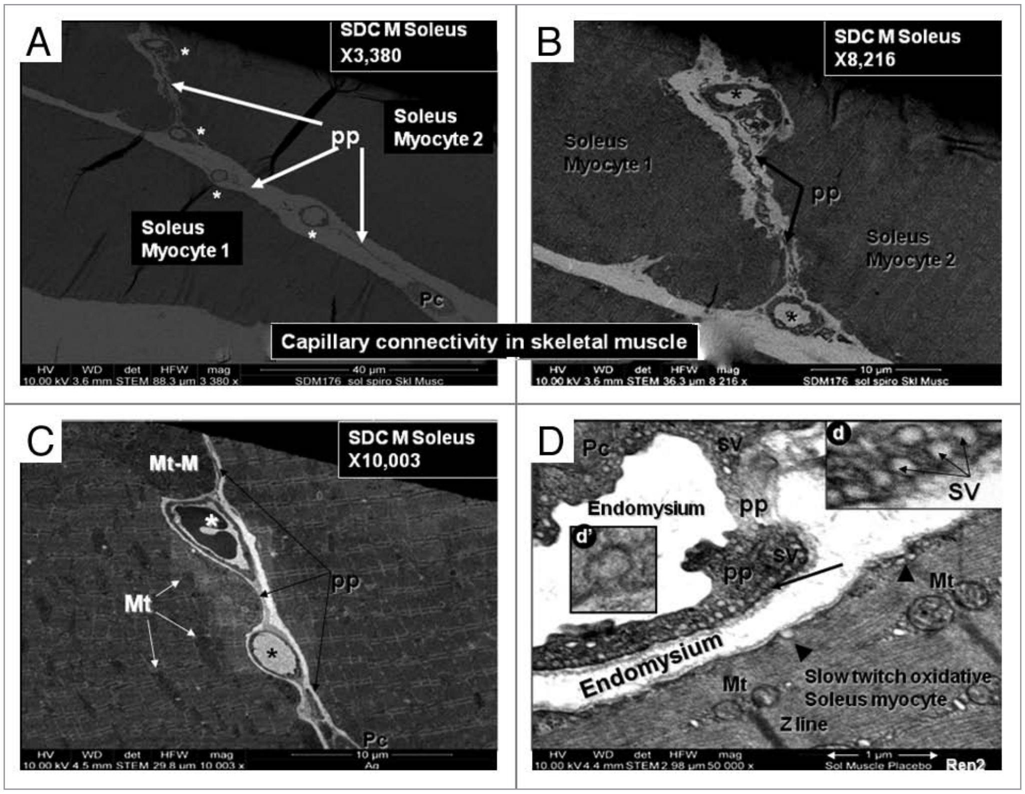

Figure 5. Capillary connectivity via long cytoplasmic pericyte processes in soleus muscle. (A) demonstrates microvascular capillaries (asterisks) in the endomysium between two soleus myocytes in the Sprague Dawley control model (SDC). Note the long cytoplasmic pericyte processes (pp) (arrows) connecting the capillaries. Magnification x3,380. Bar = 40 μm. (B) depicts the endomysial pericyte processes at higher magnification connecting two capillaries (asterisks) via long pericyte processes (pp) (arrows) between two soleus myocytes. Magnification x8,216. Bar = 10 μm. (C) demonstrates two capillaries (asterisks) connected via pericyte processes (pp) (arrows) within the endomysium. Note the mitochondrial mound (Mt-M) and the intermyofibrillar mitochondria (Mt) in the soleus muscle. Also note how the capillaries (asterisk) seems to be embedded within lacunar-shaped structures in the soleus myocyte. Magnification x10,003. Bar = 10 μm. (D) depicts a pericyte process (pp) within the endomysium of the Ren2 model extending close to the soleus myocyte. Note the pp secretory vesicles (SV) lined up in a row enabling direct communication with the soleus myocyte shown at higher magnification in insert (d) (arrows). Insert (d') further portrays a caveolae of the pp. The soleus sarcolemma also depicts receptive caveoli arrowheads within the myocyte allowing for direct communication between the pp and the myocyte within the endomysium. These organelles may allow for direct paracrine communication between pericyte processes and the myocytes. Magnification x50,000. Bar = 1 μm.

and compensatory hyperinsulinemia play an important role in the development of T2DM.[38-42] Human skeletal muscle biopsies in the MetS and T2DM have revealed important cellular and organelle remodeling changes including pericyte deterioration and apoptosis, capillary rarefaction (reduced capillary density), decrease in subsarcolemmal mitochondria, intramyocellular lipid deposition and decreased ratios of slow twitch oxidative/fast twitch glycolytic skeletal muscle fiber types.[11,12,38-43] While each of the above remodeling changes is of significance, our focus will necessarily be concentrated on microvasculature pericyte deterioration—loss and capillary rarefaction.

Capillary rarefaction of skeletal muscle tissue has been observed and accepted to be associated with HTN, MetS, IR and T2DM; however, a direct cause and effect has not been established and remains an area of continuing research. Indeed, pericyte degeneration was observed 40 years ago in human skeletal muscle biopsies[12] and those observations are reminiscent of our recent TEM observations regarding pericyte loss—degeneration—apoptosis and the unique capillary connectivity via pericytes observed in our rodent models of MetS and T2DM. The elegant study of insulin-resistant male Pima Indians without overt T2DM demonstrating capillary rarefaction (1987) brought this fundamental abnormality to the attention of clinicians and researchers.[42]

Observations of a unique pericyte-endothelium, pericyte-pericyte and pericyte-myocyte connectivity—communication in skeletal muscle in rodent models (i.e., Ren2, db/db and HIP models) have resulted in rethinking how capillary rarefaction might develop within the skeletal muscle and pancreatic tissues. Since the pericyte is quite vulnerable to the multiple metabolic toxicities associated with IR in the MetS, prediabetes and overt T2DM, its dysfunction—degeneration and/or loss (apoptosis—pericyte dropout) may precede capillary loss similar to findings in the retina and the islet.

The presence of pericyte ghost cells and apoptotic pericytes with their retracted cytoplasmic processes in the endomysium (inter-myocyte interstitium) and islet exocrine interface in IR and T2DM models is reminiscent of abnormalities observed in the retinal microcirculation (**Fig. 9**).

Additionally, in skeletal muscle, multiple secretory vesicles and caveoli were observed in endomysial (intermyocyte

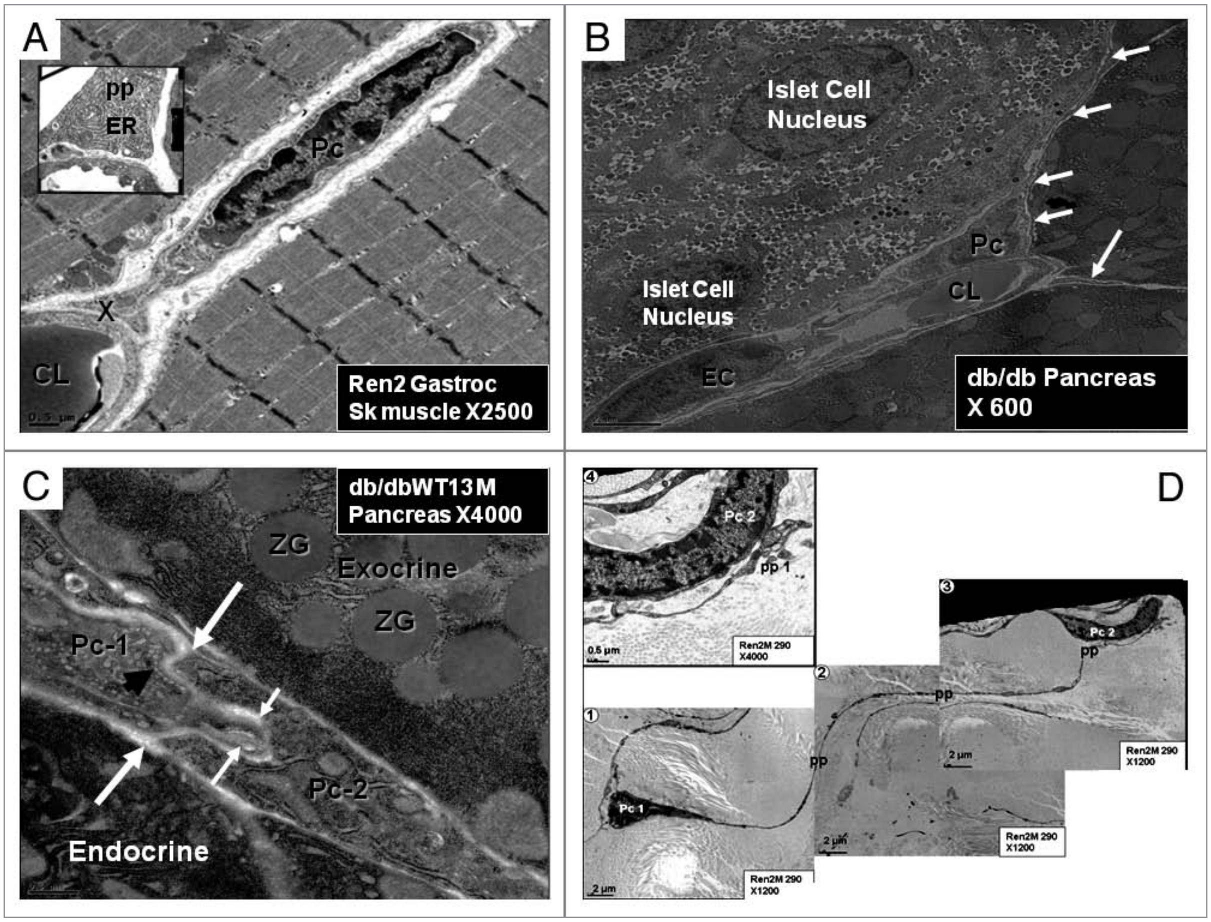

Figure 6. Pericyte-endothelial cell—pericyte-pericyte connections in pancreas and skeletal muscle of rodent animal models with cardiometabolic syndrome and type 2 diabetes mellitus. (A) depicts the pericyte (Pc) endothelial capillary connection in the gastrocnemius muscle of the Ren2 animal model. This connection appears similar to foot plate connections in neural tissue and insert portrays an exploded view of this pericyte process (pp) connection. Note the marked redundancy of the endoplasmic reticulum (ER). Magnification x2,500. Bar = 0.05 μm. (B) demonstrates the interaction between the Pc and the EC surrounding the capillary lumen (CL) within the endoacinar matrix of the db/db pancreas. Note cytoplasmic pericyte processes (arrows) within the endoacinar matrix. Magnification X600. Bar = 2 μm. (C) portrays the cytoplasmic pericyte processes of one pericyte (Pc-1) connecting and communicating with another pericyte (Pc-2) (arrows) in the islet exocrine interface (IEI) of the db/dbWT control mouse model pancreas. Note the presence of secretory vesicles within the Pc-1cytoplasmic process (arrowhead). Magnification x4,000. Bar = 0.5 μm. (D) demonstrates Pc-Pc connection of one pericyte (Pc-1) to another pericyte (Pc-2) in the Ren2 model. Inserts 1–3 depicts the marked elongation of the pp, which ranges from 40–60 μm. Magnification X1,200. Bar = 2 μm. Insert 4 demonstrates a higher magnification image of the connection between Pc-1 and Pc-2 via the cytoplasmic pericyte process (pp). Magnification x4,000. Bar = 0.5 μm.

interstitial) pericytes (**Fig. 5D**) and could play a very important role in the delivery of multiple molecules such as insulin, endothelial nitric oxide synthase—endothelial nitric oxide, lipoprotein lipase and vascular endothelial growth factors to skeletal muscle myocytes. While this has not yet been demonstrated, it certainly supports the notion that pericytes may be playing a far more important role than just being a supportive vascular mural cell.

Pericytes as Adult Mesenchymal Stem Cells or Perivascular Stem Cells

Mesenchymal stem cells (MSC) are known to reside in virtually all post-natal organs and have been suggested to have a perivascular niche, which points to the pericyte as a possible undifferentiated precursor cell capable of differentiating into multiple cell types.[44] Recently, there has been increasing interest in the pericytes' role as an adult mesenchymal stem cell with one author[45] strongly suggesting the possibility that all mesenchymal stem cells may be pericytes, while being careful to note that not all pericytes are stem cells.[44-46] In addition to the postnatal pericytes' known involvement in postnatal angiogenesis, they are known to be capable of differentiating into pancreatic stellate cells, islet stellate cells, fibroblasts-myofibroblasts, chondrocytes, osteoblasts, adipocytes, skeletal myocytes, Leydig cells, neural-linage cells, neural macrophages and VSMC.[16-18,21,22,45-54]

Pericyte ghost cells and apoptosis were readily identified in the diseased animal models studied (**Fig. 9B–D**); however, endothelial cell apoptosis was not identified. We hypothesize that the pancreatic islet and skeletal muscle pericytes may be more susceptible to injurious islet and endomysial OS injury than the

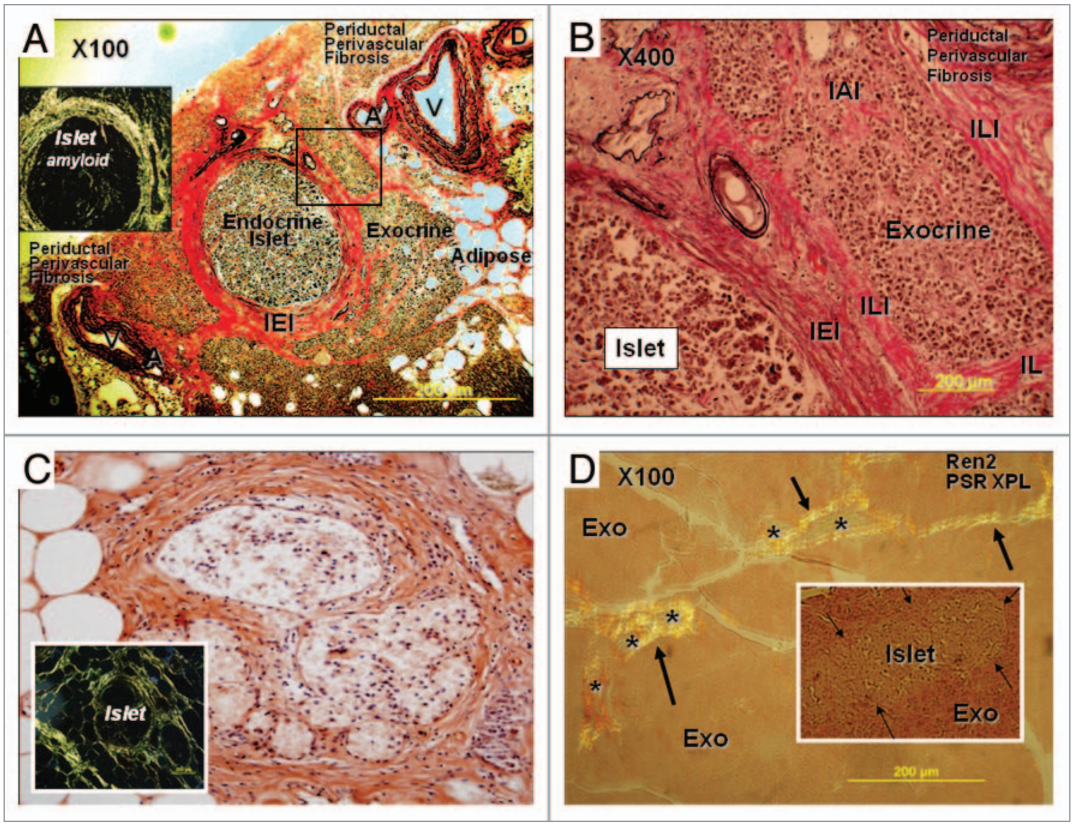

Figure 7. Late end-stage fibrosis in human pancreatic failure vs. early rodent model fibrosis. (A) illustrates the marked fibrosis of end-stage pancreatic failure in a 58 y/o female patient with type 2 diabetes mellitus (T2DM). This photoshop enhanced Verhoeff's Van Gieson (VVG) stained image demonstrates the marked fibrosis in all of the matrices of the pancreas i.e., intra-islet, islet exocrine interface, endoacinar, interlobular, perivascular and periductal matrix by the crimson red staining of collagen. Also note the arteriosclerotic changes and adipose deposition in conjunction with acinar loss and atrophy. This extensive fibrosis of the Pancreatic Superhighway matrix (**Fig. 3**) results in the loss of communication between the endocrine and exocrine pancreas in T2DM. Magnification x100. Bar = 200 μm. Artery = A, vein = V, duct = D and islet exocrine interface = IEI. Insert depicts the presence of intra-islet and islet exocrine interface amyloid deposition, which is known to occur concurrently with fibrosis in humans with the metabolic syndrome and T2DM. Micrograph stained with Congo-Red and viewed with crossed polarized light, which allows the gold colored islet amyloid to be detected. Original magnification x400. (B) demonstrates at higher magnification, the boxed in area from (A). Note the crimson red staining of collagen in the regions of the islet exocrine interface (IEI), the interlobular interstitium (ILI) and the interacinar interstitium (IAI). Magnification x400. Bar = 200 μm. (C) depicts collagen deposition–fibrosis within the islets and the peri-islet–islet exocrine interface of the human pancreas utilizing picrosirius red staining and bright field microscopy. Magnification X400. Insert demonstrates this same islet in crossed polarized light and note how types I and III collagen stain a golden color with crossed polarized light. Minimized insert also magnified x400; bar = 200 μm. Images in (A–C) demonstrate the end-stage fibrosis of pancreatic failure found in metabolic syndrome and T2DM. (A–C) modified with permission.[24] (D) illustrates the early collagen deposition of fibrosis found in the young Ren2 model. The perivascular, periductal (asterisks) and interlobular fibrosis stained yellowish gold (arrows) appears to occur prior to and more robust than peri-islet–islet exocrine interface fibrosis. Magnification x100. Bar = 200 μm. In contrast, the insert of an exploded view demonstrates the lack of peri-islet–islet exocrine interface fibrosis (arrows) as compared to the interlobular perivascular and periductal fibrosis. Original magnification x100 and exploded. There appears to be a temporal and spatial deposition of collagen in this early fibrosis found in the Ren2 model. Images adapted with permission.[19]

endothelial cell similar to retinal pericytes. Robust production of ROS associated with the MetS and T2DM (glucotoxicity, angiotensin II excess, oxidative-redox stress, ROS and islet wounding associated with amyloid deposition) may result in pericyte apoptosis; however, less robust production of these ROS might result in pericyte hypertrophy, hyperplasia, increased α-SMA staining, increased matrix metalloproteinase(s) (MMP) expression and migration from the endothelium.

Connexin 43 shedding and loss of N-cadherin (adherens junctions) due to MMP activation (resulting from robust ROS generation) could result in pericyte migration. This migratory potential of pericytes from the intra-islet region or the exocrine pancreas to the IEI and potential fibrogenic differentiation could help to explain the peri-islet fibrosis found in humans as well as the adipocyte replacement within the pancreas (**Fig.** 7). Furthermore, if the pericyte is destroyed due to apoptosis, its potential as a perivascular MSC could be entirely lost and contribute to islet capillary rarefaction[26] and decreased islet and skeletal muscle blood flow.

Translation to Clinical Medicine

We have been able to identify at least two new microvascular beds (pancreas and skeletal muscle), in which the pericyte may

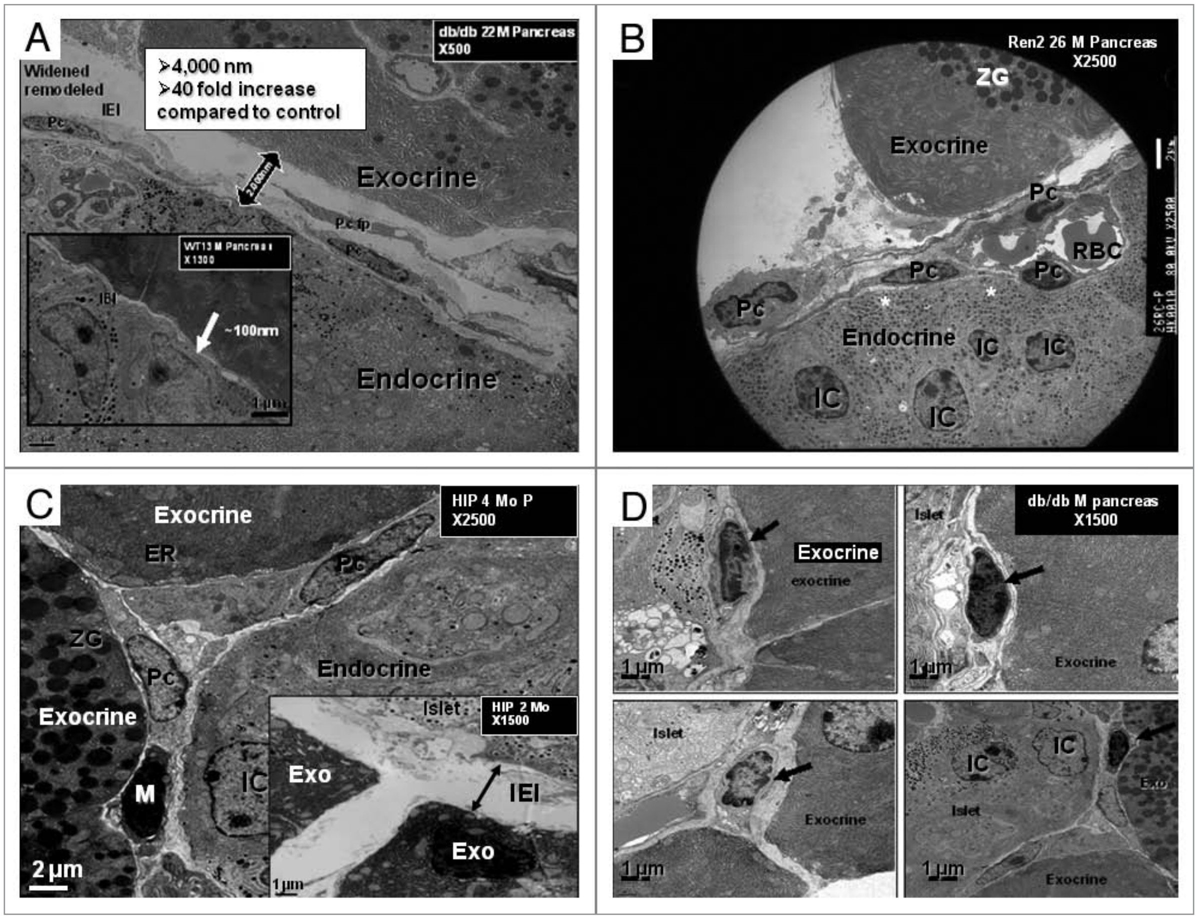

Figure 8. Widening and hypercellularity of the islet exocrine interface. (A) demonstrates the 40-fold widening of the islet exocrine interface (IEI) found in the young db/db model of type 2 diabetes mellitus (T2DM) as compared to its lean wild-type non-diabetic littermate aged 7 weeks (insert). Note the four pericytes (Pc) within the widened IEI. Magnification x500. Bar = 2 μm. (B) depicts the IEI hypercellularity with multiple Pc in the young (9-week-old) Ren2 model. IC, islet cells; Pc, pericyte; RBC, red blood cell; ZG, zymogen granules. Magnification x2,500. Bar = 2 μm. (C) portrays pericyte (Pc) and monocyte (M) infiltration of the IEI in the 4-month-old HIP model with insert demonstrating widening of the IEI (insert) similar to the db/db model and the Ren2 model in (A and B). Each of the three animal models studied demonstrate a widening and infiltration of Pc and inflammatory monocytes. Magnification x2,500. Bar = 2 μm. Insert magnification x1,500. Bar = 1 μm. (D) depicts specific inflammatory monocyte infiltration (arrows) within the IEI of the 7-week-old db/db model of T2DM. There were no inflammatory cells found in any of the control models examined (db/dbWT lean littermate controls). Magnification x1,500. Bar = 1 μm in each plate. Modified with permission.[24]

undergo an important early pathological adaptation (pericytopathy—dysfunction, apoptosis and loss of cellular longevity) similar to the previously studied retinal microcirculation in DR. Translation of these findings to clinical medicine would be greatly assisted by earlier diagnosis of impaired glucose tolerance or prediabetes in those who we know are at high risk for the development of T2DM. Aggressive management of the prediabetic state with diet and exercise are critically important in order to decrease ROS and delay the onset of T2DM. However, in order to delay the progression to overt T2DM we may need to develop and incorporate novel diagnostic markers to identify those at very high risk and develop and apply strategies to prevent or delay pericyte degeneration, dysfunction and loss due to apoptosis (pericytopathy).

Importantly, antipericyte autoantibodies have recently been identified in patients with T2DM.[49,55] We hypothesize that antipericyte autoantibodies could be helpful in deciding which patients with prediabetes (impaired glucose tolerance) require earlier and more aggressive pharmacologic intervention in addition to the usual diet and lifestyle measures currently recommended. This is a novel concept since we are focusing on the pericyte changes in several tissues in the prediabetic state (retinal, pancreatic and skeletal).

Pericyte dysfunction, degeneration and loss—apoptosis (pericytopathy) could expose the innate immune system to foreign pericyte proteins and allow the acquired immune system to form antipericyte autoantibodies. If antipericyte autoantibodies were found to be associated with the impaired glucose tolerance—prediabetes stage, then this would help to determine which patients might benefit from more aggressive and earlier pharmacologic intervention in addition to diet and exercise currently recommended.

The skeletal muscle trinity. There exists a trinity involving three key factors important to the development of skeletal muscle

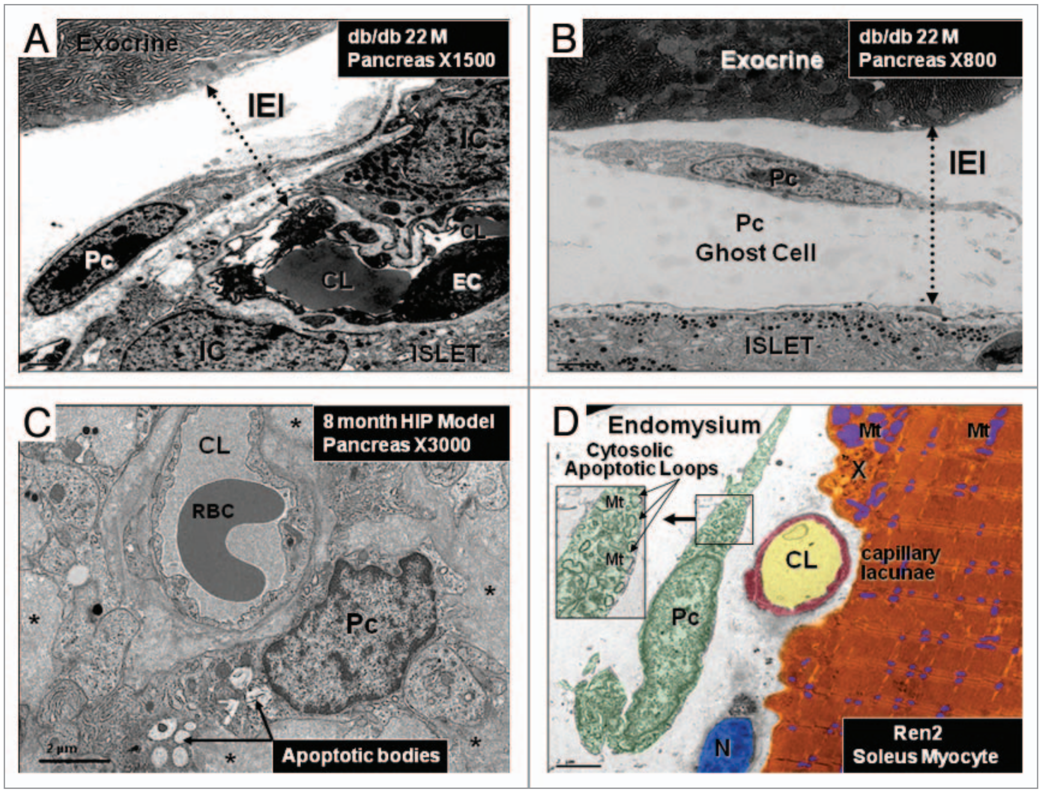

Figure 9. Pericyte degeneration in the islet exocrine interface, intraislet and endomysium of Ren2 soleus skeletal muscle. (A) depicts a normal electron dense pericyte (Pc) in the widened islet exocrine interface (IEI) (dashed double arrow) of the db/db model of type 2 diabetes mellitus (T2DM). CL, capillary lumen; EC, endothelial cell; IC, islet cell. Magnification X1,500. Bar = 2 μm. (B), in contrast to (A), demonstrates a proapoptotic Pc ghost cell in the widened IEI (dashed double arrow) from the same db/db model of T2DM. Note that this Pc is much less electron dense with less visible—less electron dense cytoplasmic organelles such as the endoplasmic reticulum. Magnification x800. Bar = 2 μm. (C) demonstrates the close relationship of an apoptotic Pc to an islet capillary with intracellular apoptotic bodies (arrows) and degeneration of cytoplasmic organelles surrounded by islet amyloid (*) in the 8-month-old human islet amyloid polypeptide (HIP) rat model prior to the development of islet capillary rarefaction. Magnification x3,000. Bar = 2 μm. (D) depicts an apoptotic Pc in the endomysial region of the soleus skeletal muscle of the Ren2 model at 9 weeks of age. Note the loss of cytoplasmic organelles and the decrease in electron density. Exploded insert depicts the cytosolic apoptotic loops (arrows) characteristic of apoptosis. Note how the microcirculatory capillary is nested within a lacunae of the soleus muscle. Endomysium, white; Pc, green; capillary lumen (CL), yellow; endothelium, red; myocyte nucleus (N), blue; mitochondria (Mt), purple; soleus skeletal muscle, rust in color. X marks a region of mitochondrial degeneration in the mitochondrial mounding of the soleus skeletal muscle. Magnification x800. Bar = 2 μm.

capillary rarefaction and progression from IR to clinical diabetes: The earliest factor is the intracellular pericyte mitochondrial loss due to excessive ROS resulting in pericyte apoptosis and loss, the second factor is the subsarcolemmal mitochondrial loss in skeletal muscle and the third factor is the endothelial cell or capillary rarefaction not only in skeletal muscle but also in the islet (**Fig. 11**). While this trinity does not include the importance of liver—hepatic IR as did DeFronzo's classic Lilly lecture "1987: Triumvirate," it importantly includes the significant role of the pericyte.[56]

Conclusion

Translation of young rodent model ultrastructural pericyte findings in the MetS and T2DM allows one to have an early snapshot image of what may have occurred during early cellular and tissue remodeling prior to end stage fibrotic and remodeling changes found in overt T2DM in humans (**Fig. 7**). We have focused on the cellular changes of the microcirculatory pericyte and how it may be importantly involved in remodeling changes regarding the microcirculation and the interstitium in the endocrine and exocrine pancreas and the major site of IR: the skeletal muscle.

Indeed, the pericyte is an old cell; however, its current role in the early remodeling of the pancreas and the skeletal muscle has allowed new insights regarding its evolving importance in islet fibrosis, intra-islet capillary rarefaction concurrent with IEI angiogenesis and capillary tube formation, collagenosis—fibrosis and adipogenesis due to its endogenous and innate adult MSC-like properties.

Pericyte degeneration in skeletal muscle as known to exist in human patients with T2DM and its loss (capillary rarefaction) in obesity, IR and HTN in relation to skeletal muscle myocyte mitochondrial loss may be of significant importance in regards to skeletal muscle IR and resultant impaired glucose uptake.

There are multiple metabolic toxicities in the MetS, obesity, HTN, IR and T2DM largely due to the excess production of ROS and this OS results in cellular–tissue injury or wounding to the islet, exocrine pancreas and skeletal muscle tissues at an early

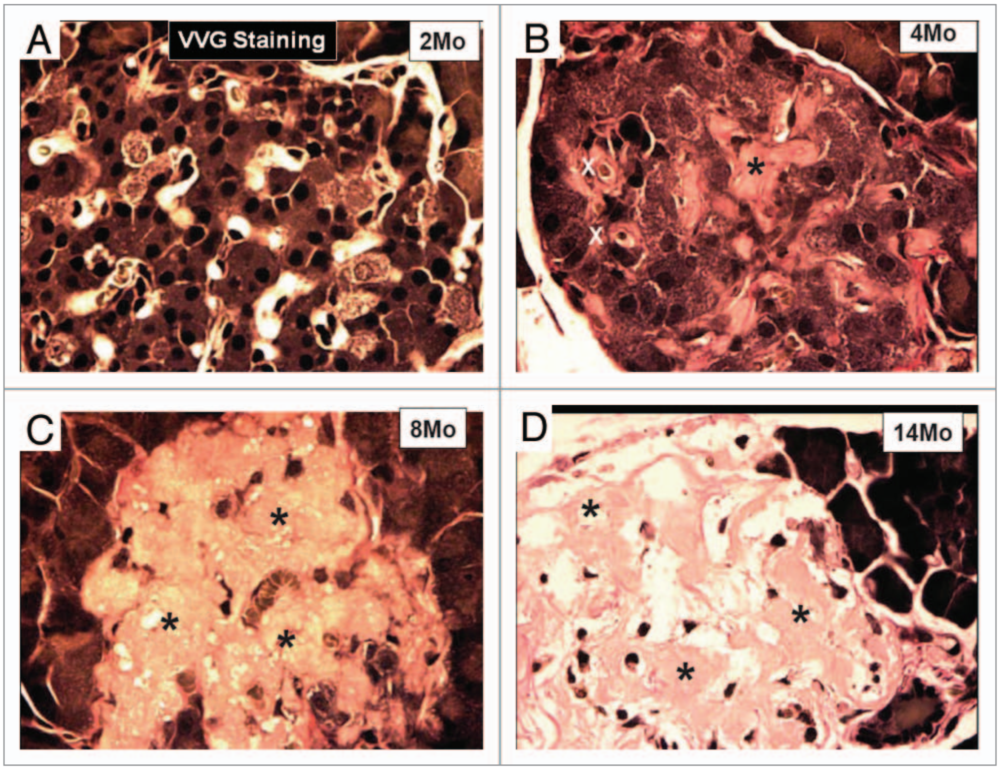

Figure 10. Progressive islet amyloid deposition in the aging hip rat model. (A–D) depict the progressive deposition of human islet amyloid within the islets of transfected Sprague Dawley rat with human amyloidogenic amylin, utilizing the Verhoeff van Gieson (VVG) Stain. Note the early pericapillary diffusion barrier created as human islet amyloid encompasses the intraislet capillaries (X) and deposits between the more centrally located β-cells of the islet (asterisk) in (B). Also note the progressive deposition of islet amyloid in the eight- and 14-month-old models in (C and D).

stage in the development of T2DM. Additionally, there are many other ubiquitous environmental stressors including microbes, allergens, ultraviolet radiation and pollutants including cigarette smoke, ozone and polycyclic aromatic hydrocarbons as well as emotional stress and depression.[57]

Since these syndromes and diseases have reached pandemic proportions, we should be willing to think "outside the box" and attempt to better understand the importance of these novel findings regarding the pericyte and its role in this early remodeling in the islet and skeletal muscle. This cell is ubiquitous and is very sensitive to the oxidative—redox stress, such that, its degeneration, dysfunction and loss—apoptosis and/or its plastic differentiation into other cell types may be very important in the development and progression of T2DM resulting in pericytopathy, skeletomyopathy resulting in capillary rarefaction in the pancreas and skeletal muscle tissue. The cellular redox state is quite complicated and there exists a double-edged sword effect for both ROS and antioxidants in that, physiologic ROS serve as important signaling molecules whereas excess ROS are damaging. Importantly, antioxidants when supplied in excess supplemental forms may become pro-oxidants when supplied in a pro-oxidant milieu such as occurs when there is excess nutrient stress and physical inactivity, which seems to be driving the current obesity, metabolic syndrome and T2DM epidemic—pandemic.[57-60] Future ultrastructural studies of the pericyte in the myocardium, kidney and aorta are warranted.

Acknowledgements

This research was supported by NIH (R01 HL73101-01A1), Veterans Affairs Merit System 0018 (J.R.S.).

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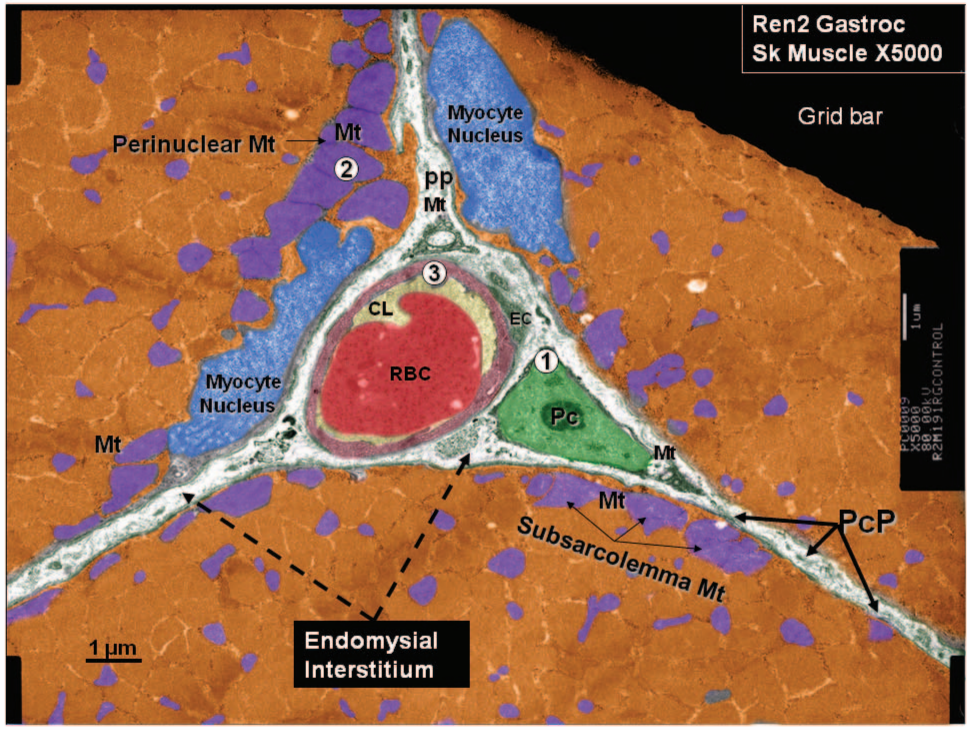

Figure 11. The skeletal muscle trinity: pericyte, mitochondria and capillary loss: rarefaction. This Photoshop-colorized image of the Ren2 gastrocnemius skeletal muscle demonstrates three crucial losses (trinity) involved in capillary rarefaction found in both the islet and skeletal muscles of patients and animal models with metabolic syndrome and type 2 diabetes mellitus. Number 1, (first) to be lost is the (green) pericyte (Pc), Number 2 (second) the subsarcolemmal and perinuclear mitochondria (purple) and number 3, the (third) to be lost the capillary with yellow capillary lumen (CL) and red blood cell (red) representing capillary rarefaction within the endomysial interstitial matrix (dashed arrows) of the gastrocnemius skeletal muscle (rust). These losses may contribute significantly to the development of skeletal muscle insulin resistance, which are known to be important in the compensatory hyperinsulinemia by the beta-cells of the islet. These losses may contribute to the development of impaired glucose tolerance and overt type 2 diabetes mellitus when the compensatory hyperinsulinemia can no longer be sustained due to beta cell secretory deficit or loss. Also note the green pericyte process (PcP) (arrows). These similar changes are also likely to be manifest within the pancreatic islet (**Fig. 7C**) prior to islet rarefaction. Magnification x5,000. Bar = 1 μm.

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