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

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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 pericyte was initially described by Rouget in 1873 3 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. They felt the role of pericytes in 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’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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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 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 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 products (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 proatherosclerotic 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.
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 postnatal 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. 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.

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.30 In the islet
receptor kinase (ERK1/2) and P38 mitogen activated protein kinase (P38 MAPK) signaling pathways. 22,34

A Unique Capillary Connectivity via Pericytes—“Contineocytes”

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
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 contiguous (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.

**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 (Leptm/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/db) non-diabetic, wild-type (WT) littermate controls (db/dbWT).

### 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 Willbrand factor; LDL-C R, low density lipoprotein cholesterol receptor.

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
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) anti-body 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).

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 micro-circulation 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

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Skeletal Muscle Pericyte Story

Skeletomypathy–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
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 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 biopsies33 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.12

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Recently, there has been increasing interest in the pericytes' role as an adult mesenchymal stem cell with one author strongly suggesting the possibility that all mesenchymal stem cells may be pericytes, while being careful to note that not all pericytes are stem cells. 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.

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 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.

Recently, there has been increasing interest in the pericytes' role as an adult mesenchymal stem cell with one author strongly suggesting the possibility that all mesenchymal stem cells may be pericytes, while being careful to note that not all pericytes are stem cells.

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.
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 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...
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...
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 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 nestled within a lacuna 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.
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, skeletomypathy 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.

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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).
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 animals models with metabolic syndrome and type 2 diabetes mellitus. Number 1, (first) to be lost is the (green) pericyte (PcP), 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 similar changes are also likely to be manifest within the pancreatic islet (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 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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