Review

**Vasa vasorum in plaque angiogenesis, metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: a malignant transformation**

Melvin R Hayden*1 and Suresh C Tyagi2

Address: 1Department of Family and Community Medicine, University of Missouri Columbia, Missouri, PO BOX 1140 Lk. Rd. 5–87, Camdenton, Missouri 65020 USA and 2Department of Physiology and Biophysics, University of Louisville, School of Medicine, 500 South Preston Street, University of Louisville, Louisville, Kentucky 40292 USA

Email: Melvin R Hayden* - mrh29@usmo.com; Suresh C Tyagi - s0tyag01@louisville.edu

* Corresponding author

### Abstract

**Background:** Vascularization is an exciting and complex mechanism involving angiogenesis and arteriogenesis. The metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM) are associated with multiple metabolic toxicities, which result in reactive oxygen species (ROS) due to an elevated tension of oxidative-redox stress and an accelerated atherosclerosis termed atheroscleropathy.

**Results:** This atheroscleropathy is associated with accelerated angiogenesis within the vulnerable, thin-cap fibro-atheroma, prone to rupture resulting in acute coronary syndromes (ACS). The resulting intimaopathy with its neovascularization due to angiogenesis of the adventitial vasa vasorum (Vv) is prone to intraplaque hemorrhage (IPH). These IPH are associated with destabilization of the vulnerable plaques resulting in plaque erosion and plaque rupture resulting in ACS. In atheroscleropathy the adventitial Vv invades the plaque in a malignant-like fashion and concurrently is associated with chronic inflammation, as macrophages are being deposited within the shoulder regions of these vulnerable plaques. These angiogenic Vv provide a custom delivery vascular network for multiple detrimental substrates, which further accelerates the growth of these vulnerable plaques and atheroscleropathy. There exists a vascularization paradox in MS and T2DM, in that, angiogenesis within the plaque is induced and arteriogenesis is impaired.

**Conclusion:** This review will attempt to provide a database of knowledge regarding the vascularization process (angiogenesis and arteriogenesis) and its mechanisms to better understand the increased cardiovascular risk and the increased morbidity and mortality associated with MS and T2DM.

### Historical background and introduction

Atheroma and atherosclerosis date to the times of the ancient Egyptians (mummies had atherosclerosis and calcification of coronary arteries). Fallopius (1575) described a degeneration of the arteries into bone and at this time the process was felt to be a natural result of the aging process. Crell (1749) published a book regarding hardening of the coronary arteries. He felt that the
inflammation noted within plaques produced pus that separated the muscular layer from the internal lining of the diseased artery. He noted that when the pus hardened it formed a scaly like change on the lining of these vessels. At approximately this same time Boerhaave suggested that hardening of the arterial wall occurred when the small arteries that feed the muscular layer constricted and hardened (ossified), which is the first description of the vasa vasorum (the vessel within the vessel) directly involved in the angiogenic process [1].

Morgagni (1761) noted tears in the soft portion of the intimal surface of the walls of the aorta (the first description of plaque rupture) and made an important pathological and clinical observation. He noted the increased size of the heart in patients with extensive ossifications and that they had complained of chest pains while living. Hodgson (1815) described the macrophage in atherosclerotic lesions. He further described that the atherosclerotic process occurred within the intima. The idea that atherosclerosis is a chronic inflammatory disease is not necessarily new even though there is excitement today in the current literature as we continue to better understand this process [2,3].

Cruveihier (1833) referred to the atherosclerotic process as an arteritis. He felt that blood clots formed to repair the artery and that ossification of the vasa vasorum resulted in bony plaques formed to prevent aneurysms. Rokitansky (1841) is remembered for his thrombogenic theory. Virchow (1856), considered by many to be the father of pathology, was of the opinion that substances permeate the wall of the arterial vessel wall (endarteritis deformans). Vogel (1847) first identified cholesterol as being a major component of the atherosclerotic plaques. As can be seen the theories of atherosclerosis are legion and several have prevailed throughout history. The thrombogenic theory of Rokitansky, the inflammatory theory of Hodgson, Virchow and others, the insudation theory of Rossle and Doerr, and the lipid theory of Vogel have persisted throughout time. As can be seen in just this brief historical outline, the atherosclerologists of today have a proud heritage upon which to build for the future [1].

In 1999 the late Jeffrey M. Isner (a leader in the field of biorevascularization with pVEGF 165 gene transfer) authored an editorial in the journal *Circulation* entitled: Cancer and Atherosclerosis: The Broad Mandate of Angiogenesis, which triggered this discussion of atheroma and its malignant transformation [4].

The metabolic syndrome (MS), prediabetes (PD), and overt type 2 diabetes mellitus (T2DM) are associated with an accelerated atherosclerosis termed atheroscleropathy (figures 1,2). This atheroscleropathy adds to the rapidity of the malignant transformation due to multiple metabolic toxicities, which are associated with multiple injurious stimuli to the endothelium with associated endothelial dysfunction.

### Angiogenesis induced

It is important to compare and contrast atheroma and atherosclerosis as being a benign and malignant condition respectively: Atheroma implies a benign wound healing response to injury with resolution and fibrous change rather than progression. In contrast: Atherosclerosis implies a malignant transformation with chronic inflammation, fibroproliferation and angiogenesis (table 1).

In 1971, Folkman introduced the hypothesis that tumors were angiogenic dependent [5]. Since atheroma is a benign healing process it is prudent to consider how it transforms to become malignant and the number one cause of death and disability.

The working definition of atherosclerosis:

**Atherosclerosis is a systemic dysfunctional endothelial, focal occurring, chronic inflammatory, fibro-proliferation**
tive, prothrombotic, angiogenic, multifactorial disease of the arterial intima caused by the retention of modified low-density lipoproteins, hemodynamic, and reductive-oxidative (redox) stress [6-10].

Atheroscleropathy = accelerated atherosclerosis in MS, PD, and T2DM.

There is no question that atherosclerosis is a systemic dysfunctional endothelial disease. It is focal, in that, lesions have a tendency to occur at predictable anatomic sites of the arterial tree. It predictably occurs at bifurcations, side branches, and opposite flow dividers at areas of low endothelial shear stress and turbulent blood flow. There is an orderly cephalad progression over time starting with the iliacs and progressing cephalad to the aorta, coronaries, carotids and cerebral vessels.

The PDAY (Pathobiological Determinants of Atherosclerosis in Youth) study and the Korean autopsy study revealed that the atheromatous process begins early in youth and young adulthood [11,12]. By the fifth and sixth decades the devastating clinical effects of this malicious disease are witnessed and will increase as our population

**Figure 2**

The atheroscleropathy associated with MS, PD, and T2DM has many deleterious pathways. There are multiple deleterious pathways associated with MS, PD, and T2DM. Atheroscleropathy is pro oxidative-redox stress, prothrombotic, pro-fibrotic, and pro-inflammatory. Each of these mechanisms and the disease process of atheroscleropathy promote a pro-angiogenic environment and associated with a diabetic vascularization paradox, in that, plaque angiogenesis is induced and arteriogenesis is impaired.
ages (as the "baby boom" generation transitions to the "senior boom" generation).

As the eccentric atheroma intima thickens, there is a relative ischemia of the vessel wall, which is a potent inducer of the adventitial angiogenic vasa vasorum ([13]). The chronic inflammation that runs concurrently serves to magnify this angiogenesis to the point that it appears to be uncontrolled as if a malignancy (table 1). The chronic inflammation (with its associated tissue factor) along with endothelial cell dysfunction contributes to the prothrombotic state of the atherosclerotic plaque. The retention of modified low-density lipoproteins is felt to be a key pathogenic event and possibly an absolute requirement for lesion development and progression ([14,15]). The hemodynamic stress is a prerequisite, as atherosclerosis does not develop within the venous system due to a low pressure – low shear stress environment. Also, pulmonary arteries do not develop atherosclerosis unless pulmonary hypertension is present ([6-10]).

There is an accelerated atherosclerosis (atheroscleropathy) associated with metabolic syndrome (MS), prediabetes (PD), and overt type 2 diabetes mellitus (T2DM). Plaque angiogenesis and intraplaque hemorrhage may be associated with unstable vulnerable plaques and contribute to plaque destabilization.

Currently the vulnerable plaque has been defined as containing the following: 1. Large lipid core. 2. Thin fibrous cap. 3. Inflammatory changes at the shoulder of the fibrous cap. 4. Decreased smooth muscle cells within the fibrous cap. [2,16] (figure 3)

An additional element within the vulnerable plaque should be considered: Number 5.

5. Increased angiogenesis within the intima and media.

Adventitial derived Vv promoting plaque angiogenesis is an important aspect of the vulnerable plaque. The important association of plaque angiogenesis and inflammation is rapidly emerging. Morphologically, the adventitia is the...
last new frontier involved in the development of vulnerable plaques. Early on in the descriptions of atherosclerosis the term arteritis was used to define this process and pathologists pointed to the chronic (mononuclear – monocytes, lymphocytes, and rare mast cells) inflammatory reaction within the adventitia.

Barger’s now classic work in 1984 entitled: "Hypothesis: Vasa vasorum and neovascularization of human coronary arteries” has made clinicians and researchers aware of the importance of the Vv in nourishing the arterial vessel wall in health and disease [17,18]. He was able to show with the injection of white silicone polymer into the coronaries of atherosclerotic vessels a "malignant like" infiltration of microvessels into the media and intima of the atherosclerotic diseased segments. He was able to show that this neovascularization (angiogenesis) of the vessel wall was originating from the adventitial vasa vasorum. In the healthy segments of the same coronary artery there was no involvement of angiogenesis (figure 4).

Later it was revealed that neovascularization could arise from the lumen as well as the adventitial vasa vasorum. Kumamoto et al. [19] a decade later in 1995 was able to show in diseased atherosclerotic epicardial arteries that intimal vessels originated 28 times more frequently from the adventitial vasa vasorum than those originating from the lumen. He was also able to reveal that intimal-medial neovascularization was closely associated with the inflammatory reaction within the plaque, established early in the atherosclerotic process, and capable of regression.

Most authors feel the intimal-medial neovascularization arises more frequently from the adventitial vasa vasorum. For the sake of this discussion and the vulnerability of the atherosclerotic plaque it does not make a tremendous difference as both adventitial and luminal microvessels are both very fragile and prone to leak and rupture creating intraplaque hemorrhage. These intraplaque hemorrhages accelerate plaque vulnerability and are associated with plaque rupture and acute coronary events.
the progression of increasing numbers of microvessels within the atherosclerotic vessel wall. Vessel wall remodeling including angiogenesis is important in the development of the vulnerable unstable atherosclerotic plaque and an important factor in the development of acute coronary syndromes (ACS).

Angiogenesis in the setting of the vulnerable plaque is a double-edged sword. It is the body’s natural protective response to ischemic injury of the vessel wall providing oxygen and metabolic nourishment as the intima undergoes a positive outward remodeling and thickening, while at the same time may contribute to plaque growth through the response to injury mechanism to IPH (table 2) (figure 3). As the numbers of these “malignant like” microvessels increase within the plaque, the numbers of IPH increase as a result and contribute to the instability of the atherosclerotic plaque.

Even though the IPH may be clinically silent, it may result in:

1. Rapid plaque growth due to increase in the size of the plaque, as well as, the necrotic lipid core.
2. Serve as an angiogenic stimulus, thus auto-amplifying the continued vasa vasorum (angiogenic process) further increasing the chance for IPH.
3. Serve as an antigenic stimulus, thus auto-amplifying the continued intraplaque inflammatory response.
4. Activate the inflammatory macrophages at the shoulders of the plaque causing them to secrete their matrix metalloproteinases (MMPs) or collagenases causing a weakening and thinning of the protective fibrous cap as well as possible digestion of the fibrous cap resulting in erosion, fissuring, rupture, with platelet adhesion, aggregation, and ensuing thrombus formation with acute coronary syndrome.

Since 2002 and during 2003 there has been an increasing interest in the vasa vasorum and the adventitia regarding plaque vulnerability and plaque rupture. Fuster V et al. have done much to rejuvenate and expand Barger’s original exciting findings in 1984 [20].

**A focus on plaque angiogenesis (vv) and the MS, PD, and T2DM**

In 2003, Purushothaman KR et al. have presented data that neovascularization is the most powerful independent predictor for plaque rupture in vulnerable plaques (p=0.0001) followed by disruption of the internal elastic lamina (p=0.01), and fibrous cap thinness (p=0.02) [21]. This exciting publication has also prompted this current review and has extended the important role of the adventitia and plaque angiogenesis in our understanding of remodeling of the arterial vessel wall in macrovascular disease and the development of the vulnerable plaque in MS, PD, and T2DM.

Just as atheroscleropathy has been used to describe the accelerated atherosclerotic process, the term intimopathy may be used to describe the accelerated positive outward remodeling and neovascularization of the intima and media in MS, PD, and T2DM. This neovascularization is derived from the adventitial vasa vasorum (Vv) and may also be termed intimal microangiopathy, which is similar to the retinopathy found in the retinal vascular bed.

The Vv is called into the intima and media by a process of angiogenesis, which is increased in MS, PD, and overt T2DM. As can be seen from table 1 this occurs at an accelerated rate in stages IV and V (table 2). There are at least

---

**Table 2: The 10-point process of Angiogenesis:**

(See FIGURE 4)

1. Endothelial cell activation and Proliferation.
2. Local Vasodilatation.
3. Increased vascular permeability.
4. Accumulation of extravascular fibrin* PAI-1
5. Proteolytic degradation of basement membrane. MMP-9 MMP-2
6. Thin cytoplasmic processes are extended from the endothelial cell.
7. Directed migration into surrounding ECM toward the Angiogenic Stimulus (IPH or Ischemia).
8. E. Cells. elongate and align to form a capillary Sprout.
9. E. Cell. Division proximal to the migrating tip.
10. Reconstitution of the basement membrane.
   - PAI-1 inhibits fibrinolysis.
   - PAI-1 inhibits uPA and tPA and PLASMIN production:
   - PAI-1 inhibits the conversion of latent MMPs to active MMPs
   - THUS interfering with remodeling and arteriogenesis
three mechanisms driving the angiogenic response in atheroscleropathy and intimopathy – intimal microangiopathy associated with MS, PD, and T2DM:

1. The initial angiogenic response by the adventitial Vv (via angiogenesis) is stimulated by hypoxia and ischemia as the intima and media undergoes thickening (exceeding the ability of simple diffusion of nutrients from the lumen [usually greater than 0.35 millimeters]) and positive outward remodeling [13]. This hypoxia induces the adventitial Vv to undergo angiogenesis due to Hif-1 and VEGF stimulation [22].

2. In MS and T2DM there exists a microangiopathy within the adventitial derived Vv microvessels. There is increased vascular permeability (leaky micro-vessels) of red blood cells (RBCs) and plasma proteins (comparable to the proteinuria in diabetic nephropathy) into the interstitium of the ECM due to the microangiopathy, which instigates an inflammatory response of monocytes and macrophages [6,7,23,24]. The inflammatory response is excessive in MS, PD, and T2DM and is further increased by the inflammatory signals induced by the increase of RBC plasma membrane extravasation. The inflammatory macrophages are a source of VEGF and other positive regulators of angiogenesis.

3. As the numbers of angiogenic vessels (Vv) increase, the probability of intraplaque hemorrhage increases with extravasated RBCs. The adventitial Vv microvessels within the atherosclerotic plaque are very fragile and prone to rupture as previously described. The inflammatory infiltrate runs concurrently with the development of the intimopathy (neovascularization) as oxidized LDL cholesterol, glycated LDL-cholesterol, and glycoxidated LDL-cholesterol (all of which are forms of modified LDL-cholesterol) also induce an antigenic stimulus and calls in the inflammatory macrophage, which is known to accumulate within the shoulder region of vulnerable atherosclerotic plaques.

Kолодиге FD et al. have just published an important article revealing the importance of the RBC plasma membranes and their deposition within the vulnerable atherosclerotic plaques [25]. The RBC plasma membrane deposition within the necrotic core, in addition to, free cholesterol, macrophage infiltration, and enlargement of the necrotic core is currently felt to be an additional atherogenic stimulus and concurrently increases the risk of plaque destabilization and rupture with ensuing ACS. This new information contributes to the importance of plaque angiogenesis, IPH, and aid in the understanding of the important role they play in the development of ACS.

**Synngism between the inflammatory infiltrate and the vasa vasorum**

The inflammatory infiltrates of the vulnerable shoulder region are responsible for:

1. Recruitment of more monocytes (fueling the inflammatory process).

2. Synthesis of tissue ACE and Tissue Factor (TF), which are known to be direct positive regulators of angiogenesis.

3. Promoting a thinning of the fibrous cap. (IFN gamma of the t-lymphocytes). [26]

4. Increasing the apoptosis of the smooth muscle cell (SMC). (IFN gamma of the t-lymphocyte). [26]

5. Promoting the increased modification of LDL-cholesterol.

6. Promoting the arrival of the t-lymphocyte to the shoulders of the plaque.

7. TNF alpha, IL-1, and IFN gamma by the t-lymphocyte.

8. An increased synthesis of VEGF and FGF.

**Summary:** 1. Tissue Factor. 2. Tissue ACE. 3. MMPs. 4. Cytokines and growth factors (figure 3) [6].

**The fate of the smc and macrophage are opposed**

It is interesting to note that while there is synergism between the vasa vasorum and the inflammatory infiltrate there exists an opposing phenomenon between the SMC and the macrophage. The SMC is initially damaging (causing a progression of the atheroma and atherosclerotic plaque through fibroproliferation) and later in the atherosclerotic process becomes protective providing the strength of the fibrous cap [27]. The macrophage is initially protective (removing or modifying the injurious stimuli i.e. modified LDL-cholesterol to promote healing) and later becomes important in accelerating the disease process (the inflammatory process) by promoting instability and plaque rupture.

**Vasa vasorum: a custom delivery system**

The vasa vasorum acts as a custom-delivery system for the following substrates:

1. Substrates of the Renin Angiotensin Aldosterone System.

2. Substrates of native LDL-cholesterol and modified LDL-cholesterol.
3. Substrates for angiogenesis. The endothelial progenitor cells, as well as, the endothelial cell itself via migration associated with the 10-point process of angiogenesis (table 2) (figure 5).

4. Supply the route for the second wave of inflammatory cells to the intima and vulnerable shoulder regions of the plaque. Fueling the inflammatory process. The first wave originating from the endothelial luminal surface.

5. Supply the direct route for the adventitial fibroblasts and TGF-beta allowing for intimal plaque expansion.

During the past few years there have been important articles published concerning the importance of angiogenesis within the unstable atherosclerotic plaque. Kwon reported (with micro computer tomography in the coronary porcine model) rapid, extensive development of vasa vasorum angiogenesis in coronary vessels. These were induced by a high fat diet in three months [28,29]. Burke and Virmani revealed that plaque rupture in sudden death is related to vasa vasorum angiogenesis in addition to the chronic macrophage inflammatory response [30]. O’Brien was able to show that adhesion molecules (VCAM, ICAM and E-Selectin) expression was in the endothelial lining of the angiogenic vasa vasorum [31]. Thus, later in the atherosclerotic process the angiogenic vasa vasorum may be more responsible for the delivery of the previously discussed harmful molecules and substrates than diffusion through the traditional endothelial lumen surface early in the atherosclerotic process.

Recently de Boer and van der Wal [32] substantiated the above by staining for endothelial cells within the vulnerable plaque and concluded that angiogenesis and expression of adhesion molecules by the vasa vasorum may sustain the influx of inflammatory cells with subsequent plaque destabilization. They as O’Brien found that E-Selectin, ICAM and VCAM were up regulated. In addition they found the expression of CD40 to be increased. This custom delivery system of angiogenic vessels (Vv) acts to deliver the harmful substrates directly to the vulnerable plaque in addition to supplying a route for the chronic inflammation.

This is quite reminiscent of the angiogenic vessels noted in malignant tumors, which provides nourishment for the exponential growth and a vascular route for the metastasis of tumor cells to other organs.

In summary, atherosclerosis is malicious, resistant to treatment, occurring in severe form, frequently fatal, locally invasive, and destructive with growth, resulting in chronic inflammation, fibroproliferation, and angiogenesis (table 1).

**Biomechanical instability may be related to angiogenesis within the vulnerable plaque: “the slapped plaque effect”**

Muller in 1994 [33] revealed to the atherosclerotic community that the malignant atheroma (vulnerable thin-cap fibrous atheromatous plaque) had a tendency to rupture with certain activities of daily living. He was able to relate cardiovascular events to certain triggers:

**SEASONAL:** winter

**TIME of DAY:** mornings

**DAY OF THE WEEK:** Mondays

**RELATED TO:** exertion, anger, and fear (the fight or flight syndrome).

Exertion, anger, and fear are all related to a surge in adrenalin-epinephrine, norepinephrine. These vasoactive molecules when excreted would have a tendency to concentrate in areas where there are increased capillary densities such
as the vulnerable, angiogenic-laden plaques as compared to the healthy segments of coronary arteries. Plaques have tendency to develop in an eccentric fashion with frequent layering of one active atheroma over another. The fixed eccentric plaque with its increased angiogenesis cannot constrict in response to this increased concentration of vasoconstrictive molecules. The vasa vasorum does communicate to the opposing side of the eccentric plaque demonstrated by Barger’s work. The vasoactive molecules could stimulate the smooth muscle cells within the opposing healthy media opposite the fixed plaque and result in a slapping of the fixed diseased wall vessel. This would result in increased biomechanical stress to the unstable, vulnerable, angiogenic laden, rupture prone atherosclerotic plaque and contribute to IPH.

Recently, Stefanadis C [34] and Madjid M [35] have been able to demonstrate that the vulnerable plaque has a higher temperature than stable plaques with a temperature probe. The increased temperature of the probe was associated with elevations in C-reactive protein (CRP), serum amyloid A, and macrophage content. The authors contend that they are measuring indirectly the activity of plaque inflammation, when in fact; they may be measuring the increased temperature as a result of the increased angiogenic blood flow seen by the probe through a vulnerable and thinned fibrous cap.

To summarize, the constant injury and response to injury to the arterial vessel wall causes this natural repair and healing process (remodeling) to go awry with plaque angiogenesis and a malignant transformation (table 1).

**Shared similarities in vascular beds: diabetic intimopathy and retinopathy**

The term intimopathy is used to describe the remodeling within the arterial vessel wall and includes ECM expansion and neovascularization of the adventitial vasa vasorum. This intimopathy is comparable to the angiogenic neovascularization seen in diabetic retinopathy and carries a similar risk when discussing IPH and retinal hemorrhage, each having a detrimental outcome. In the case of retinopathy and retinal hemorrhages there is reduced vision and blindness, whereas in intimopathy there is IPH, destabilization of the vulnerable atherosclerotic plaque, and plaque rupture with ACS and excessive remodeling of the involved plaque [10].

**Arteriogenesis impaired**

MS, PD, and T2DM are associated with impaired coronary collateral vessel formation via the process of arteriogenesis [10,36].

The formation of coronary collateral vessels (arteriogenesis) is a compensatory mechanism secondary to repetitive or chronic epicardial arterial blockage by arterial vasospasm or thrombosis. The process of arteriogenesis (collateral vessel formation) begins with small (20 micron diameter vessels) preexisting, quiescent, minimally functioning arterioles and remodels them into larger (1–2 millimeter diameter vessels) more functional arterioles capable of carrying more oxygen enriched blood to ischemic tissue (a mini auto-coronary artery bypass). The shear stress associated with a pressure gradient as a result of epicardial coronary vasospasm or thrombosis is responsible for inducing the arteriolar remodeling process. In contrast, angiogenesis begins with capillaries and ends with more capillaries (figure 6,7). Important in this remodeling process are the shared cytokines, chemokines, growth factors, the inflammatory monocyte, and the extracellular matrix metalloproteases (MMPs) (table 4) [10].

Recently Waltenberger J et al. were able to delineate the importance of the monocyte. They were able to demonstrate that monocyte migration was impaired in diabetes and associated with impaired collateral formation [37,38]. Panutsoopoulos D et al. were also able to demonstrate an impairment of monocyte migration with an associated increase in basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) mRNA [39]. While the monocyte is an important cell associated with arteriogenesis and its impairment in
migration will have a detrimental effect on remodeling collateralization there may also be a defect associated with MS, PD, and T2DM affecting the remodeling of the extracellular matrix (ECM).

MS, PD, and T2DM are associated with increased PAI-1 [8-10]. PAI-1 inhibits both tissue and urokinase plasminogen activator resulting in impaired synthesis of plasmin. Plasmin is known to be very important in activating latent MMPs to active MMPs [40]. Therefore, PAI-1 excess may additionally serve to impair monocyte migration in addition to inhibiting the ECM remodeling collateralization (intima – media) of preexisting arterioles to the newly remodeled collateral arterioles (figure 7) [10].

There is a diabetes vascularization paradox in that angiogenesis is induced and arteriogenesis is impaired.

The diabetes vascularization paradox: accelerated intraplaque angiogenesis and impaired arteriogenesis

Angiogenesis and arteriogenesis are two distinct forms of postnatal vasculogenesis. Angiogenesis is induced by ischemia through the promoter Hif-1 TACGTCGCT promoter and VEGF activity, whereas arteriogenesis is impaired. Due to elevations in PAI-1. Conversion of plasminogen to plasmin by tPA – uPA is negatively affected by elevations in PAI-1. This defect in the generation of Plasmin results in decreased conversion of latent MMPs to active MMPs and impair the remodeling necessary for the required remodeling of the preexisting arterioles to larger arterioles necessary for a more functional blood flow around obstructed epicardial coronary arteries and obstructed peripheral vascular systems in MS, PD, and T2DM.
Table 3: Multiple metabolic toxicities in ms and t2dm: the a-flight acronym

| Initiator | Metabolic Defect | Metabolic mediator | Functional mediator | Consequence ROS |
|-----------|------------------|--------------------|---------------------|-----------------|
| A | AMYLIN (Co-secreted – Co-packaged within the insulin secretory granule) by the islet Beta cell. Insulin’s “Fraternal Twin” Elevated in MS, PD, and Early T2DM | Hyperamylinemia | Activation of ANG II | PKC Signal Transduction Islet Amyloid IAPP Ilelet aggregation and deposition. Beta cell apoptosis – Beta cell defect. |
|  | ANG II Via RAAS activation In MS, PD, and T2DM | Ang II Excess | Most potent stimulus for: Activation of Vascular membrane bound NAD(P)H Oxidase Enzyme | PKC Signal Transduction. Superoxide production. Uncoupling of the eNOS reaction. TGF beta-1 activation. |
|  | AGE Advanced Glycation Endproducts | AGE / AFE See Glucotoxicity (G) | Protein Cross – linking / Dysfunction | Matrix Defects Signal Transduction |
|  | AFÉ Advanced fructosylation endproducts | RAGE activation Receptor for AGE ALE | RAGE Receptor for AGE Protein Cross – linking | Matrix Defects Signal Transduction |
|  | Advanced Lipoxidation Endproducts (ALE) | Antioxidant Enzymes: Reduced – Dysfunctional | Decreased NO | Decreased NO REDOX STRESS |
|  | Antioxidant reserve compromised | Antioxidant Enzymes: | | |
|  | Absence of antioxidant network | AGING: Accumulation of multiple metabolic toxicities → ROS | Increased Ox-LDL-C, TNFalpha, Capase 3, Glomerulosclerosis. Atherosclerotic Nephropathy | Decreased NO Self perpetuating Decreased NO |
|  | F Free fatty acid toxicity | Lipotoxicity | Decreased NO Atherosclerosis | Decreased NO Increased NO ROS beget ROS Decreased NO |
|  | L Lipotoxicity | Lipid Triad | Long chain acyl-COA’s | Decreased NO Self perpetuating Decreased NO |
|  | I Insulin toxicity | ENDOGENOUS Insulin Resistance | Hyperinsulinemia | Decreased NO Atherosclerosis |
|  | Inflammation toxicity. "Inflammatory Cycle" | Activity of the innate immune system: IL-6, IL-8, TNF alpha | Activation of the innate immune system: C- Reactive Protein Serum Amyloid A Fibrinogen | NF kappa B Cellular Adhesion Molecules: ICAM, VCAM, and MCP-I |
|  | Insulin deficiency | OVERT T2DM | GLUCOTOXICITY POLYOL SORBITOL PATHWAY | REDUCTIVE STRESS NADH > NAD+ PSEUDOHYPOXIA |
|  | G Glucotoxicity | Glycation / AGE | See above | See above |
|  | Antioxidant Enzymes: | | | |
|  | Antioxidant reserve compromised | Antioxidant Enzymes: | | |
|  | Absence of antioxidant network | AGING: Accumulation of multiple metabolic toxicities → ROS | Increased Ox-LDL-C, TNFalpha, Capase 3, Glomerulosclerosis. Atherosclerotic Nephropathy | Decreased NO Self perpetuating Decreased NO |
|  | F Free fatty acid toxicity | Lipotoxicity | Long chain acyl-COA’s | Decreased NO Self perpetuating Decreased NO |
|  | L Lipotoxicity | Lipid Triad | Long chain acyl-COA’s | Decreased NO Self perpetuating Decreased NO |
|  | I Insulin toxicity | ENDOGENOUS Insulin Resistance | Hyperinsulinemia | Decreased NO Atherosclerosis |
|  | Inflammation toxicity. "Inflammatory Cycle" | Activity of the innate immune system: IL-6, IL-8, TNF alpha | Activation of the innate immune system: C- Reactive Protein Serum Amyloid A Fibrinogen | NF kappa B Cellular Adhesion Molecules: ICAM, VCAM, and MCP-I |
|  | Insulin deficiency | OVERT T2DM | GLUCOTOXICITY POLYOL SORBITOL PATHWAY | REDUCTIVE STRESS NADH > NAD+ PSEUDOHYPOXIA |
|  | G Glucotoxicity | Glycation / AGE | See above | See above |
|  | Antioxidant Enzymes: | | | |
|  | Antioxidant reserve compromised | Antioxidant Enzymes: | | |
|  | Absence of antioxidant network | AGING: Accumulation of multiple metabolic toxicities → ROS | Increased Ox-LDL-C, TNFalpha, Capase 3, Glomerulosclerosis. Atherosclerotic Nephropathy | Decreased NO Self perpetuating Decreased NO |

(continued...
induced by the shear stress promoter by the shear stress responsive element (SSRE): GAGACC (figure 6) [41].

The atherosclerotic plaque angiogenic adventitial Vv within atherosclerosis is excessive and associated with MS, PD, and overt T2DM. The Vv are induced by ischemia as the intima-media undergoes positive outward remodelling. Additionally, the Vv angiogenesis is further induced by the inflammatory process within the shoulder region of the plaque, which is associated with the known angiogenic factors: Tissue ACE, tissue factor, cytokines and growth factors (TNF alpha, VEGF, FGF). MS, PD, and T2DM are associated with multiple metabolic toxicities, which are responsible for reactive oxygen species (ROS) production (table 3). This elevated tension of redox stress contributes to the ischemia within the plaque and induces even more angiogenesis within the plaque.

In contrast arteriogenesis is induced and promoted by shear stress in normoxic conditions (table 4). Redox stress and ROS are elevated in the preexisting (un-remodeled) arteriolar endothelial milieu in MS, PD, and T2DM and is associated with endothelial dysfunction with decreased eNOS activity and decreased generation of eNO. This could result in an impaired initial vasodilatation and permeability ordinarily induced by increased shear stress in the arteriogenic process. Terjung RL et al. and Matsunaga T et al. have reported that arteriogenesis is eNOS and eNO dependent [42,43]. Thus, the impairment in eNOS and eNO (due to redox stress and ROS) [8] could impair the initiation and progression of arteriogenesis and result in a negative effect on remodeling collateralization.

Given that collateral formation is inhibited by NOS inhibition, dysfunction or knockout, one can hypothesize that eNOS dysfunction and – or decreased eNO may decrease arteriogenesis through the following mechanism: a decrease in arteriolar vasodilation would impair the normal arteriolar vasodilatation due to eNOS and eNO and decrease the pressure gradient between normal and ischemic tissue. This would impair the normal increased flow and the shear stress responsive element in the collateral vessel undergoing remodeling collateralization, as well as, decreasing endothelial cell permeability [44]. Future work is needed to establish if reversing endothelial dysfunction associated with MS, PD, and T2DM could restore improved arteriogenesis [10]. Thus it appears that a blunted endothelial NO production as noted in MS, PD, and overt T2DM could temper vascular remodeling and arteriogenesis [45].

Excess in redox stress or ROS and PAI-1 elevation could have an overall negative effect on arteriogenesis in MS, PD, and overt T2DM (table 4).

Another possibility is the existence of early advanced glycation end products (AGE) and that these AGE would contribute not only to the impaired remodeling collateralization phase but also contribute to additional collagen cross linking within the extracellular matrix. If glucotoxicity were to be better controlled this might improve the remodeling phase of arteriogenesis in T2DM by improving redox stress, endothelial dysfunction, monocyte migration, and PAI-1 levels.

**Endotheliopathy**

MS, PD, and T2DM are associated with a diffuse endotheliopathy. This endothelial dysfunction can be related to the three vulnerable arms of the endothelial nitric oxide synthase (eNOS) reaction.
Oxidative – redox stress is elevated in MS, PD, and T2DM and results in the production of reactive oxygen species (ROS). The multiple metabolic toxicities of the A-FLIGHT acronym result in an abundance of ROS. These ROS inter-
fere with the eNOS reaction resulting in a decrease in endothelial nitric oxide (eNO). The process of uncoupling of the eNOS reaction allows this reaction to proceed with the endothelium becoming a net producer of superoxide \( \text{O}_2^- \) instead of the quintessential, protective, anti-inflammatory, antioxidant eNO. When L-arginine uncouples from the eNOS enzyme via an intact necessary cofactor tetrahydrobiopterin (BH\(_4\)) the following reaction ensues:

\[
\text{NAD(P)H} \rightarrow \text{NAD(P)H Oxidase} \rightarrow \text{NAD(P)}^+ + \text{O}_2^-
\]

The BH\(_4\) requisite cofactor itself is quite sensitive to oxidative – redox stress and can be oxidized to BH\(_2\) and BH\(_3\) which will not run the eNOS reaction and allow uncoupling. This allows the endothelium to become a net producer of superoxide instead of eNO. As can be seen it is
very important to have an adequate substrate, a proper functioning eNOS enzyme, and the necessary cofactor BH4. Folic acid is not only a methyl donor and aids in the control of elevated homocysteine but also an electron and hydrogen donor, which brings BH2 and BH3 back to the requisite completely reduced BH4. These findings would lead one to not only control the multiple metabolic toxicities (A-FLIGHT table 3) but also provide folic acid in adequate amounts to allow BH4 to run the eNOS reaction fully re-coupled to once again become a net producer of eNO [8,9].

Conclusion
MS, PD, and T2DM are associated with endothelial dysfunction and carry an elevated risk for both micro and macrovascular disease that are often present at the time of diagnosis of overt T2DM. Postnatal vascularization in a response to injury mechanism to the endothelium and the arterial vessel wall as well as the capillary bed result in most of the complications associated with these disorders.

This review has focused on the arterial vessel wall in order to better understand the development and acceleration of macrovascular disease termed: atheroscleropathy and the capillary bed in microvascular disease. The diabetic vascularization paradox has been presented in order to better understand the mechanisms involved in the finding of angiogenesis being induced and arteriogenesis impaired.

The important role of plaque angiogenesis and subsequent plaque destabilization has been presented as this mechanism plays such an important role in plaque rupture and acute coronary syndromes. The epicardial coronary vessels and the peripheral arterial vessels are involved with ischemic manifestations as a result of atheroscleropathy and impaired arteriogenesis – collateral vascular formation.

When you stand back and examine the overall vascular health in MS, PD, and T2DM it becomes obvious that treatment to prevent, slow, or halt these vascular abnormalities requires a global risk reduction approach in treatment in order to treat the multiple metabolic toxic abnormalities associated with this complex disease (figure 2) (table 3). We currently have multiple treatment regimens available to use as each metabolic abnormality is addressed clinically. The RAAS acronym is provided as a tool to assist the clinician in the treatment of these complicated multiple metabolic derangements (table 5). Each therapeutic component of the RAAS acronym will have a positive effect on the excessive angiogenesis and the impaired arteriogenesis associated with MS and T2DM atheroscleropathy [46-48].

Abbreviations
See list of abbreviations following table 6.

Competing interests
None disclosed.

Authors contributions
M. R. Hayden and S.C. Tyagi contributed equally in the inception, writing, and editing of this manuscript.

Acknowledgements
A part of this study was supported by NIH grants HL-71010 and HL-74185.

The authors wish to acknowledge the late A. Cliff Barger and Jeffrey M. Isner for their large body of work they have contributed to the importance of angiogenesis in the field of cardiovascular disease. Also, to acknowledge the large body of work Wolfgang Schaper and colleagues have contributed to the importance of arteriogenesis in the field of cardiovascular disease.

References
1. Acierno LJ: Atherosclerosis (arteriosclerosis). In The History of Cardiology Edited by: Acierno LJ. New York: Parthenon Publishing Group Inc; 1994:109-126.
2. Ross R: Atherosclerosis – an inflammatory disease. N Engl J Med 1999, 340(2):115-126.
3. Ridker P, Morrow DA: C-reactive protein, inflammation, and coronary risk. Cardiol Clin 2003, 21(3):315-325.
4. Isner JM: Cancer and atherosclerosis: the broad mandate of angiogenesis. Circulation 1999, 99:1653-1655.
5. Folkman J: Tumor angiogenesis: therapeutic implications. N Engl J Med 1971, 285(21):1182-1186.
6. Hayden MR, Tyagi SC: Atherosclerosis: Implications of angiotensin II and the AT-1 receptor. In Angiotensin II Blockade: Physiological and Clinical Implications Edited by: Dhalla NS, Zahradka P, Dixon IMC, and Beamish RE. Boston MA. Kluwer Academic Publishers; 1998:233-243.
7. Hayden MR, Tyagi SC: Arterial vascular remodeling: The endothelial cell’s central role. Massoni Medicine 1998, 95(5):213-217.
8. Hayden MR, Tyagi SC: Intimal redox stress: Accelerated atherosclerosis in metabolic syndrome and type 2 diabetes mellitus. Atheroscleropathy. Cardiovasc Diabeteal 2002, 1(1):3.
9. Hayden MR, Tyagi SC: Is type 2 diabetes mellitus a vascular disease (atheroscleroathy) with hyperglycemia a late manifestation? The role of NOS, NO, and redox stress. Cardiovasc Diabeteal 2(1):2.
10. Hayden MR, Tyagi SC: Arteriogenesis: Angiogenesis within Unstable Atherosclerotic Plaque – Interactions with Extracellular Matrix. Curr Interv Cardiol Rep 2000, 2(3):218-227.
11. Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman WP 3rd, Herderick EE, Cornhill JF: Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 1999, 281(8):727-735.
12. Enos WF, Holmes RH, Beyer J: Landmark article, July 18, 1953: Coronary disease among United States soldiers killed in action in Korea. Preliminary report. JAMA 1986, 256(20):2859-2862.
13. Williams JK, Heistad DD: [The vasa vasorum of the arteries]. J Mol Vasc 1996, 21(Suppl C):266-269.
14. Williams KJ, Tabas I: The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 1995, 15(5):551-561.
15. Williams KJ, Tabas I: The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol 1998, 9(5):471-474.
16. Lee RT, Libby P: The unstable atheroma. Arterioscler Thromb Vasc Biol 1997, 17(10):1859-1867.
17. Barger AC, Beeuwkes R 3rd, Lainey LL, Silverman KJ: Hypothesis: vasa vasorum and neovascularization of human coronary
arteries. A possible role in the pathophysiology of atherosclerosis. N Engl J Med 1984, 310(3):175-177.
18. Barger AC, Bessette RR 3rd: Rupture of coronary vasa vasorum as a trigger of acute myocardial infarction. Am J Cardiol 1990, 66(14):41G-43G.
19. Kumamoto M, Nakashima Y, Sueishi K: Intimal neovascularization in human coronary atherosclerosis: Its origin and pathophysiological significance. Hum Pathol 1995, 26:450-456.
20. Fuster V, Corti R, Badimon JJ: The Mikamo Lecture 2002. Therapeutic targets for the treatment of atherothrombosis in the new millennium – clinical frontiers in atherosclerosis research. Circ J 2002, 66(9):783-790.
21. Furushothaman KR, Fuster V, O'Connor WN, Moreno PR. Neovascularization is the most powerful independent predictor for progression in high-risk atherosclerotic plaques. J Am Coll Cardiol 2003, 41(6 Suppl B):352-353.
22. Pugh CW, Ratcliffe PJ: Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 2000, 9(6):671-684.
23. Williamson JR, Kilo C: Hyperglycemia "pseudohypoxia" and diabetic complications. Diabetes 1993, 42:801-813.
24. Zhang Y, Clift WJ, Schoeffl GI, Higgins G: Immunohistochemical study of intimal microvessels in coronary atherosclerosis. Am J Pathol 1993, 143:161-172.
25. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, Farb A, Guerrero LJ, Hayase M, Katush R, Narula J, Finn AV, Virmani R: Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003, 349(24):2316-2323.
26. Libby P: Molecular basis of the acute coronary syndromes. Circulation 1995, 91(11):2844-2850.
27. Lafont A, Libby P: The smooth muscle cell: sinner or saint in atherothrombosis and their relation to intimal leukocyte content. J Am Coll Cardiol 2000, 35(1):272-278.
28. Kwon HM, Sangiorgi G, Ritman EL, McKenna C, Holmes DR Jr, Schwartz RS: Images in Cardiology Imaging the coronary microcirculation: 3D micro-CT of coronary vasa vasorum. Int J Cardiovasc Intervent 1999, 2(1):79.
29. Burke AP, Farb A, Malcom GT, Liang Y, Smialek JE, Virmani R: Plaque rupture and sudden death related to exertion in men with coronary artery disease. JAMA 1999, 281(10):921-926.
30. O'Brien KD, McDonald TO, Chait A, Allen MD, Alpers CE: Neovascular expression of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte content. Circulation 1996, 93(6):672-682.
31. de Boer OJ, van der Wal AC, Teeling P, Becker AE: Leucocyte recruitment in rupture prone regions of lipid-rich plaques: a prominent role for neovascularization? Cardiovasc Res 1999, 41(2):441-449.
32. Muller JE, Abela GS, Nesto RW, Toffler GH: Triggers, acute risk factors and vulnerable plaques: the lexicon of a new frontier. J Am Coll Cardiol 1994, 23(3):809-813.
33. Stefanidis C, Diamantopoulos L, Derrunjel J, Economou E, Tsimis E, Toutouzas K, Vlachopoulos C, Toutouzas P: Heat production of atherosclerotic plaques and inflammation assessed by the acute phase proteins in acute coronary syndromes. J Mol Cell Cardiol 2000, 32(1):43-52.
34. Madjid M, Naghavi M, Malik BA, Litovsky S, Willerson JT, Casscells W: The thermal detection of vulnerable plaque. Am J Cardiol 2002, 90(10C):36L-39L.
35. Abaci A, Oguzhan A, Kahraman S, Erayl NK, Unal S, Arinc H, Ergin A: Effect of diabetes mellitus on formation of coronary collateral vessels. Circulation 1999, 99(17):2239-42.
36. Waltenerber J: Impaired collateral vessel development in diabetes: potential cellular mechanisms and therapeutic implications. Cardiovasc Res 2001, 49(3):554-560.
37. Waltenerber J, Lange J, Kranz A: Vascular endothelial growth factor-A-induced chemotaxis of monocytes is attenuated in patients with diabetes mellitus: A potential predictor for the individual capacity to develop collaterals. Circulation 2000, 102(2):185-190.
38. Panatopulos D, Zafiroopoulos A, Krambouitis E, Kochiadakis GE, Igoumenidis NE, Spanidios DA: Peripheral monocytes from diabetic patients with coronary artery disease display increased bFGF and VEGF mRNA expression. J Transl Med 2003, 1(1):6.
39. Tyagi SC, Kumar S, Cassetas S, Parker JL: Temporal expression of extracellular matrix metalloproteinases and tissue plasminogen activator in the development of collateral vessels in the canine model of coronary occlusion. Can J Physiol Pharmacol 1996, 74(8):983-995.
40. Schaper W, Scholz D: Factors regulating arteriogenesis. Arterioscler Thromb Vasc Biol 2003, 23(7):1143-1151.
41. Prior BM, Lloyd PG, Ren J, Li Z, Yang HT, Laughlin MH, Terjung RL: Arteriogenesis: role of nitric oxide. Endothelium 2003, 10:207-216.
42. Wartier D, Wehrach DW, Moniz M, Tessmer J, Chilian WM: Ischemia-induced coronary collateral growth is dependent on vascular endothelial growth factor and nitric oxide. Circulation 2000, 102(25):3098-3103.
43. Brevechi LS, Chang DS, Tog GL, Sarkar R, Messina LM: Overexpression of endothelial nitric oxide synthase increases skeletal muscle blood flow and oxygenation in severe rat hind limb ischemia. J Vasc Surg 2003, 38(4):820-826.
44. Yang HT, Zan Y, Abraham JA, Terjung RL: VEGF(121)- and bFGF-induced increase in collateral blood flow requires normal nitric oxide production. Am J Physiol Heart Circ Physiol 2001, 280(3):H1097-H1104.
45. Sukhova GK, Williams JK, Libby P: Statins reduce inflammation in atheroma of nonhuman primates independent of effects on serum cholesterol. Arterioscler Thromb Vasc Biol 2002, 22(7):1452-1458.