Recent Highlights of *ATVB* Diabetes Mellitus

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Keywords

diabetes complications; diabetes mellitus, type 1; diabetes mellitus, type 2; experimental diabetes mellitus

The defining feature of diabetes mellitus is elevated levels of blood glucose. The majority of cases of diabetes mellitus result from 2 major etiologic mechanisms.[1] First, type 1 diabetes mellitus is an autoimmune disease in which absolute deficiency of insulin because of immune-mediated destruction of pancreatic β cells causes hyperglycemia. Second, type 2 diabetes mellitus (T2D) is characterized by insulin resistance. The main risk factors for T2D include obesity, excess caloric consumption, and reduced physical activity. A strong genetic component contributes to vulnerability to T2D as well. In addition to type 1 diabetes mellitus and T2D, diabetes mellitus associated with pregnancy, so-called gestational diabetes mellitus, is often a forbearer of T2D. Epidemiological data indicate that both type 1 diabetes mellitus and T2D are on the rise throughout the world.[2,3] The complications of diabetes mellitus reduce health and life span in affected individuals and add a substantial financial burden to already taxed healthcare delivery systems. Because cardiovascular disease is a major cause of morbidity and mortality in diabetes mellitus,[4,5] identification of the fundamental biochemical and molecular pathways that lead to diabetic complications is essential. In this context, several recent publications in *ATVB* have provided novel insights into mechanisms that cause diabetes mellitus, its complications, and the failure of vascular and wound repair in the disease. This article reviews recent studies published in *ATVB* on diabetes mellitus and its complications. From experiments and trials in human subjects, rodents, and swine models, as well as in primary cells exposed to high glucose, diabetes mellitus–relevant conditions, this article summarizes many of these recent publications within a brief context of the literature.

T2D: Reducing the Risk

As noted above, the significant long-term impact of T2D on life- and health span has led to many endeavors to block the development of diabetes mellitus as one means to prevent the complications. Goldberg and Mather[6] reviewed the design and results of the Diabetes

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Disclosures: None.

Prevention Program in which individuals highly vulnerable to the development of T2D were enrolled and randomized to lifestyle intervention, metformin or standard care. The study ultimately concluded that >2.8 years of follow-up, the lifestyle intervention and metformin treatments resulted in 58% and 31% fall in the incidence of T2D, respectively. Cardiovascular risk factors also were significantly reduced, at least in part because of treatment for lipid abnormalities and hypertension. In a second study, the type of physical activity, walking or running, was assessed for the ability to reduce T2D risk. Both forms of physical activity were found to reduce diabetes mellitus risk. Interestingly, running offered no significant benefit over walking regiments.[7] Hence, it was concluded that equivalent energy expenditures by moderate (walking) and vigorous (running) exercise produced similar risk reductions for T2D.

Basic Mechanisms of Cellular Perturbation and Relevance to Diabetes Mellitus

Endothelial dysfunction has long been considered an early and fundamental perturbation that contributes to the micro-and macrovascular complications of diabetes mellitus. Based on the role of dyslipidemia in the development of endothelial dysfunction, human microvascular endothelial cells (ECs) were treated with fenofibrate, a ligand of peroxisome proliferator activated receptor-α. Fenofibrate mediated a reduction in endothelin-1 expression in ECs by 2 distinct mechanisms; first, via peroxisome proliferator activated receptor-α, fenofibrate induced transcription of the Krüppel-like factor 11 repressor, and second, by peroxisome proliferator activated receptor-α–independent actions via inhibition of glycogen synthase kinase-3 activity.[8] In other studies, the relationship between EC and cardiomyocyte interaction in conditions of high glucose was examined. Bovine coronary artery ECs were exposed to high glucose and the conditioned medium was used to treat cardiomyocytes. This resulted in a release of lipoprotein lipase from the surface of the cardiomyocytes because of increased heparanase content in the medium from the ECs, in addition to facilitation of its replenishment.[9] These studies highlighted that cross-talk between the EC and the cardiomyocyte might contribute to cardiac dysfunction in diabetes mellitus.

Smooth muscle cells also integrally contribute to the pathogenesis of diabetic complications. In a recent study, vascular smooth muscle cells from the aortas of T2D db/db mice were shown to exhibit higher levels of miR200; experiments using mimics and inhibitors of this miR showed that in diabetic conditions, this miR contributed to increased expression of proinflammatory genes, such as *Ptgs2* and *Ccl2*.[10]

Finally, a study in macrophages addressed the pathophysiologic effects of stearic acid, as free fatty acids are established risk factors in T2D for cardiovascular disease. The accumulation of these fatty acids in macrophages resulted in activation of inflammatory signaling and induction of endoplasmic reticulum stress–induced apoptosis and the M1 proinflammatory polarization.[11] The authors concluded that their experiments in macrophages provided a mechanistic underpinning for the known links between free fatty acids and risk factors in diabetes mellitus for cardiovascular disease.

Studies in primary cultured cells relevant to diabetes mellitus and its complications have many strengths; by removing confounding cell type cross-talk or antagonism in heterogeneous whole tissue, researchers may isolate the relevant signaling and transcriptional pathways to the cell of interest. The down-side is, however, that such cells are removed from the paracrine and cell–cell contact cues that may heavily modulate the overall outcomes in the tissue. In the sections to follow, we detail the numerous studies published recently in *ATVB* that tested the effects of type 1-like or type 2-like diabetes in small and large animal models.

Uncovering Mechanisms of Diabetic Complications in Rodent Models

Reactive oxygen species have been speculated to play important roles in the pathogenesis of diabetic complications. The full range of sources and impact of reactive oxygen species is yet to be discovered. Studies in db/db mice uncovered roles of reactive oxygen species perturbation in the endothelium; inhibition of bone morphogenetic protein 4 and activin receptor-like kinase-3 reduced oxidative stress in mice and in aortas and improved endothelial dysfunction.[12] In other studies, Nox4 was shown to adversely affect insulin receptor signaling in T2D.[13]

Other studies tested the interplay between inflammatory networks and endothelial dysfunction in diabetes mellitus. These studies revealed that mutation of toll like receptor 4 in T2D mice resulted in protection against endothelial dysfunction, despite the sustained development of obesity in the mouse model.[14] Roles for Krüppel like factor 11 in mediation of endothelial inflammation were shown using mice devoid of this factor.[15] In diabetic mice and in ECs exposed to high glucose, roles for the 26S proteasome in activation of the nuclear factor-kB signaling pathway in endothelial inflammation were demonstrated.[16]

As a means to image and track the microvasculature, a study in the microcirculation using synchrotron cine-angiograms revealed that early stage diabetes mellitus induced by streptozotocin is associated with localized coronary microvascular endothelial dysfunction.[17] Thus, using such technology, it might be possible to discern the sites, timing, and extent of the development of microvascular dysfunction in diabetes mellitus.

In specific complications, in the heart, novel roles for the heparanase-lipoprotein lipase-vascular endothelial growth factor axis were shown in amplification of fatty acid delivery, and, thereby, uncovered mechanisms underlying potential lipotoxicity, if left unchecked.[18] In wound healing, derangement of the relationship between miR-200b and transcription factors occurs in diabetes mellitus. Downregulation of miR-200b derepresses globin transcription factor binding protein 2 and VEGFR2 expression and this switches on wound angiogenesis; this process was shown to be deranged in diabetic wounds.[19] In the macrovasculature, roles for protein kinase C-β in diabetic atherosclerosis were shown.[20,21] The impact of hyperactive platelets in diabetes mellitus was illustrated, which could contribute to increased thrombotic risk.[22]

In parallel with increased inflammation, oxidative stress, and endoplasmic reticulum stress, a series of published studies showed that bone marrow angiogenic cells were dysfunctional in diabetes mellitus. Upregulation of thrombospondin 2 expression in diabetes mellitus was

shown to contribute to angiogenesis dysfunction in bone marrow angiogenic cells.[23] Hierarchically, miR27b rescued impaired bone marrow angiogenic cell angiogenesis via suppression of thrombospondin 2.[24] In other studies, bone marrow ECs in diabetes mellitus were shown to display activation of RhoA/Rho-associated kinase and Src/vascular endothelial cadherin signaling pathways in mechanisms that were redox dependent. These findings, together with inactivation of Akt, contribute to endothelial dysfunction in diabetic bone marrow.[25]

Taken together, these studies underscore the significant derangements at multiple levels of molecular and biochemical regulation, which contribute to tissue-specific complications.

Studies in Large Animal Models: Hyperglycemic Swine

In addition to more human-like atherosclerotic lesions, swine offer the unique opportunity to serially image coronary vessels because of the location and size of the lesions. Coronary angiography and 3-vessel intravascular ultrasound were performed serially in diabetic, hyperlipidemic swine and the impact of low versus high endothelial shear stress on vascular lesion formation was examined. It was shown that atherosclerotic lesions consistently exposed to low shear stress, that is, <1.2 Pa, revealed increased collagenolytic activity, in parallel with reduced collagen content and marked thinning of the fibrous cap.[26] Because these types of lesions are more prone to rupture, the studies may highlight mechanisms of infarction in diabetes mellitus. In other studies, proteomic approaches showed that in stent restenosis in stented lesions of swine expressed higher levels of adipocyte fatty acid binding protein in both diabetic and nondiabetic animals. Studies in cultured primary smooth muscle cells revealed novel roles for this factor in growth and migration in a manner dependent on reactive oxygen species.[27] Finally, near infrared spectroscopy coupled with multivessel intra-vascular ultrasound in diabetic, hypercholesterolemic pigs revealed that near infrared spectroscopy predicted the development of inflamed fibroadenoma plaques in the animals.[28] Hence, imaging techniques that may be of ultimate predictive benefit in human subjects are undergoing rigorous and serial testing in large animal models of diabetes mellitus and atherosclerosis.

Translational Studies: Mechanisms of Diabetic Complications and Potential Therapies in Human Subjects

Rounding out the recent series of studies in *ATVB* on the subject of diabetes mellitus were several key studies in human subjects that addressed important facets of vascular perturbation—from endothelial dysfunction to lipid abnormalities to the benefits of putative therapeutic interventions.

Given the importance of microvascular dysfunction in diabetes mellitus, this subject was addressed in human subjects. One question addressed, what is the specific effect of diet on endothelial function? These studies were performed in non-diabetic human subjects to determine the innate effects of dietary patterns on the vasculature. Using finger skin capillaroscopy, a dietary pattern consisting of higher consumption of vegetable oils, poultry, and fish and seafood, together with low consumption of sweets was associated with optimal

microvascular function.[29] A major question is whether these findings may be of benefit in diabetic subjects, or is the die long cast to damage the endothelium much earlier in the course of diabetes mellitus? Testing such diets in human diabetic subjects may be feasible and may highlight lifestyle intervention vascular-protective strategies in this disorder. This remains to be tested.

In mechanistic studies in endothelial dysfunction, roles for diabetes mellitus in affecting nitroglycerin-induced vasodilation were uncovered.[30] In other studies, arterioles isolated from human gluteal subcutaneous adipose revealed that mitochondrial dysfunction was elevated in the subjects with diabetes mellitus and that correction of such dysfunction improved vascular function.[31] Examination of biomarkers of impaired wound healing in diabetes mellitus revealed that levels of soluble interleukin-33 receptor ST2 were increased in diabetic versus nondiabetic subjects and that these elevated levels represented a predictive marker of 1-year mortality.[32] In peripheral arterial disease, elevated skin autofluorescence, a putative biomarker for the formation and increased accumulation of advanced glycation endproducts, was observed in human subjects with peripheral arterial disease versus control subjects even after consideration of usual cardiovascular risk factors, thereby suggesting that skin autofluorescence may reflect underlying mechanisms linked to increased vascular dysfunction.[33]

Several recent articles in *ATVB* addressed the role of lipid abnormalities in diabetes mellitus and mechanistic links to vascular disease. In men with T2D optimally treated with statins, administration of extended release niacin imparted beneficial outcomes; extended release niacin resulted in increased high-density lipoprotein apolipoprotein AI concentration by lowering the apolipoprotein AI fractional catabolic rate.[34] In human subjects with T2D, retinol binding protein 4 was found to be implicated in the pathophysiology of hypertriglyceridemia by reducing very low-density lipoprotein catabolism.[35] Finally, insulin is known to acutely inhibit both intestinal and hepatic triglyceride-rich lipoprotein production, and the inhibitory effect on very low-density lipoprotein production is known to be blunted in T2D but by unknown mechanisms. In experiments using stable isotope techniques, it was shown that in T2D, chylomicron production is resistant to the normal acute suppressive effects of insulin, thereby potentially explaining, at least in part, the known dyslipidemia observed in diabetes mellitus.[36]

Coronary artery calcification and carotid intima-media thickness are 2 surrogate markers of atherosclerosis. Recent work showed that even in nondiabetic subjects, higher levels of glycosylated hemoglobin showed a modest and independent association with increasing coronary artery calcification.[37] In a large study performed in 7 centers in Finland, Sweden, The Netherlands, France, and Italy, no independent relationships between 25(OH)D (vitamin D) and segment-specific or composite intima-media thickness measures were observed in the overall cohort.[38] Even in analyses stratified by diabetes mellitus state, no consistent or independent effects of vitamin D levels on carotid intima-media thickness were noted. In subjects vulnerable to the development of diabetes mellitus, so-called prediabetes mellitus, administration of pioglitazone for an average follow-up time of 2.3 years revealed slowed progression of carotid intima-media thickness.[39] Interestingly, the effects of pioglitazone were likely to be direct on the vasculature, as the effects of the agent were independent of

improvements in hyperglycemia, insulin resistance, dyslipidemia, and systemic inflammation in the prediabetic subjects.

Finally, these human-based studies and the animal-based experimentation addressed mechanisms leading to increased risk for atherosclerosis and its consequences in diabetes mellitus. The question arises, do diabetic subjects display unique responses to plaque hemorrhage? Haptoglobin (Hp) is a protein that plays roles in hemoglobin clearance after intraplaque hemorrhage. There are 2 alleles of the *HP* gene, that is, Hp-1 and Hp-2 alleles. In plaques from human diabetic atherosclerosis, it was reported that plaques with Hp-2-2 displayed increased hemorrhage, iron content, and reduced CD163 expression compared with controls. These Hp-2-2 plaques demonstrated increased heme-oxygenase-1 protein, myeloperoxidase gene, and protein.[40] In parallel with increased inflammation, in Hp-2-2, decreases in the anti-inflammatory IL10 gene were noted, together with increased macrophage content, expression of vascular cell adhesion molecule-1, smooth muscle actin scores, and neovessel density. These data suggested that Hp-2-2 in diabetes mellitus was associated with impaired hemoglobin clearance after rupture, in parallel with increased oxidative, inflammatory, and proangiogenic stresses in atherosclerotic lesions.

Summary

In summary, the significant rise in types 1 and 2 diabetes mellitus is poised to impart grave consequences on health and lifespan of the world's population if left unchecked. Recent publications in *ATVB* highlight progress toward the identification of the fundamental mechanisms, and their interplay, that contribute to derangements in endothelial function, oxidative and inflammatory stresses in diabetes mellitus to mediate complications. Studies reported from primary cells contribute to elucidation of the key signaling mechanisms underlying complications and are buttressed by hypothesis-driven research in animal models. Using genetic modulation strategies, modulators of miRs, pharmacological agents and siRNA knockdown strategies, observations and associations progress to causality in diabetes mellitus. In large animal models of diabetes mellitus and atherosclerosis, efforts to improve imaging and diagnostic capabilities with respect to atherosclerotic plaques hold promise for extrapolation to human subjects, given the similarity between the 2 species in vessel size and anatomic characteristics. The messages from these studies are brought home to the human subject in multiple papers that both suggest means to reduce diabetes mellitus risk and to mitigate the complications. Surely, we are getting closer to identifying fundamental causative pathways in complications—the challenge is to develop therapeutic strategies for rigorous testing in human subjects. *ATVB* will continue to track the promise of basic, translational, and clinical research in diabetes mellitus and its complications.

Acknowledgments

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