recapitulates human AVF maturation, including decreased maturation and patency in female mice, we hypothesize that sex hormones mediate sex differences during AVF maturation.

**Methods:** C57Bl/6 mice (aged 9-11 months) were treated with sham or aorticaval AVF surgery, some were pretreated with gonadectomy 1 week before surgery. AVF diameter was measured via ultrasound examination (days 0-21). Blood was collected for fluorescent activated cell sorting, and tissue examined using immunofluorescence or enzyme-linked immunosorbent assay (days 3,7). Wall thickness was assessed using histology (day 21).

**Results:** At baseline, female and male mice had similar inferior vena cava (IVC) diameters (P = 0.61) and wall thicknesses (P = 0.49). After AVF creation, female mice had an increased normalized IVC diameter (P = 0.0012), flow velocity (P = 0.0013), and shear stress (P = 0.002, days 3-21; n = 10); however, there was no difference in wall thickness (female: 15.8 ± 0.2 μm; male: 12.4 ± 2.3 μm. P = 0.68; n = 10). There were also increased circulating CD11b+ macrophages in female compared with male mice (31.8% ± 4.0% vs 18.7% ± 1.9%. P = 0.01; day 3, n = 22) and CD4+ T cells (57.9% ± 2.9% vs 34.5% ± 6.1%. P = 0.001). In the AVF wall there were increased CD4+ cells on immunofluorescence in females (22.5 ± 1.5 cells/high-power field vs 15 ± 2 cells/high-power field, P = 0.06; day 6; n = 4) as well as increased IL-10 immunoreactivity (5.96 ± 0.44 ng/g vs 2.49 ± 0.47 ng/g, P = 0.067). In mice with gonadectomy, baseline IVC diameter, velocity, and shear stress were similar between female and male mice. After gonadectomy, male mice had increased AVF wall thickness than intact males (24.2 ± 1.6 μm vs 12.4 ± 2.3 μm, P = 0.014), conversely, female mice had decreased thickness than intact females (6.1 ± 0.6 μm vs 15.8 ± 0.2 μm. P = 0.005). There was loss of differences among circulating immune cells after gonadectomy (CD11b+ vs 0.088; CD4+ vs 0.700).

**Conclusions:** Sex differences in hemodynamics and inflammation are present during venous remodeling, whereas these differences disappear after gonadectomy. Sex hormones mediate AVF maturation and suggest that hormone receptor signaling may be a target to improve AVF maturation.


### 22-VIRC-505-AHA-VD

**MOF Expression Regulates Interferon β in Diabetic Wound Macrophages and Impairs Tissue Repair**

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**Objective:** Following tissue injury, monocytes are recruited to the site of injury and differentiate into proinflammatory macrophages (M&delta;). As wound healing progresses, these cells transition to an anti-inflammatory phenotype and promote tissue repair. Conversely, in type 2 diabetic (T2D) wounds the transition of M&delta; to an anti-inflammatory phenotype does not occur. This causes M&delta; to remain in a chronic inflammatory state, effectively preventing wound resolution. The molecular mechanisms controlling M&delta; plasticity are not fully understood. Our prior work has focused on epigenetic-based mechanisms that lead to persistent expression of inflammatory genes. Specifically, we have identified that the histone acetyltransferase MOF is involved in regulating wound M&delta; phenotype.

**Methods:** Using mice that are deficient in M&delta; in their monocytes/macrophages (M&delta;Lyzz2−/−), we identified that tumor necrosis factor α receptor signaling induces MOF transcription in wound macrophages and that MOF is increased in T2D wound M&delta;. We also found that MOF acetylates interferon regulatory factor 3 (IRF3) in M&delta;, resulting in repression of downstream genes, including interferon β (IFNB).

**Results:** This is important; we have previously shown that IFNB expression is decreased in T2D wounds and that IFNB is critical for the downregulation of inflammatory genes in M&delta; during the transition from a proinflammatory to an anti-inflammatory phenotype.

**Conclusions:** Taken together, these data suggest that tumor necrosis factor α-induced MOF expression in wound macrophages regulates IFNB via IRF3.

**Figure 1:**


### 22-VIRC-499-AHA-VD

**Upregulation of the Absent-in-melanoma 2 Inflammasome Correlates with Markers of DNA Damage and Cell Cycle Dysregulation in Peripheral Arterial Disease**

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**Background:** We showed that myoblasts (Mb) in peripheral arterial disease (PAD) differentiate poorly and have evidence of inflammasome activation. Absent-in-melanoma 2 (AIM2), an inflammasome and mediator of pyroptosis, binds damaged DNA, which also triggers the G2/M DNA damage checkpoint including CDC2 and its regulator, polo-like kinase 1 (PLK1). Dysregulated checkpoints can cause faulty replication, but a link between checkpoint function and AIM2 is not known. We hypothesize that AIM2 expression in PAD Mb correlates with increased DNA damage and a dysregulated G2/M DNA damage checkpoint.

**Methods:** Cells were harvested from ischemic and perfused muscle obtained during amputations for PAD. Mb (Pax7+/MyoD+) were isolated using sequential plating and cell sorting. Mb from healthy donors (PAD−) were purchased (Cook MyoSite). AIM2, γH2AX (DNA damage marker), PLK1, and CDC2 were measured with immunofluorescence (five fields) with an anti-γH2AX antibody.

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images/dish). Mb were treated with the PLK1-inhibitor BI2536 (10 nmol/L; 1 hour) to assess checkpoint responsiveness. Analysis of variance with post hoc analysis confirmed statistical significance (\( \alpha = 0.05 \)).

**Results:** \( \gamma \)H2AX expression was elevated in all PAD groups compared with PAD\(^{-} \) Mb (n = 3-4/PAD group). While PLK1 expression was similar across groups, CDC2 was lower in all ischemic PAD groups relative to perfused PAD Mb. BI2536 attenuated CDC2 expression in perfused PAD Mb (P = 0.06), whereas Mb in ischemic PAD remained unresponsive. AIM2 expression was significantly higher in ischemic Mb compared with perfused and PAD\(^{-} \) Mb, correlating with high \( \gamma \)H2AX and low CDC2. Results are summarized in the figure.

**Conclusions:** Here, we show that in PAD Mb, AIM2 correlates with DNA damage, attenuated DNA damage checkpoint protein expression, and diminished CDC2 responsiveness to BI2536. Thus, AIM2 may promote pyroptosis in the setting of defective DNA damage mechanisms that would otherwise prompt apoptosis. Whether these pathways are reversed by revascularization is a topic of further study.

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**22-VIRC-487-AHA-VD**

**Modifiable Mesenchymal Stem Cell Defects in Veterans with Diabetes Mellitus**

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**Background:** Diabetes mellitus (DM) affects 34.2 million Americans. DM impairs the body’s reparative machinery leading to early onset chronic illness (peripheral arterial disease [PAD], neuropathy, nephropathy, and retinopathy, the latter being hallmarks of microvascular disease). Patients with DM and PAD have increased risk of major amputation. Mesenchymal stem cells (MSCs) are reparative cells found in all tissues providing paracrine and trophic support for new tissue. This study’s objective was to identify intracellular and epigenetic mechanisms of how DM impairs MSC function and test if these defects are modifiable with culture rejuvenation.

**Methods:** MSCs obtained from bone marrow of 13 consecutive male veterans undergoing lower limb major amputation were cultured in 10% fetal bovine serum or 5% human platelet lysate for culture rejuvenation. MSCs obtained from bone marrow of 13 consecutive male veterans undergoing lower limb major amputation were cultured in 10% fetal bovine serum or 5% human platelet lysate for culture rejuvenation. Groups were DM and PAD (n = 8) and PAD with no DM (n = 5). Intra-cellular signaling was analyzed with multiplexed enzyme-linked immunosorbent assay. Epigenetic differences were identified by ATAC-seq, sequencing.

**Results:** DM and PAD MSCs had modifiable AKT signaling defects with platelet lysate (Fig 1) and a discrete DNA profile identified in their introns and intergenic regions (Fig 2). MSCs from PAD alone had increased transcription factor binding at Wnt and cGMP-PKG pathways (P = 0.04). DM and PAD MSCs had increased binding at MAPK (P = 0.01) and Rap1 (P = 0.01) pathways.

**Conclusions:** DM is a complex disease disrupting reparative mechanisms and can lead to major complications. MSC dysfunction in DM may have common mechanisms throughout the body. We have identified potentially druggable pathways that may provide therapeutic solutions to relieve chronic illness for endogenous MSCs and to expand MSC donor pool for regenerative medicine strategies.

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**22-VIRC-491-AHA-VD**

**Phosphodiesterase 10A Regulates Medial Artery Calcification**

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**Background:** Vascular calcification results from deposition of calcium hydroxyapatite crystals in the vessel wall. It is highly prevalent in patients with chronic kidney disease (CKD), diabetes, and peripheral artery disease. In lower extremity arteries, elevated calcification levels are associated with an increased risk of ischemic events including amputation. The cyclic nucleotides cyclic adenosine monophosphate and cyclic guanosine monophosphate, controlled by distinct cyclic nucleotide...