based RNA in situ sequencing method to unravel their distinct location within these plaques.

Conclusions: Taken together, our datasets, methods and different animal-models demonstrate that combining bulk with scRNA-seq data and spatially resolved sequencing methods are powerful tools to identify and characterize novel lncRNAs being expressed by a certain cell-type in the disease progression.

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22-VIRC-529-AHA-VD

Systematic Review and Mendelian Randomization of Plasma Biomarkers to Predict Their Causal Role in Peripheral Artery Disease Pathophysiology

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Background: Observational analyses have described hundreds of biomarkers for peripheral artery disease (PAD). These studies can be limited by sample size, lack of replication, residual confounding, and reverse causality. To assess this, we performed a systematic review of the literature and leveraged genetic approaches to causal inference.

Methods: We performed a systematic literature review for terms related to PAD and/or biomarkers using PubMed, the Cochrane database, and Embase, followed by manual review to extract biomarkers and their direction of effect. To test for evidence of causality we used two-sample Mendelian randomization. We developed genetic instruments for the biomarkers by mapping them to genome-wide association studies (GWAS) of circulating biomolecules agglomerated in the IEU Open GWAS project. We tested the association of the genetic instruments with PAD using summary statistics from a GWAS of 31,307 individuals with and 211,753 individuals without PAD in the VA Million Veteran Program. We used the Wald ratio or inverse variance weighted Mendelian randomization; weighted median and weighted mode methods were applied as sensitivity analyses.

Results: After manual review, we identified 159 unique papers mentioning 268 unique PAD biomarkers. We mapped 76 biomarkers to genetic data, 19 of which were nominally associated with PAD ($P < .05$). After accounting for multiple testing (false discovery rate of <0.05), 12 remained significant, of which only 7 had concordant directions of effect with published reports: ApoB, ApoA1, high-density lipoprotein-associated cholesterol, triglycerides, Von Willebrand factor, cadherin-5, and b2-microglobulin.

Conclusions: This systematic review paired with genetic causal inference illuminates key biomarkers causally relevant to PAD, and highlights discrepancies between observational and genetic findings. This highlights the importance of rigorous analysis of observational biomarker data and the opportunity to leverage human genetics to inform these studies.

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Ketosis Prevents Abdominal Aortic Aneurysm Progression and Rupture in Male Rats

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Background: Abdominal aortic aneurysms (AAA) are common in aging populations, and its rupture is associated with high mortality. There is no effective medical therapy for the prevention of AAA expansion and rupture. Ketone bodies (KB) are argued to produce novel responses against injury. Serum KB are elevated through a ketogenic diet (KD) or the intake of exogenous KB (EKB). We hypothesize that ketosis can reduce AAA expansion and risk of rupture.

Methods: Male Sprague-Dawley rats underwent intraluminal exposure to porcine pancreatic elastase to induce AAA formation. Rats were also administered daily β-aminopropionitrile to promote AAA rupture. Control rats (n = 12) were fed a standard diet (SD). Treated rats were given either a KD (n = 9) or EKB (n = 10) starting 3 days after AAA induction. Ketosis was verified by blood beta-hydroxybutyrate level of greater than 0.5 mmol/L. The in vivo aortic diameter was evaluated by ultrasound examination.

Results: After 2 weeks, surviving animals were harvested, and AAA tissue analysis was performed. Rupture AAA was determined by necropsy. Rats treated with KD consistently remained in ketosis, while rats treated with EKB remained in ketosis for only 8 hours per day. AAA size was significantly reduced ($P < .01$ and $P < .05$) in both KD and EKB groups at weeks 1 and 2. Rupture rate was also reduced in the KD and EKB groups (22% and 40%, respectively) compared with 67% in the SD group ($P = .03$ and .12, respectively). AAA trichrome staining demonstrated greater content of collagen in KD and EKB groups when compared with SD group ($P = .08$ and .02, respectively). AAA gelatin zymography demonstrated significantly decreased active matrix metalloproteinase (MMP)-9 levels in the KD and EKB groups ($P < .05$) while total MMP-2 was attenuated in the KD group ($P < .01$). Ketosis appears to reduce AAA expansion and risk of rupture. This may have resulted from AAA increased collagen deposition as well as decreased MMP activity. These findings provide initial impetus to investigate whether ketosis in patients with small AAAs can prevent expansion and risk of rupture.

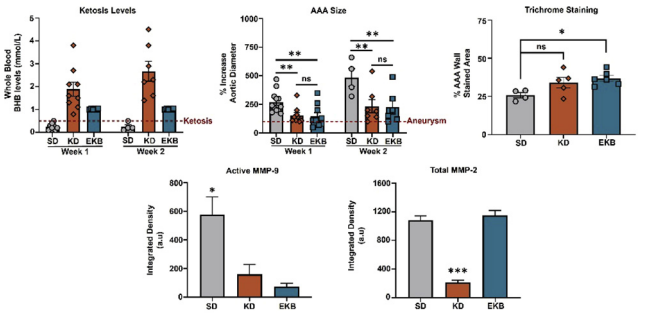

Fig.

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22-VIRC-616-AHA-VD

Effects of Human Angiotensinogen and Human Renin in Proximal Tubule Cells on Development of Atherosclerosis in Hypercholesterolemic Mice

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Background: This study determined whether angiotensinogen (AGT) interacted with renin in renal proximal tubule cells (PTCs) to promote atherosclerosis.

Methods: Since hepatocyte-produced AGT can be filtered by glomeruli and retained in renal PTCs, we first determined whether hepatocyte-derived AGT interacts with renin in PTCs to promote atherosclerosis. Transgenic mice expressing human renin in PTCs driven by a kidney androgen-related protein promoter (KAP-hREN) in an $LDLR^{-/-}$ background were used. To induce synthesis of human AGT in hepatocytes, an adeno-associated viral vector (AAV) containing human AGT with a liver-specific promoter was injected intraperitoneally. Three groups of male littermates were administered testosterone to activate human renin expression in PTCs: (1) wild-type mice administered null AAV, (2) KAP-hREN transgenic mice administered null AAV, and (3) KAP-hREN transgenic mice administered AAV containing human AGT. Two weeks after administration of testosterone and AAVs, mice were fed a Western diet for 6 weeks. Induction of human AGT in liver and human renin in