or mixed cell populations. By studying the effect of IFNβ/α activity ratio on individual monocytes, we can determine the functional impact of the IFN ratio and suggest the cellular mechanisms that underlie response/non-response to TNFi therapy in RA.

METHODS/STUDY POPULATION: We used single cell analysis to investigate whether monocyte gene expression differs significantly between RA patients according to their pre-TNFi serum IFN-β/α ratio. Single classical (CL) and non-classical (NC) blood-derived monocytes were isolated from 15 seropositive RA subjects prior to biologic therapy. Subjects were grouped by pre-TNFi serum IFN-β/α ratio into two groups, those with a high IFN-β/α ratio (≥1.3, n = 6) and those with a low IFN-β/α ratio (<1.3, n = 9). 87 target genes were analyzed. Genes that varied significantly between the groups by categorical analyses were tested in multivariate logistic regression models.

RESULTS/ANTICIPATED RESULTS: Every participant was seropositive for rheumatoid factor and antibodies to cyclic citrullinated peptide. Among the participants in the groups, there were no significant differences in age or DAS scores (P >0.05). The treatments were comparable and none were being treated with biologic therapy. There were striking differences in monocyte gene expression between patients with pre-treatment blood IFNβ/α activity <1.3 and ≥1.3. Expression of (1) key type I IFN pathway genes (JAK1, STAT2, IFT2, IFIH1, PRDM1); (2) IL12; (3) CD36; and (4) CTLA4 were the strongest differentiators between groups (P <0.0001 for each, corrected for multiple comparisons).

DISCUSSION/SIGNIFICANCE OF IMPACT: In this study we were able to measure gene expression in single monocytes from seropositive RA patients prior to biologic treatment. Within-cell co-expression patterns demonstrate biological differences in monocytes of RA patients with an IFNβ/α ratio ≥1.3, the ratio of I IFNs which predicts non-response to TNFi. The data suggest that there may be differential IFN production and pathway activation in patients who do not respond to TNFi. The increased expression of CD36 in monocytes from RA patients with high IFNβ/α activity may be a reflection of increased "foam cells" in the inflamed tissue of patients who do not respond to TNFi. Enrichment of CTLA4 in those with high serum IFNβ/α suggests that CTLA4-Ig may be less likely to be an effective alternative for someone who is not likely to respond to TNFi. Current work includes determining whether the peripheral blood findings reflect altered cellular composition, type I IFN production and signaling in the synovium. Significance: This work will help to develop a more individualized approach to therapy in RA and determine an immunological basis of response/non-response to TNFi.

Dynamic Afterload Cardiac Microtissue Model To Examine Molecular Pathways of Heart Failure
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OBJECTIVES/SPECIFIC AIMS: This project aims to determine the key molecular pathways that link increased myocardial wall stress to cardiomyocyte hypertrophy and subsequent heart failure. We will use a cardiac microtissue (CMT) model with dynamically tunable cantilever stiffness to examine changes in CMT hypertrophy and electro-mechanical properties in response to increased afterload (cantilever stiffness). Subsequently, we will determine if inhibition of pro-hypertrophic or anti-hypertrophic pathways alter the hypertrophic response to increased afterload. Primary outcomes for this study are static/dynamic force, minimum electric field strength (VT), maximum capture rate (MCR), average cell area, and tissue cross-sectional thickness, and secondary outcomes are degree of myoblast activation and apoptosis. METHODS/STUDY POPULATION: CMT platforms will be fabricated using iron-doped polydimethylsiloxane (PDMS) to create magnetically tunable cantilevers. Cantilever stiffness will be increased with the application of an external magnetic field. Cantilever stiffness will be measured using a capacitance probe, where the force required to deflect both the cantilever and calibration probe is in accordance with Hooke’s Law. Human induced pluripotent stem cell cardiomyocyte (hiPSC-CMs) will be cultured and matured as 3D CMTs. In-vitro static/dynamic force generation will also be calculated by measuring the deflection of the cantilevers and applying Hooke’s law. CMTs will be paced using carbon electrodes to obtain VT and MCR. Structural data will be obtained using immunostaining and confocal microscopy. Finally, we will use pharmacologic inhibitors to inhibit molecular pathways that we identified in prior genetic screens such as ABCC8 (anti-hypertrophic mediator) and CIQ6K6F9 (pro-hypertrophic mediator). We will examine each of these pathways in low- and high-stiffness conditions. RESULTS/ANTICIPATED RESULTS: We believe increased afterload will cause significant hypertrophy, measured by increases in CMT cross-sectional thickness, cardiac myocyte area, myofibroblast activation, and myocyte apoptosis. In addition, we expect to see increases in static/dynamic force, increased voltage threshold, and decreased maximum capture rate. Preliminary results show a 64.3% increase in force generation when stiffness is increased by approximately 30%, and a 44.4% decrease in force generation when stiffness is decreased by approximately 30%. Finally, we expect that inhibiting a pro- or anti-hypertrophic molecular pathway will weaken or strengthen the hypertrophic response to increased afterload, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: To our knowledge, our lab is the first to create a dynamically tunable afterload system in the cantilever CMT model. This advance provides us with a robust platform to determine the molecular pathways that cause increased myocardial wall stress to result in cardiomyocyte hypertrophy and heart failure, which remain a critical knowledge gap in our understanding of cardiovascular disease. With more precise understanding of these pathways, we will equip ourselves with the knowledge to develop novel therapeutic agents to prevent the development or progression of heart failure.

Effects of Early Life Stress on Adult Behavioral and Neural Outcomes in Rats
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OBJECTIVES/SPECIFIC AIMS: Early life stress is known to greatly impact neurodevelopment during critical periods, conferring risk for various psychopathologies, including the onset and exacerbation of schizophrenia and anxiety disorders. The endocannabinoid system is highly integrated into the stress response and may be one means by which early life stress produces such deleterious effects. Using a naturalistic, ecologically valid animal model, this study explored interactions between the stress response and endocannabinoid systems within the cerebellum, a region dense with the CB1 endocannabinoid receptor.
Effects of Local Interleukin-6 on Mitochondrial Physiology in Skeletal Muscle

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OBJECTIVES/SPECIFIC AIMS: In the context of skeletal muscle, IL-6 plays a major role in muscle quality. The goal of this project was to study the influence of systemic IL-6 on skeletal muscle mitochondrial physiology, most notably mitochondrial function (respiration and ROS production) and mitochondrial content. METHODS/STUDY POPULATION: To determine the influence of interleukin-6 (IL-6) on skeletal muscle mitochondria, high-resolution respirometry was performed to simultaneously measure oxygen consumption (JO2) and ROS production in differentiated myotubes incubated with increasing IL-6 (0, 10, 50, 100 ng/mL) for 18 hours in serum free conditions. To evaluate the impact of IL-6 on mitochondrial content we performed western blots on cell lysates from treated cells, measuring proteins of the mitochondrial electron transport chain (ETC) using a cocktail antibody and PGC-1α/PGC-18 for mitochondrial biogenesis. To determine the role of mitochondrial ROS production on JO2 and mitochondrial content, we co-treated differentiated myotubes for 18 hours with 50 and 100ng/mL IL-6 and the mitochondrial specific antioxidant, MitoQ and performed respirometry for mitochondrial functional measurements and western blots for mitochondrial content. Statistical significance was evaluated by using a 2-tailed Student’s t-test and two-way ANOVA. Post hoc all-group analyses were conducted to determine which groups were different when the model was significant. RESULTS/ANTICIPATED RESULTS: Mitochondrial functional measurements show increased JO2 and increased ROS production in an IL-6 dose-dependent manner. Targeting mitochondrial ROS production with 0.5μM MitoQ attenuated IL-6 induced increases in JO2 and ROS production. Complexes I and II (CI, CII) of the ETC increased significantly in an IL-6 dose-wise fashion, and co-treatment with MitoQ normalized increases at 100ng/mL IL-6. 100ng/mL IL-6 significantly increased protein expression of PGC-1α and PGC-18. Co-treatment with MitoQ normalized IL-6 induced increase in PGC-1α. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data suggest that when treated chronically at a high dose, IL-6 increases mitochondrial respiration, ROS production, and content. Targeting mitochondrial ROS production normalizes these mitochondrial adaptations. The present study provides new insights into mitochondrial physiology in the context of inflammation. Therapeutically targeting mitochondrial ROS production may impact skeletal muscle quality in certain populations.

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European Ancestry as a Risk Factor for Atrial Fibrillation in Puerto Rican Hispanics

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OBJECTIVES/SPECIFIC AIMS: Consequently, we have decided to evaluate the presence of single nucleotide polymorphism (SNP) previously associated with AF on a European-descent population in an attempt to first identify the most common loci present in the PRH population and then search for specific PRH SNP associated with AF. METHODS/STUDY POPULATION: A secondary analysis of a Puerto Rican population sample (n = 120) from The Pharmacogenetics of Warfarin in Puerto Ricans Study will be performed. We will implement data from the 1000 genome project to establish a control group of healthy PRH population. Will evaluate the presence of 111 known single nucleotide polymorphisms associated with AF in Europeans and determine the frequency in PRH population sample, and validate predictability of such SNPs. Using admixture informatic markers (AIM) analysis will determine the percentage of admixture by Yoruba, Native American and Iberic-European. Statistical analysis will include the use of the Pearson Product-Moment Coefficient correlation analysis and multivariate linear regression. For admixture will use Maximum Likelihood Estimation and Markov Chain Monte Carlo models. RESULTS/ANTICIPATED RESULTS: A higher frequency of AF associated European single nucleotide polymorphisms, and an overall higher percentage of European admixture will be associated with atrial fibrillation in Puerto Rican Hispanic patients.