HUVECs and downregulated the expression of genes involved in alloantigen presentation through the class I major histocompatibility complex (IRF1, TAP1, B2M), and reduced the activation and pro-inflammatory polarization of co-cultured allogeneic lymphocytes. Consequently, RNA-Seq analysis of these lymphocytes showed that treatment with MXene nanosheets downregulated genes responsible for transplant-induced T-cell activation, cell-mediated rejection, and development of allograft vasculopathy. Furthermore, gene set enrichment analysis revealed significant negative enrichment of genes involved in interferon alpha/beta and interferon gamma signaling. Finally, in an in vivo rat model of allograft vasculopathy, treatment with Ti3C2Tx MXene nanosheets reduced lymphocyte infiltration and preserved medial smooth muscle cell integrity within transplanted aortic allografts.

CONCLUSION: These findings support the potential of Ti3C2Tx MXene nanosheets for prevention and treatment of allograft vasculopathy and other inflammatory diseases. This research also opens the door to development of Ti3C2Tx MXene technologies for other immune-sensitive regenerative medicine applications.

Canadian Institutes of Health Research - Fellowship, Canadian Institutes of Health Research (CIHR)

P012
INCREASED EICOSAPENTAENOIC ACID TO ARACHIDONIC ACID (EPA/AA) RATIO IN BRAIN ENDOTHELIAL CELLS ASSOCIATED WITH REDUCED EXPRESSION OF NEUTROPHIL DEGRANULATION PROTEINS DURING INFLAMMATION

S Sherratt, P Libby, D Bhatt, P Mason

Beverly, Massachusetts

BACKGROUND: Endothelial cell (EC) dysfunction results in inflammation and increased atherothrombotic risk, including stroke. The omega-3 fatty acid (FA) eicosapentaenoic acid (EPA) improves arterial compliance in a manner predicted by circulating EPA/AA ratios, an indicator of cardiovascular (CV) risk. Treatment with icosapent ethyl (IPE), a formulation of highly purified EPA, reduced composite CV events, including stroke, in REDUCE-IT that correlated with on-treatment EPA levels. We measured the effects of EPA on the EPA/AA ratio and protein expression in endothelial cells from brain ECs (BECs) following cytokine challenge.

METHODS AND RESULTS: BECs were challenged with IL-6 (12 ng/mL) for 2 hours and then treated with EPA (40 μM) or equivolume vehicle and incubated for 24 hours. Samples were allocated for FA analysis or proteomic analysis. Total cellular FAs were extracted, derivatized to FA methyl esters (FAME), and analyzed using gas chromatography. Total protein content was also measured and used to normalize fatty acid content. Global proteomic analysis was performed using LC/MS to measure relative expression levels of proteins between treatment groups. Significant ($p < 0.05$) changes in expression between treatment groups (>1-fold) were analyzed using differential enrichment analysis of proteomics data (DEP). Biological pathways were analyzed using proteins that survived the cutoff criteria via gene set enrichment analysis (GSEA). EPA treatment significantly modulated proteins in the "neutrophil degranulation" pathway (GO:0043312) with pathway adjusted-p values of $3.68 \times 10\text{-}13$. EPA significantly altered expression of 66 proteins in this pathway, including increasing peroxiredoxin-6 and heat shock protein 90, relative to IL-6. These changes in protein expression in BECs correlated with large, significant increases in the EPA/AA ratio from 0.025 ± 0.002 to 1.72 ± 0.20 (>67-fold increase, $p < 0.001$).

CONCLUSION: EPA favorably modulated proteins in brain ECs related to neutrophil degranulation in a manner that correlated with increases in the EPA/AA ratio. The anti-inflammatory benefits may contribute to reduced stroke risk with IPE as demonstrated in outcome trials.

Amarin Pharma Inc., Elucida Research LLC

P013
INTESTINAL ADAPTATION TO SHORT-TERM, EXTREME FAT CONSUMPTION ALTERS TRIGLCYERIDE-RICH LIPOPROTEIN SECRETION AND INTESTINAL LIPID HANDLING IN MALE AND FEMALE MICE

N Morrow, C Locatelli, N Trzaskalski, C Klein, A Hanson, I Tripathi, A Clément, I Lorenzen-Schmidt, E Mulvihill

Ottawa, Ontario

BACKGROUND: Dysregulated postprandial metabolism including elevated chylomicron remnants, contributes to atherogenesis. The ketogenic diet is a patient-accessible metabolic health intervention whereby nutritional ketosis is achieved by consuming predominantly fat (>70% of kcal) with restricted carbohydrates and protein. It has been established that intestinal enterocytes package dietary triglycerides (TG) into chylomicrons for transport into circulation and store excess TGs as cytosolic lipid droplets (CLDs) such that nutrient absorption is maximized.

METHODS AND RESULTS: The current study aimed to characterize the impact of diet composition on postprandial intestinal lipid handling in male and female wild-type mice. We evaluated intestinal cytosolic lipid droplets, TG secretion rates, and intestinal morphology with short-term, high-fat Western diet (WD) and an extreme fat, low protein, carbohydrate-restricted ketogenic diet (KD) before developing obesity. For three weeks, mice previously fed a grain-based standard laboratory diet (GBD) were switched to a WD or KD. Both male and female KD-fed mice displayed significantly higher plasma TG levels in response to an olive oil gavage than WD- and GBD-fed mice. At fasting, intestinal TG mass was significantly higher in both male and female

mice fed the KD than GBD- and WD-fed mice, providing increased substrate for chylomicron formation and secretion. Interestingly, KD feeding significantly enhanced intestinal-TG secretion rates in male but not female mice and KD-refeeding after a 12-hour fast led to significant jejunal TG accumulation in female mice compared to GBD- and WD-refeeding but not in male mice suggesting female mice. KD feeding lengthened the small intestine in male mice, whereas in female mice, jejunal villi length increased compared to GBD- and WD-fed mice.

CONCLUSION: Overall, KD feeding promotes functional changes to lipid mobilization and distinct morphological alterations to the small intestine compared to the WD diet over three weeks of feeding. Moreover, changes to intestinal lipid handling in response to KD feeding manifest differently in male and female mice. The contribution of elevated postprandial lipid secretion observed with a ketogenic diet on metabolic health and its impact on atherogenesis remains to be determined.

Canadian Institutes of Health Research - Doctoral Research Award, Canadian Institutes of Health Research - Operating Grant, Ontario Graduate Scholarship

Trainee Research Award Finalist—Basic Science

P014
KETONE ESTER THERAPY REDUCES CARDIAC INFLAMMATION AND CARDIAC DYSFUNCTION IN SEPSIS

S Soni, M Martens, S Takahara, H Silver, M Ferdaoussi, J Dyck

Edmonton, Alberta

BACKGROUND: Sepsis is the body's reaction to an infection that often causes lasting cardiac damage and multi-organ injury due to a dysregulated inflammatory response. Currently, there are no effective treatments to reduce inflammation during sepsis and assist in preventing the lasting cardiovascular damage. Not only are sepsis outcomes worse for cardiovascular disease patients, but the permanent organ damage to sepsis survivors also makes them more susceptible to diseases such as heart failure. Thus, therapeutic strategies to reduce the inflammatory response in sepsis are needed to mitigate the development of cardiovascular disease and improve the outcomes and quality of life for cardiovascular patients who survive sepsis. Herein, we tested the efficacy of a therapy that increases circulating ketones via ketone ester supplementation. Ketones are small molecules that are normally produced by the liver and are elevated during low-carbohydrate states, such as fasting. While ketones are classically known to be metabolic substrates that produce ATP, they also have non-metabolic effects, such as inhibiting inflammation. Thus, we hypothesized that ketones have anti-inflammatory effects which will protect against sepsis-induced cardiac dysfunction in a mouse model of sepsis.

METHODS AND RESULTS: To determine the effects of ketone therapy in sepsis, 8-week-old mice orally received vehicle or a clinically tested ketone ester (KE) for 3 days. On day 3, mice were injected with saline or lipopolysaccharide (LPS), and cardiac function, cardiac inflammation, as well as systemic inflammation and multi-organ injury were assessed 24 hours post-injection. Vehicle-treated LPS mice had higher blood ketones compared to non-septic controls, suggesting that ketones may be important as an innate defense mechanism. This response was further increased in KE-treated LPS mice. While vehicle-treated LPS mice had an induction of cardiac and systemic inflammation (e.g., IL-1β, IL-6), most inflammatory markers were significantly lower in KE-treated LPS mice. Similarly, KE-treated septic mice had lesser cardiac dysfunction than vehicle-treated septic mice. These anti-inflammatory effects were also observed in other vital organs such as the kidney and liver thereby demonstrating that KE therapy had global protective effects. Lastly, ketolytic enzymes were reduced or unchanged in vehicle- and KE-treated septic mice, potentially ruling out a normalization of ketone metabolism as a mechanism by which KE treatment may improve function.

CONCLUSION: Together, these data show that ketone therapy may be a novel translational approach to reducing cardiac and systemic inflammation, as well as cardiac dysfunction in a model of sepsis.

Canadian Institutes of Health Research - Doctoral Research Award, Canadian Institutes of Health Research (CIHR), Heart and Stroke Foundation of Canada

P015
LARGE ANIMAL MODEL OF DONATION AFTER CIRCULATORY DEATH AND NORMOTHERMIC REGIONAL PERFUSION FOR CARDIAC ASSESSMENT

K Khalil, R Ribeiro, J Alvarez, M Badiwala, S Der Sarkissian, N Noiseux

Montréal, Québec

BACKGROUND: The increase in demand for cardiac transplantation throughout the years has fueled interest in donation after circulatory death (DCD) to expand the organ donor pool. However, the DCD process is associated with the risk of cardiac tissue injury due to the inevitable period of warm ischemia. Normothermic regional perfusion (NRP) allows for an in-situ organ assessment, allowing the procurement of hearts determined to be viable.

METHODS AND RESULTS: Here, we described a clinically relevant large animal model of DCD followed by NRP. Circulatory death was established in anesthetized pigs by stopping mechanical ventilation. After a preset warm ischemia period, an extracorporeal membrane oxygenator (ECMO) was used for a NRP period lasting at least 30 min. During this reperfusion period, the model allowed the collection of various myocardial biopsies and blood samples