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

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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

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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

for initial cardiac evaluation. Once NRP was weaned, biochemical, hemodynamic, and echocardiographic assessments of cardiac function and metabolism were performed before organ procurement. DCD hearts initially reperfused with NRP demonstrated similar functional recovery following transplantation when compared to conventional beating heart donation preserved with cold storage. Following DCD, there is a severe decline in cardiac index, however, these organs demonstrated similar functional recovery post-transplantation when compared to conventionally transplanted hearts (beating heart donation followed by cold storage). Cerebral oximetry measurements dropped significantly after the withdrawal of life-sustaining treatments and remained stable throughout the NRP procedure. This confirmed the absence of adequate cerebral perfusion during NRP when the supra-aortic vessels were clamped. Lung compliance measurements throughout the experiments showed no significant changes from baseline during the NRP procedure.

CONCLUSION: This large animal model of DCD followed by thoracoabdominal NRP can be a reliable method to assess the cardiac function of a donor heart within the donor and establish if the organ can be transplanted. This protocol closely simulates the clinical scenario previously described for DCD and NRP in heart transplantation and has the potential to facilitate studies aimed at decreasing ischemia-reperfusion injury and enhancing cardiac functional preservation and recovery. It has the potential of being an adequate pre-clinical model that can be used to investigate novel pharmacologic and non-pharmacologic interventions that might improve cardiac functional recovery and have been previously validated only in small-animal models.

P016
MITRAL VALVE CHORDAE TENDINEAE DEVELOP INDEPENDENTLY FROM LEAFLET TISSUE DURING FETAL DEVELOPMENT

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BACKGROUND: During fetal development of the mitral valve, chordae tendineae are thought to derive from the leaflet after it's delamination from the ventricular wall. However, preliminary work on the fetal bovine heart has shown the presence of branched chordae tendineae well before leaflet delamination (Fig. 1A). We hypothesized that the fetal chordae and leaflet tissue are developing along distinct timelines in the bovine heart. To test this hypothesis, we examined the anatomical development and extracellular matrix composition of the bovine mitral valve chordae and leaflet during fetal development and in adult.

METHODS AND RESULTS: Fetal bovine hearts were harvested from a local slaughterhouse and crown-rump length was used to determine gestational age. Samples ranged from 83-270 d.g. (full term = 290d.g.). Anterior leaflets were imaged then chordae number and leaflet area measured in ImageJ. Immature and mature collagen contents were determined using Sircol™ Soluble and Insoluble Collagen assays (Biocolour, Carrickfergus, UK). Age-paired tissue samples underwent Blyscan Sulfated Glycosaminoglycan (sGAG) assays (Biocolour) to determine sGAG content. Both chordae tendineae number and leaflet area increased linearly during gestation. While leaflet area continued to increase into adulthood the chordae number remained unchanged after birth. Mature, insoluble collagen content increased during gestation in both chordae and leaflet, with the rate of increase higher in the leaflet than chordae (ANCOVA p = 2.07e-5, Fig 1C). Despite this, average levels of mature collagen remained higher in chordae during gestation, with contents in the leaflet more than doubling post-natally (Fig 1D). Surprisingly, there was no change in the content of immature, uncrosslinked collagen during gestation in either tissue (Fig. 1E). Immature collagen was found at nearly 10-fold higher concentrations in fetal chordae than in leaflet, where low levels remained unchanged into adulthood (Fig. 1F). Unlike collagen, GAG content was similar in both tissues and remained unchanged during gestation and postnatal development.

CONCLUSION: Bovine mitral valve chordae tendineae and leaflet possess unique and independent developmental processes. Chordae increase in number along with leaflet expansion but only until birth, after which their numbers become fixed. In addition, collagen contents are strikingly different in both tissues and appear to be regulated by different mechanisms. The paradoxically low levels of immature collagen in the leaflet during fetal development suggests that the accumulation of mature collagen occurs via an upregulation of crosslinking. This parallels our observations in the maternal mitral valve, suggesting that valvular remodeling during pregnancy may be a recapitulation of fetal developmental mechanisms

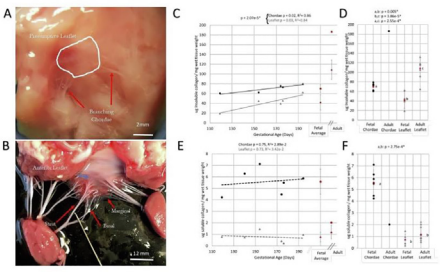

Image 1 The bovine mitral valve chordae tendineae are forming independently of the leaflet during fetal development. A,B Anatomical images of early and late fetal development show that branched chordae tendineae are present before the leaflet has matured. A)Fetal bovine mitral valve at 83 gestational days showing branched chordae tendineae before the leaflet has become opaque. B) Fetal bovine mitral valve at 270 gestational days (full-term) showing that the chordae tendineae have differentiated into their different types before birth. C, E Regression analyses of insoluble (mature) and soluble (immature) collagen in the leaflet and chordae tendineae over gestational time. D, F Scatterplots depicting variation in collagen concentration and ANOVA comparisons of overall average collagen concentrations between fetal/adult tissues. Black circles denote raw fetal chordae data (n=3 insoluble, n=6 soluble), grey triangles denote raw fetal leaflet data (n=3 insoluble, n=6 soluble), and red symbols denote fetal and adult averages (chordae n=1, leaflet n=9). Points with dissimilar letters are significantly different. C) Insoluble collagen concentration increases in both the fetal chordae (R^2=0.86, p<0.05) and leaflet (R^2=0.84, p<0.05) during gestation. ANCOVA analysis reveals there is a significant difference between the fetal chordae and leaflet regression slopes (p<0.05). D) The average insoluble collagen concentration was greater in the fetal chordae than in the leaflet (p<0.05). Compared to the adult leaflet, the insoluble collagen concentration was significantly lower in the fetal chordae and leaflet (p<0.05). E) Soluble collagen concentration does not change during gestation in the fetal chordae and leaflet (p>0.05). F) The average soluble collagen concentration was greater in the fetal chordae than in the leaflet (p<0.05). Compared to the adult leaflet, the soluble collagen concentration was greater in the chordae (p<0.05) but not significantly different in the fetal leaflet (p>0.05).

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