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BIOL 4990

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Honours Proposal

Title:

Integrity and sodium dynamics of the endothelial glycocalyx and impact of water-loading.

Supervisors:

Dr. Mark Rakobowchuk - Primary Supervisor

Dr. Heidi Huttunen-Hennelly – Secondary Supervisor

Introduction:

About 1 in 12 Canadian adults over the age of 19 live with diagnosed heart disease, and of these, about 14 die every hour[1]. High blood pressure (BP), referred to as hypertension, contributes to cardiovascular disease (CVD) and kidney disease[13]. In CVD and kidney disease, the inner lining of the blood vessel called the endothelium is often dysfunctional. Endothelial dysfunction leads to the inability to dilate your arteries which causes CVD[14]. The endothelial cells forming the vessel layer are coated with a complex polysaccharide matrix, the endothelial glycocalyx (EG), responsible for cell signalling, mechanotransduction, and other key roles[2]. The mechanotransduction function of the EG enables local flow-mediated dilation (FMD) by the mechanical forces produced through blood flow which alters blood vessel diameter[2]. Each increase in diameter, vasodilation, arises through the production of vasodilators such as nitric

oxide (NO)[15]. NO is demonstrated to limit the vascular calcification which leads to CVD[3]. The dysfunction of the EG has detrimental effects on the vascular system and initiates CVD.

The EG is primarily made up of proteoglycans containing core proteins with glycosaminoglycans (GAG). These GAGs are negatively charged, and form chains attached to the core proteins[2]. The negative charge on the GAGs enables interaction with positively charged molecules like sodium (Na)[3]. The EG is thought to serve as a protective buffering coating for endothelial cells from rapidly altering Na levels in plasma by binding and buffering this ion during hypertonic periods[5]. An increased chronic exposure of endothelial cells to Na, from diet, overloads the endothelial cells resulting in endothelial dysfunction[10]. With 58% of Canadians consuming over the recommended maximum amount of Na per day, the impacts of Na on EG function are widespread[12].

The consumption of water has been demonstrated to precipitate hyponatremia in some exercise situations[11]. In these instances, the Na-containing sweat is expelled and water which is lower in Na is consumed which leads to net loss in Na. During water ingestion, the increase in plasma volume will acutely lower plasma Na concentration. The decrease in Na concentration will alter the concentration and osmotic gradient of Na causing sodium mobilization from different compartments to the plasma. Using predictive formulas, Wouda et al. established a discrepancy between plasma Na concentrations and hematocrit not in line with current knowledge of plasma homeostasis[4]. The discrepancy leads to the questioning of the compartment that can mobilize Na, possibly the EG, to alleviate an acute hypotonic load[4]. There is currently no work assessing the buffering capacity of the EG and the alleviation of high EG Na levels by a hypotonic load.

EG integrity and endothelial function are variable between individuals. To test integrity and function simultaneously, specific methodology must be used. The vasodilatory and vasoconstrictive ability of the brachial artery will be measured to determine the function of the endothelium. The EG integrity has been demonstrated to be strongly correlated to erythrocyte glycocalyx[8]. Therefore, by measuring the integrity of the erythrocyte glycocalyx, the EG integrity can be determined. Additionally, a urine sample will allow for the determination of Na levels that are being excreted. By tracking the Na dynamics, the buffering capacity of the EG and its ability to serve as a Na storage and buffer for the vasculature can be demonstrated.

Objectives:

This research project will investigate the hypothesis that the decreased endothelial function from high Na diet will be restored after an acute hypotonic load. Additionally, the integrity of erythrocyte glycocalyx will predict a decrease in function of the endothelium with high Na diets. Finally, the hypothesis that the glycocalyx acts as a Na store and buffer separate of the two-compartment model will also be investigated through the tracking of Na dynamics.

Materials and Methods:

Healthy young participants will be recruited with rigorous exclusion criteria and will complete four days of testing each spaced by one week. Participants will receive an information form with all the details of the testing and project at least 24 hours in advance of the first testing session. Participants will also be asked to not consume any food or water for 4 hours before each testing session. The first day will begin with the reading and signing of an information and consent form followed by baseline participant measures. Age, sex, height, mass, BP, heart rate (HR) will be measured. Following baseline measurements, participants will begin the testing protocol which will be identical at all sessions. The experimental conditions are administered in a double-blind setup. Participants will receive condition salt (S) or placebo (P) on weeks 1 or 3 of testing and consume XXX grams of salt or sugar daily. Participants will ingest pills for a total of seven days where weeks 2 or 4 of testing occur on the final day. Participants will not ingest any pills between weeks 2 and 3 to washout the potential Na stored following condition S.

At testing, participants are instrumented with ECG electrodes, a BP monitor and a respiratory belt. They then rest for 20 minutes, and baseline data will be recorded during the last 10 minutes. Following the rest, the participant will undergo ultrasound imaging of their carotid

artery while conducting the Valsalva maneuver. Participants will then have endothelial function of their brachial artery assessed by FMD. Along with measures of FMD, venous blood samples will be taken at the same time points and hematocrit assessed to determine plasma concentration corrections. Whole blood will be centrifuged, the red blood cells tested for a measure of EG integrity by sedimentation rate[9], and plasma stored for ELISA work and sodium concentration analyses. A urine sample will be collected in a private place following each blood sample and used to assess changes in Na concentrations.

The participant will then be seated upright and water-load by consuming an individually tailored volume of water adjusted to their body mass and asked to consume the entire volume within 20 minutes. Beat-to-beat BP, respiration rate, and HR will be recorded following the water-loading. Endothelial function will be assessed followed by blood and urine sampling directly and 30 minutes following the water-loading.

Outcome variables (FMD, EG integrity, Na levels, EG shedding products) will be assessed using appropriate statistical analyses. A 2-way repeated measures ANOVA with Condition (S vs P), and Time (pre, post, post+30) will be used.

An ethics application to the TRU Research Ethics Board will be submitted. It is expected to be approved by October 2024. Biosafety training approval has already been provided.

Expected Results:

I expect that salt loading will reduce the endothelium function, and an acute hypotonic load will return endothelium function to baseline preceding salt loading. I expect the erythrocyte glycocalyx test to predict the varying changes in endothelial function and sodium storage capacity.

Timeline:

June-July 2024	Method development.
July 2024	Finalize techniques and methods.
September 2024	Ethics approval.
October 2024 – January 2024	Data collection.
February 2025	Data analysis.
March 2025	Thesis writing and dissemination preparation.
April 2025	Thesis defence.

Budget:

Item	Cost
1. Sodium probe	$1,066
2. 1L urine collection bottles (x20)	$200
3. Blood tubes, pipette tips, microcentrifuge tubes, conical tubes	$100
4. Bovine serum albumin	$500

All of the above expenses will be covered by the Professional Development funding of Dr. Mark Rakobowchuk. All other equipment used is already in laboratory.

References:

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