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### Plant-Based Eating, A Novel Approach For Arresting Erythrocyte Dysfunction, Redox Dysregulation, and Vascular Injury Influenced by Type 2 Diabetes Mellitus

Walter Craig

*Cal Poly Humboldt*, [wgc11@humboldt.edu](mailto:wgc11@humboldt.edu)

Lukas Coppen

*Cal Poly Humboldt*, [wgc11@humboldt.edu](mailto:wgc11@humboldt.edu)

Brian K. Blackburn

*Cal Poly Humboldt*, [wgc11@humboldt.edu](mailto:wgc11@humboldt.edu)

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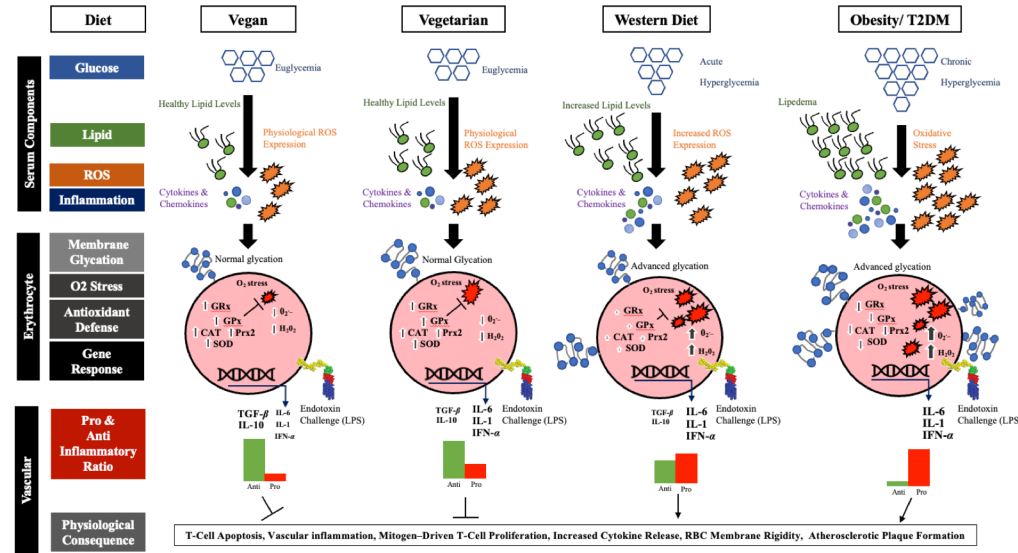
## Introduction

Type 2 Diabetes Mellitus (T2DM) is a multifactorial condition that has been implicated in vascular complications, increased oxidative stress, and chronic inflammation. While the most common metabolic abnormality associated with diabetes is hyperglycemia, there are also aberrations in circulating carbohydrate, fat, and protein concentrations that impose grave alterations on Red Blood Cells (RBCs). These adverse modifications may further exacerbate the diabetic condition by significantly amplifying the immune response. Historically, analysis of blood samples has only been specific to plasma and serum components, while whole red blood cells were discarded, as they were believed to be limited to oxygen transport and respiratory exchange (1). Importantly, RBCs have been reported to store cytokines and chemokines, which are proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses. Specifically, these proteins direct the pro and anti-inflammatory messaging ratio responsible for the recruitment of immune cells, and the duration of the immune response (1,2). Therefore, a critical evaluation of the whole RBC superimposed with serum/plasma components may be a more vital analysis for determining how dietary macronutrient concentration in the circulation may be responsible for the acceleration of diabetic induces vascular injuries such as microvascular disease and Atherosclerosis. In vitro studies show that induced stress, via lipopolysaccharides (LPS), disrupt red blood cell formability, function increasing inflammatory response (3). Recent studies indicate that exogenous uptake from diets exacerbates changes in hematocrit resulting in alterations to ability to secrete important signaling molecules (4,5,6).

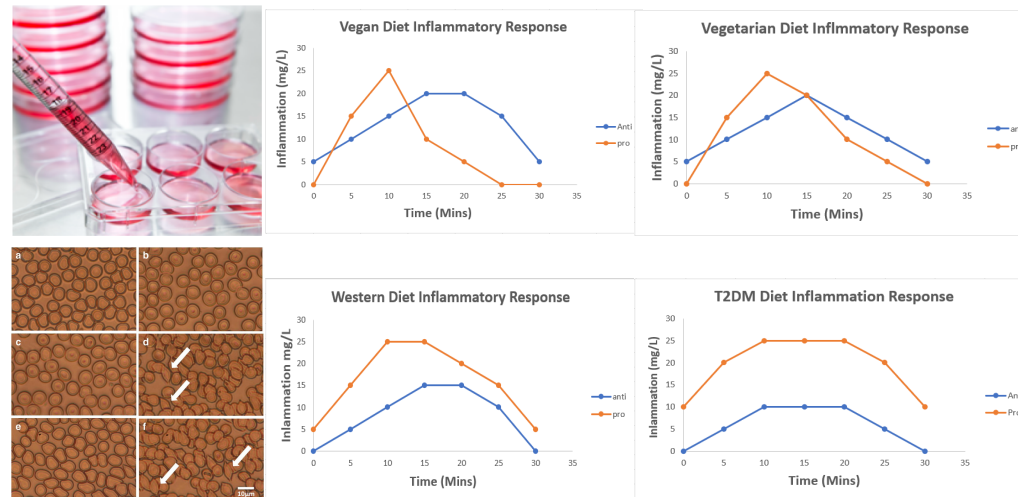
## Methods

Diets represented in this project will include a Vegan (VD), Vegetarian (VegD), Western Diet (WD), and a high-fat dietary intake commonly associate with Obesity/Type 2 Diabetes (OD/T2DM). Subjects will include 40 adult participants with a subject number of ten (n=10) in each tier. The inclusion criteria will consist of self-identified dietary habits provided by the subjects in each population aligning with the categories in the study and participants will be excluded if they are on any lipid/cholesterol or glucose limiting medication. Informed consent will be obtained before experimental procedures occur and participants will be required to perform a 12-hour fast before the initial blood draws occurs. Once admitted into the study, a 5ml of a whole blood draw (1 unit) will be acquired by a registered nurse. Whole blood samples will then be processed and suspended in stabilizing media and then frozen at -80 C. Whole blood samples will then be incubated with LPS for 24 hours and supernatant will be collected. Inflammatory signatures associated with each diet type will be determined via commercially available ELISA Assay kit.

## Serum Mediated Erythrocyte Dysfunction & Vascular Complications



## Erythrocyte Mediated Inflammasome Response to LPS Challenge



## Results

Measured anti-inflammatory cytokine TNF-B and IL 10 release was significantly expressed in the VG and VegD blood samples compared to WD and OD/T2DM groups. Due to the increased anti-inflammatory response to a 24-hour LPS challenge, proinflammatory cytokine expression was minimized and suppressed between 25 to 30 minutes. Interestingly, the VD inflammatory bout was approximately 5 minutes shorter than the VegD RBC response, although both groups demonstrated a similar peak of proinflammatory cytokine expression at peaked (25 mg/L). This would suggest that an all plant based diet, completely absent of any animal products is better capable at immunosuppression and more protective against vascular inflammation that the VegD diet, which is typically associated with the composition of some animal products. Contrary to the plant-based diets, the WD and OD/T2DM exhibited considerable unique outcomes. In these samples, anti-inflammatory cytokines were significantly diminished (15 mg/L) resulting in robust proinflammatory cytokine release a robust inflammatory response. The inflammatory response generated by RBCs occurred for 30 minutes in the WD and OD/T2DM groups.

## Discussion

We hypothesize that RBCs in whole blood samples, dependent on four distinctive dietary patterns, will express diverse pro and anti-inflammatory cytokine ratios when challenged with LPS. We anticipate that VD and VegD, due to favorable physiological serum levels of glucose, lipids, ROS, and vascular inflammation typically associated with a plant-based diet, will preserve critical RBC antioxidant defenses, thus eliminating the potential of oxidative stress maturation, a robust pro-inflammatory response and vascular injury. We further speculate that these combined effects associated with plant-based eating may have a critical influence on RBC's capacity to neutralize pro-inflammatory cytokine damage by releasing heightened concentrations of anti-inflammatory cytokines. However, we speculate RBCs primed in human serum profiles commonly linked to intensified lipids, glucose, and vascular inflammatory mediators (WD & OB/T2DM) will demonstrate contrasting inflammatory profiles that favor proinflammatory signaling thereby directly contributing to vascular inflammation and vascular injury commonly related to T2DM.

For references and additional information concerning this investigation, contact [Walter.Craig@humboldt.edu](mailto:Walter.Craig@humboldt.edu)