Correlations between glycocalyx quality, adipose tissue NOX activity, and adipose tissue microvascular blood flow

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ABSTRACT

Background: Cardiovascular disease (CVD) is the leading cause of death in America. On the biological level, researchers have identified endothelial dysfunction as playing a key role in CVD development. Lining the interior portion of endothelial cells (ECs) are glycocalyx proteins, which protect the ECs from hyperglycemia and excessive shear stress from red blood cells. A low density of glycocalyx proteins has been associated with CVD, although low glycocalyx density may also occur in metabolic diseases. A main cause of endothelial dysfunction in people with type 2 diabetes (T2D) is high reactive oxygen species (ROS) production. However, it remains unclear if glycocalyx quality correlates with ROS production and markers of T2D risk other than increased blood glucose, such as plasma insulin and glycerol concentrations. In addition, glycocalyx function studies remain limited in humans.
Methods: We recruited young, healthy males and females between the ages of 18-45 years for testing. The Glycocheck system, a camera and computer analysis software duo, was used. The Glycocheck camera, placed sublingually, allowed for assessment of glycocalyx quality and capillary function through the measurement of red blood cell velocity and glycocalyx density. These microvascular assessments were performed first under fasted conditions and repeated during a hyperinsulinemic-euglycemic clamp. Simultaneously, local ROS concentrations and interstitial glycerol (an indicator of fat metabolism) in adipose tissue were measured by utilizing microdialysis.
Data is being collected and analyzed in our laboratory to determine the correlations between glycocalyx density and markers of cardiometabolic disease risk.

BACKGROUND

Lipolysis

- When a meal is consumed under normal conditions, insulin is secreted, leading to triglyceride storage in adipose tissue and the suppression of lipolysis.
- With obesity, insulin fails to suppress lipolysis adequately, leading to the dysregulation of lipolysis, the development of insulin resistance, and endothelial dysfunction.

NOX and ROS

- Elevated lipolysis leads to the activation of NADPH oxidase (NOX), which further stimulates lipolysis via a reactive oxygen species (ROS) signaling pathway, which further reduces the antilipolytic effect of insulin, resulting in a vicious cycle of dysregulated lipolysis and insulin resistance.
- Elevated ROS concentrations directly increase plasma glucose concentrations, as an increase in one causes an increase in the other, resulting in a vicious cycle of heightened ROS and glucose production.
- The vicious cycle of of hyperglycemia, NOX activation, and ROS production contributes to oxidative stress within endothelial cells, leading to endothelial dysfunction.

Endothelial cells and the glycocalyx

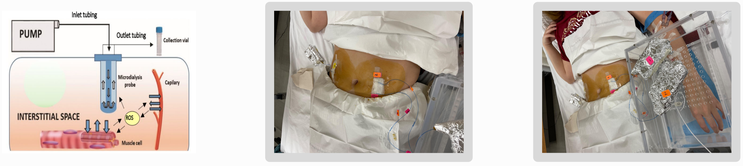
- Endothelial cells compose the inner lining of blood vessels and are responsible for containing blood cells within the vascular space, separating blood cells from extra-vascular tissues, vasoconstriction, and vasodilation.
- The glycocalyx, a protein and carbohydrate complex, lines the interior/luminal side of endothelial cells, protecting vessels from damage and maintaining the integrity of the endothelial cells.
- A decline in the glycocalyx density and quality can lead to the destruction of endothelial cells, resulting in the development of cardiometabolic diseases.
- It currently remains unknown how NOX mediated lipolysis affects glycocalyx quality and adipose tissue microvascular health.

METHODS

Six sedentary, healthy individuals underwent microdialysis, a hyperinsulinemic-euglycemic clamp, and glycocheck procedures.

Microdialysis

- Microdialysis was used in the abdominal adipose tissue of participants to measure *in vivo* ROS, glycerol, and microvascular function within the region.
- Four probes were inserted, each perfused with a control solution of saline, ethanol, Amplex Ultrared, superoxide dismutase (SOD) and horseradish peroxidase, combined with Apocynin, and either Isoproterenol or atrial natriuretic peptide (ANP). Dialysate was collected every 15 minutes.



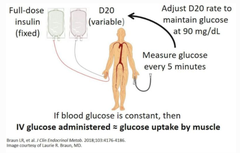
Figures show how microdialysis functions *in vivo* (left) and the application of the microdialysis technique in participants for this study (middle and right).

Microdialysis perfusate

- Amplex Ultrared, SOD, and horseradish peroxidase were used to measure hydrogen peroxide and superoxide (ROS) levels.
- Ethanol was used to measure microvascular blood flow.
- Apocynin was used to measure local NOX-mediated ROS production, as it is a NOX inhibitor.
- Isoproterenol, a beta adrenergic agonist, and ANP, a local non-canonical lipolysis agonist, were used to measure lipolysis.

Hyperinsulinemic-euglycemic clamp

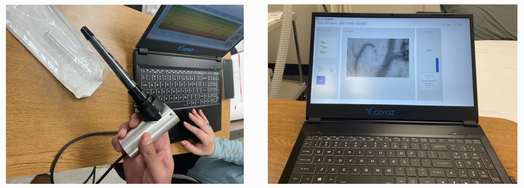
- The clamp procedure was used to asses the suppressive effects of insulin on lipolysis.
- Insulin and glucose were administered intravenously, to clamp the participants glucose level at 90 mg/dL.



The figure depicts a diagram of the clamp procedure. On one side of the participant, a two port IV is inserted into the anteubital space by a certified medical provider. One port administers insulin at a fixed rate, while the other port administers a 20% glucose solution that varies in dosage, depending on the participants glucose level. On the other side of the participant, a single port IV is inserted into the antecubital region. From this port, blood draws are taken every five minutes to measure the participant's blood glucose level. Based upon the participant's glucose level, the amount of glucose administered via the two port IV will either be altered or kept constant.

Glycocheck

- The Glycocheck system, a camera and computer software duo, was used to assess the density, function, and structure of the glycocalyx proteins, capillary density, capillary blood flow, and blood flow velocity, within the sublingual microvessels of the participant.
- Collections of twenty four data points were taken under fasted (control) conditions and clamp conditions.



Figures show the Glycocheck camera that is placed sublingually (left) and the Glycocheck computer system that collects and analyzes the microvessel and glycocalyx data (right).

RESULTS

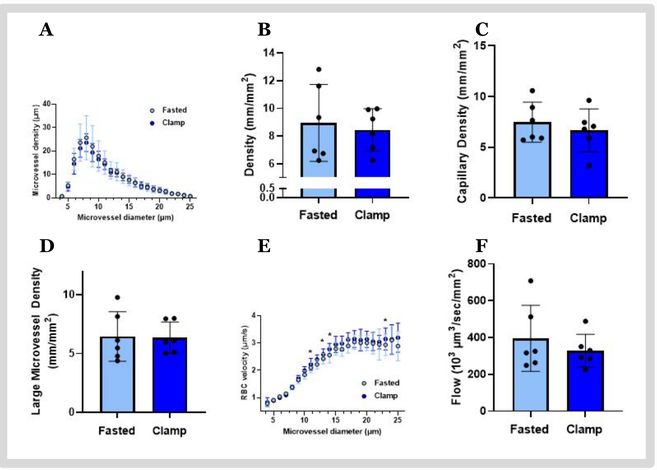


Figure 1. Group by microvessel diameter and group comparisons of fasted and clamp participants. Data was analyzed using a paired t-test to analyze microvascular densities for each microvessel diameter (4-25μm) (A), and red blood cell (RBC) velocity for each microvessel diameter (4-25μm) (E). Data was analyzed using a paired t-test to identify differences in microvascular density of all microvessels (B), microvascular density of capillary microvessels sized 4-6μm (C), microvascular density of large feeding microvessels sized 10-25μm (D), and blood flow in all microvessels (F). *P < 0.05 vs Fasted. Data are means ± SE. *n* = 6 participants.

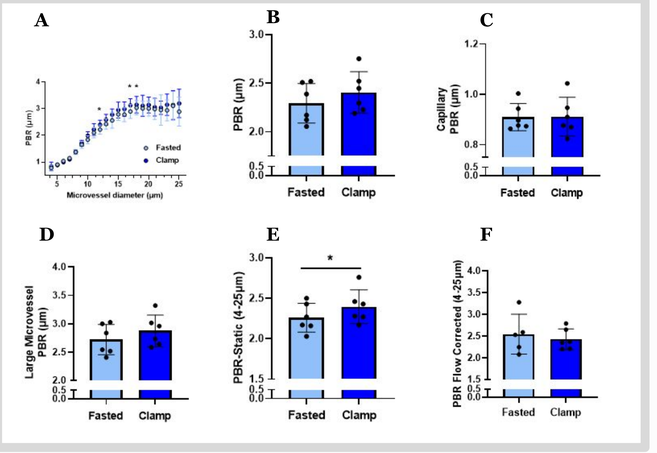


Figure 2. Group by microvessel diameter and group comparisons of fasted and clamp participants. Data was analyzed using a paired t-test to analyze perfused boundary region (PBR) for each microvessel diameter (4-25μm) (A). Data was analyzed using a paired t-test to identify differences in PBR in all microvessels (B), PBR of capillary microvessels sized 4-6μm (C), PBR of large feeding microvessels sized 10-25μm (D), static PBR in all microvessels (E), and flow corrected PBR in all microvessels. *P < 0.05 vs Fasted. Data are means ± SE. *n* = 6 participants.

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ACKNOWLEDGEMENTS

Thank you to Cesar Meza and Dr. Robert Hickner for serving as my mentors during my Honors in The Major Thesis and for guiding me through the process of conducting my own research. Thank you to Dr. Dan Machin for allowing me to use the Glycocheck system and to Jacob Zheng for guiding me through data analysis with the Glycocheck.