NOX and Creatine Monohydrate Supplementation Impact Microvascular Blood Flow

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Abstract

Consumption of high-carbohydrate (HC) or high-fat (HF) meals are known to increase reactive oxygen species (ROS), which underlie the development of cardiovascular disease (CVD). NADPH oxidase (NOX) is a primary source of ROS in the vasculature, but the effects of NOX-generated ROS on *in vivo* microvascular blood flow following a HC or HF meal are unclear. Recent studies indicate creatine monohydrate (CM) may reduce ROS levels and improve blood flow. **PURPOSE:** The primary aim of this study was to determine if NOX-derived ROS impairs microvascular blood flow in response to a HC or HF meal and to establish whether 5 days of CM could reduce in vivo ROS concentrations and improve microvascular blood flow in response to a HC or HF meal. **METHODS**: Young, healthy males and females (n = 6; age: 28 ± 6 yrs.; BMI: 27.4 ± 6.0 kg/m^2) were studied. Microdialysis was utilized to measure local skeletal muscle (vastus lateralis) ROS concentrations and microvascular blood flow at rest and for 4 hours after consumption of either a HC (150 g of glucose) or HF (66 g of fat) meal. One microdialysis probe was perfused with a control saline solution containing 5 mM ethanol (CON) and a second probe perfused with CON plus apocynin (APO; NOX inhibitor). Microvascular blood flow was assessed by the ethanol outflow-to-inflow ratio (o:i), which is inversely related to blood flow. Microdialysis procedures were repeated after 5 days of CM supplementation (20 g/day). Due to limited sample size, HC and HF groups were combined for data analysis. **RESULTS**: APO significantly lowered ROS concentrations post HC/HF consumption (H_2O_2 µM mean ± SD, 1.33 ± 0.60) compared to CON (1.94 ± 0.74, p = 0.049). Microvascular blood flow was significantly higher in APO post HC/HF consumption (o:i, APO = 0.60 ± 0.15, CON = 0.69 ± 0.11, p = 0.009). Following 5 days of CM supplementation, ROS concentrations (POST: 3.22 ± 1.76, PRE: 1.94 ± 0.86, p = 0.025) and microvascular blood flow (ethanol o:i, POST = 0.58 ± 0.26; PRE =0.74 ± 0.13, p = 0.038) were significantly increased at 180 mins post HC/HF consumption. **CONCLUSION**: NOX plays a large role in microvascular blood flow changes following the administration of a HC/HF meal. Further, CM supplementation improves microvascular blood flow which may indicate CM may be effective for the prevention of CVD.

Introduction

Endothelial dysfunction, a disorder that impairs the functioning of the endothelial cells lining the vasculature, is a major public health issue that contributes to a shorter lifespan and an increased risk of cardiovascular disease - the leading cause of death worldwide. A key characteristic of endothelial dysfunction includes impairments to blood flow and elevations in oxidative stress. All these characteristics are further exacerbated by obesity, aging, sedentary lifestyles, and sugar-rich diets. Oxidative stress occurs when the body's natural defense mechanisms are outmatched by the accumulation of reactive oxygen species (ROS). NADPH oxidase (NOX), a major source of ROS in endothelial cells, is known to contribute to oxidative stress and endothelial dysfunction (La Favor et al., 2016). NOX is activated by a variety of factors, including high levels of glucose and free fatty acids, both of which are commonly elevated in obesity and metabolic disorders. Current recommendations to reduce oxidative stress involve engaging in regular physical activity, losing weight, and reducing sugar intake. Despite these efforts, endothelial dysfunction continues to increase, highlighting the need for simple, effective solutions. Creatine monohydrate (CM), a commonly used sports performance supplement, may reduce oxidative stress and improve blood flow. CM has demonstrated significant antioxidant properties against ionized radicals, and supplementation has been linked to a reduction in oxidative stress after high-intensity exercise in humans (Lawler et al., 2002). To date, researchers have had to resort to the utilization of indirect surrogates, or in-vitro (outside a living organism) tissue measurements of ROS to assess oxidative stress. However, our lab has a novel microdialysis technique that can be used to measure in-vivo (in human) production of ROS. Therefore, to further explore the effects of NOX and CM, our lab conducted a study to determine whether 5 days of supplementation can reduce in-vivo ROS concentrations at rest and in response to a HC or HF meal. The study will utilize a novel microdialysis technique to directly measure ROS production, as opposed to indirect surrogate measures or in-vitro tissue measurements. The hypothesis is that NOX produced ROS will hinder blood flow and that five days of CM supplementation will lead to reduced ROS and improved blood flow.

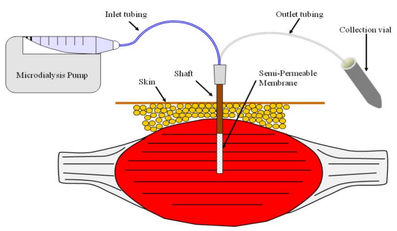
Research Questions

- What is the impact of NOX on microvascular blood flow following a meal?
- Does CM supplementation lower ROS concentrations and increase microvascular blood flow at rest and following a HC/HF meal?

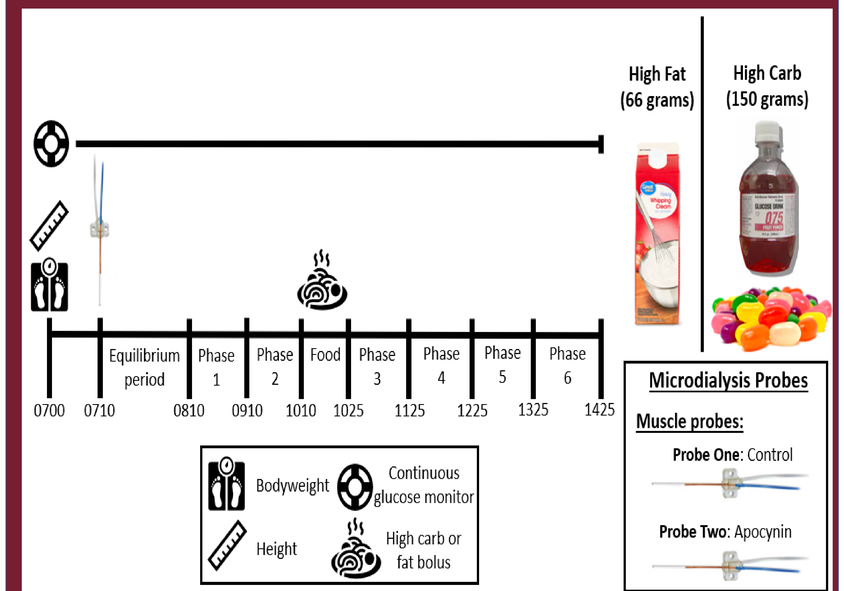
Methods

•A pre-post study design was implemented. Participants completed a baseline visit in which bodyweight, height, and body composition was obtained. Following the baseline visit, participants came in for a testing day in which two microdialysis probes were inserted into the vastus lateralis. One microdialysis probe contained a control solution of ethanol and saline while the other probe contained control solution plus 1mm Apocynin (NOX inhibitor). Dialysate samples were collected at baseline and up to 4 hours following the consumption of a HC (150 g of carbohydrate; 600 kcal) or HF (66 g of fat; 600 kcal) meal. All dialysate samples were immediately assessed in a fluorometer to determine ROS concentrations. The remainder of each dialysate was stored at 4˚C and analyzed for ethanol concentration within 24 hours, which is displayed as a percent change in ethanol outflow/inflow ratio that reflects microvascular blood flow. After the completion of the visit, participants were sent home with 5 days of CM supplementation (20 grams per day). Following the 5 days, participants returned for a second experimental day, in which the same procedures were performed.

Microdialysis



Study Design



Results

Figure 1

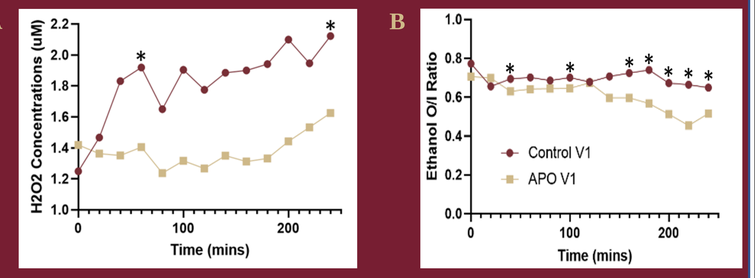


Figure 1. (A) APO compared to the control probe significantly lowered ROS concentrations at 60 mins. (B) The APO probe compared to the control probe showed a significantly increased microvascular blood flow at 40 mins (*P < 0.05).

Figure 2

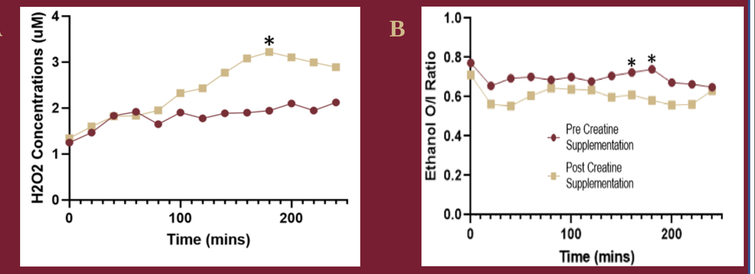


Figure 2. (A) ROS concentrations were significantly increased following 5 days of CM supplementation compared to pre supplemented conditions at 180 mins. (B) Microvascular blood flow was improved at 160 mins and 180 mins post carb/fat consumption (*P < 0.05).

Clinical Implications/Conclusion

This research can play an important role in the mitigation of vascular health diseases by pinpointing areas of focus for treatments (such as NOX), which can decrease the burden associated with treating at risk patients. Further, the implications of this area of research suggest that CM can improve microvascular blood flow and could possibly be used in the future to reduce the risk of endothelial dysfunction.

References

- La Favor, J. D., Dubis, G. S., Yan, H., White, J. D., Nelson, M. A., Anderson, E. J., & Hickner, R. C. (2016). Microvascular endothelial dysfunction in sedentary, obese humans is mediated by NADPH oxidase: influence of exercise training. *Arteriosclerosis, thrombosis, and vascular biology*, *36*(12), 2412-2420.
- Lawler, J. M., Barnes, W. S., Wu, G., Song, W., & Demaree, S. (2002). Direct antioxidant properties of creatine. *Biochemical and biophysical research communications*, *290*(1), 47-52.