Creatine Monohydrate Supplementation Increases Skeletal Muscle Microvascular Blood Flow

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Background

ROS

- Reactive oxygen species (ROS) are highly reactive molecules produced by cellular metabolism.
- Chronically elevated levels of ROS have been shown to increase risk for cardiovascular disease.
- Consumption of high-carbohydrate (HC) or high-fat (HF) meals are known to increase concentrations of ROS.

Creatine Monohydrate

- Given the pivotal role that ROS plays in the pathogenesis and progression of cardiovascular diseases there is an urgent need to identify novel potential interventions that can attenuate ROS concentrations and enhance blood flow.
- Recent evidence has shown that creatine monohydrate (CM), a popular ergogenic aid, may lower ROS levels and improve vascular function

Purpose

To determine if NOX-derived ROS impairs skeletal muscle microvascular blood flow at rest and in response to a HC or HF meal. Furthermore, to investigate whether five days of CM supplementation can reduce *in-vivo* ROS concentrations and increase skeletal muscle microvascular blood flow at rest and in response to a HC or HF meal.

Methods

Participants

- Seven participants (3 males, 4 females, 26 ± 4 years, 27.1 ± 5.4 kg/m², 30.8 ± 9 % body fat percentage).

Microdialysis Procedures

- Microdialysis was used to monitor *in-vivo* ROS production and microvascular blood flow within skeletal muscle.

ROS measurements (Hydrogen Peroxide (H_2O_2) and Superoxide (O_2)

- Microdialysis probes were perfused with Amplex UltraRed, horseradish peroxidase, and superoxide dismutase.
- As SOD converts H_2O_2 into O_2, ROS was analyzed as the combination of H_2O_2 and O_2 concentrations.

Skeletal muscle microvascular blood flow

- Microvascular blood flow was assessed by perfusing microdialysis probes with ethanol. The ethanol outflow-to-inflow ratio is *inversely* related to blood flow

Creatine Monohydrate Supplementation

- 20 grams of CM was given to participants each day for a total of 5 days.

Methods Continued

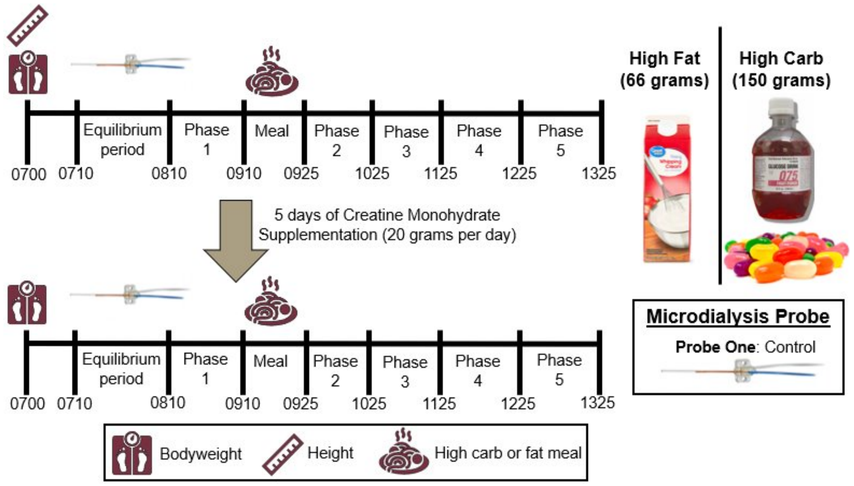
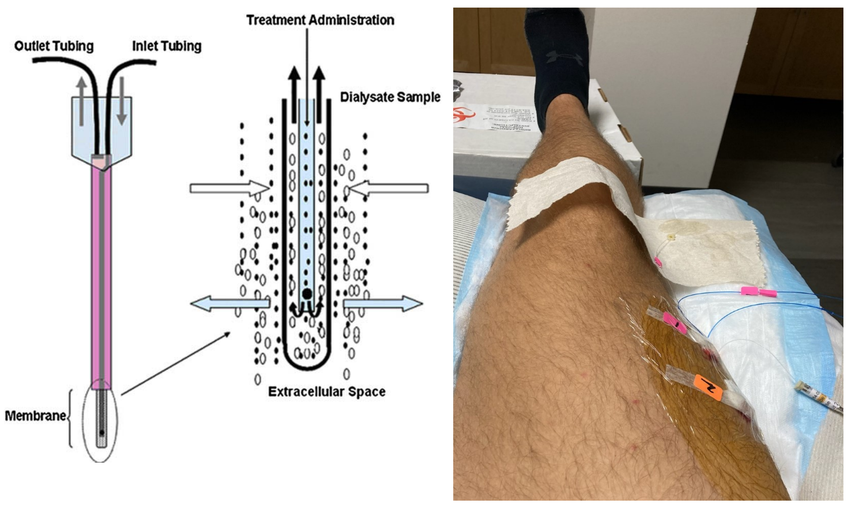


Image shows the testing visit in which microdialysis probes were inserted. Resting and up to fours post meal consumption concentrations of ROS and microvascular blood flow were taken. This visit was performed before and after taking 20 grams of CM for five days.



Images show a schematic illustration of the microdialysis technique (left) and microdialysis probes inserted into skeletal muscle (right).

Results

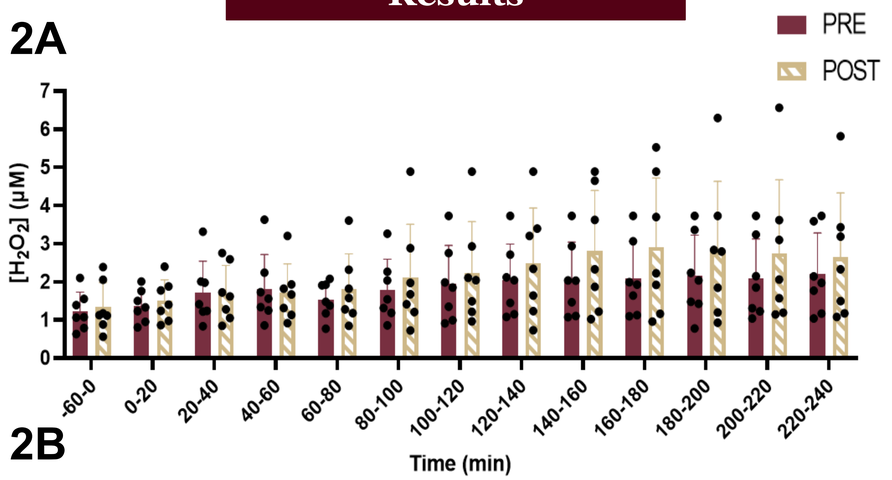


Figure 2A: H_2O_2 concentrations at baseline and up to four hours following the consumption of HC or HF meal PRE and POST CM supplementation (n=7). **Figure 2B**: Ethanol outflow/inflow ratio at baseline and up to four hours following the consumption of HC or HF meal PRE and POST CM supplementation (n=7). Marginal models were used to determine the main effect of the visit (CM supplementation). Post hoc analyses were adjusted for multiple comparisons using Bonferroni adjustment. Abbreviations: PRE, PRE-CM supplementation; POST, POST-CM supplementation; min: minutes. * represents a $P \leq 0.05$.

Conclusions

- Five days of CM supplementation did not affect *in-vivo* ROS concentrations. CM did improve skeletal muscle microvascular blood flow following the administration of a HC or HF meal.
- Future studies are needed in individuals with obesity to assess the extent to which CM supplementation could improve skeletal muscle microvascular blood flow.

References

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NOX and Creatine Monohydrate Supplementation Impact Skeletal Muscle Microvascular Blood

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Background

NOX

- NADPH oxidases (NOX) are predominant sources of reactive oxygen species (ROS) in the vasculature
- Consumption of high-carbohydrate (HC) or high-fat (HF) meals are known to increase concentrations of ROS.
- However, the effects of NOX-generated ROS on *in-vivo* skeletal muscle microvascular blood flow following a HC or HF meal are unclear.

Creatine Monohydrate

- Given the pivotal role that ROS plays in the pathogenesis and progression of cardiovascular diseases there is an urgent need to identify novel potential interventions that can attenuate ROS concentrations and enhance blood flow.
- Recent evidence has shown that creatine monohydrate (CM), a popular ergogenic aid, may lower ROS levels and improve vascular function

Purpose

To determine if NOX-derived ROS impairs skeletal muscle microvascular blood flow at rest and in response to a HC or HF meal. Furthermore, to investigate whether five days of CM supplementation can reduce *in-vivo* ROS concentrations and increase skeletal muscle microvascular blood flow at rest and in response to a HC or HF meal.

Methods

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Microdialysis Procedures

- Microdialysis was used to monitor *in-vivo* ROS production and microvascular blood flow within skeletal muscle.

ROS measurements (Hydrogen Peroxide (H_2O_2) and Superoxide ($O_2^{\cdot-}$))

- Microdialysis probes were perfused with Amplex UltraRed, horseradish peroxidase, and superoxide dismutase.
- As SOD converts H_2O_2 into $O_2^{\cdot-}$, ROS was analyzed as the combination of H_2O_2 and $O_2^{\cdot-}$ concentrations.
- NOX-mediated ROS production was assessed by local perfusion of apocynin (APO).

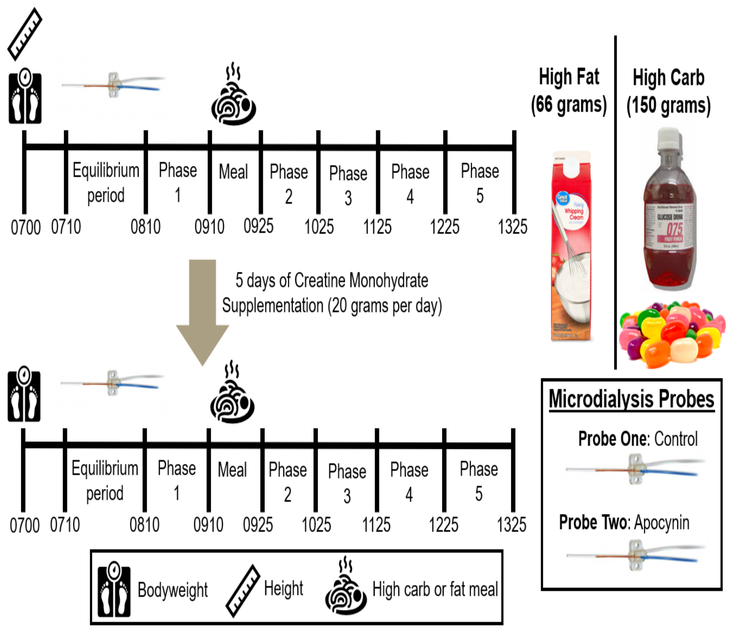
Skeletal muscle microvascular blood flow

- Microvascular blood flow was assessed by perfusing microdialysis probes with ethanol. The ethanol outflow-to-inflow ratio is *inversely* related to blood flow

Creatine Monohydrate Supplementation

- 20 grams of CM was given to participants each day for a total of 5 days.

Methods Continued



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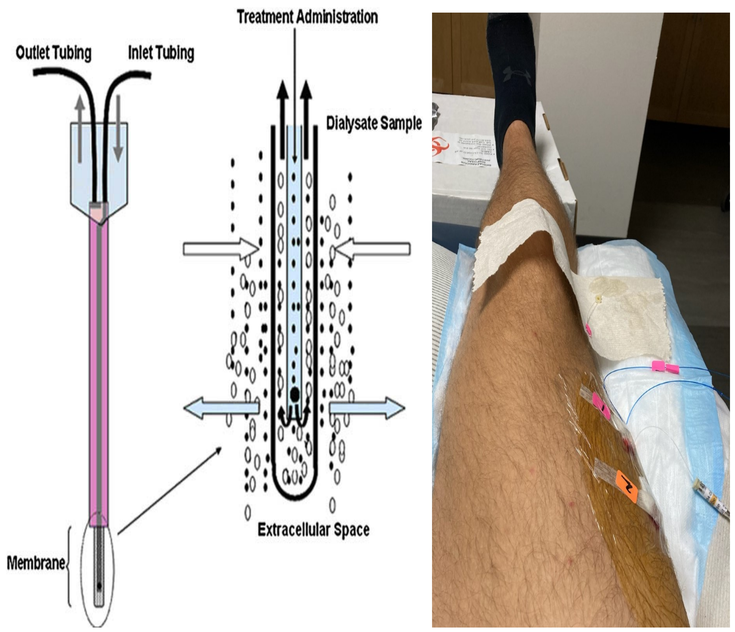

Images show a schematic illustration of the microdialysis technique (left) and microdialysis probes inserted into skeletal muscle (right).

Results

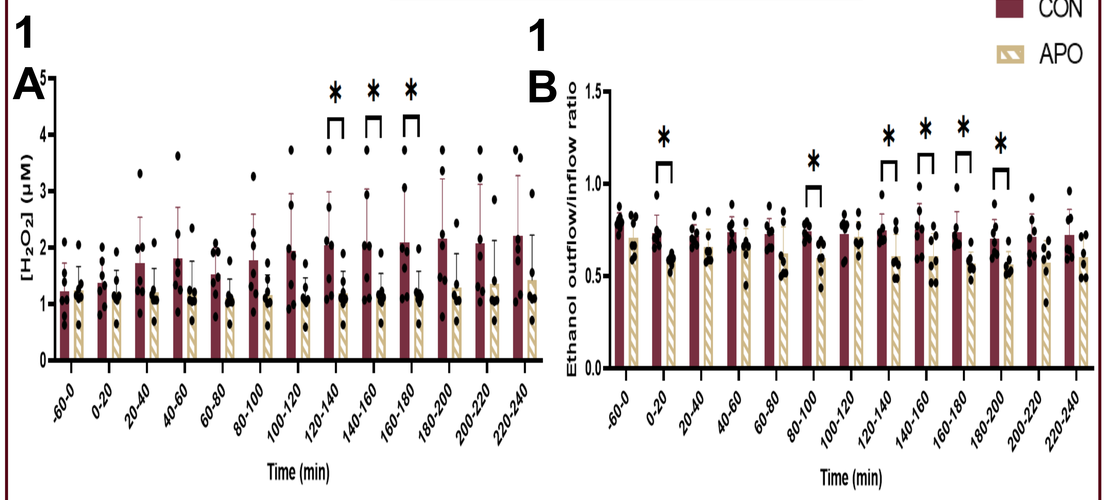

Figure 1A: H_2O_2 concentrations at baseline and up to four hours following the consumption of HC or HF meal (n=6). **Figure 1B**: Ethanol outflow/inflow ratio at baseline and up to four hours following the consumption of HC or HF meal (n=6). Marginal models were used to determine the main effect of APO. Post hoc analyses were adjusted for multiple comparisons using Bonferroni adjustment. Abbreviations: CON, control; APO, apocynin; min: minutes. * represents a P ≤ 0.05

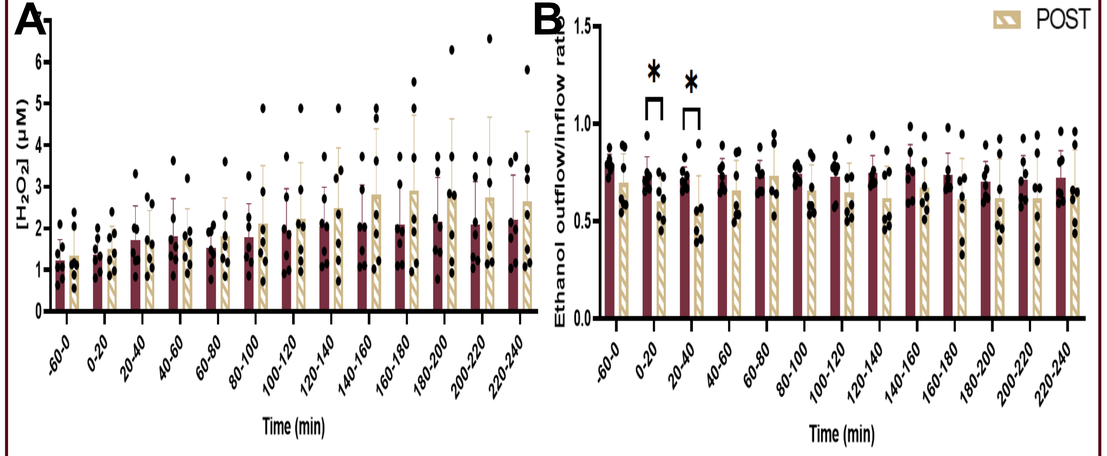

Figure 2A: H_2O_2 concentrations at baseline and up to four hours following the consumption of HC or HF meal PRE and POST CM supplementation (n=6). **Figure 2B**: Ethanol outflow/inflow ratio at baseline and up to four hours following the consumption of HC or HF meal PRE and POST CM supplementation (n=6). Marginal models were used to determine the main effect of the visit (CM supplementation). Post hoc analyses were adjusted for multiple comparisons using Bonferroni adjustment. Abbreviations: PRE, PRE-CM supplementation; POST, POST-CM supplementation; min: minutes. * represents a P ≤ 0.05.

Conclusions

- NOX inhibition increases skeletal muscle microvascular blood flow following the administration of a HC or HF meal
- NOX may be especially prominent in individuals with elevated adiposity.
- Five days of CM supplementation did not affect *in-vivo* ROS concentrations. CM did improve skeletal muscle microvascular blood flow following the administration of a HC or HF meal.
- Given the association between elevated NOX and obesity, further studies are needed in individuals with obesity to assess the effects of NOX-produced ROS on skeletal muscle microvascular blood flow following the administration of a HC/HF meal and the extent to which CM supplementation could improve skeletal muscle microvascular blood flow.