NADPH Oxidase-derived ROS Effects on Lipolysis and Cardiometabolic Diseases

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BACKGROUND

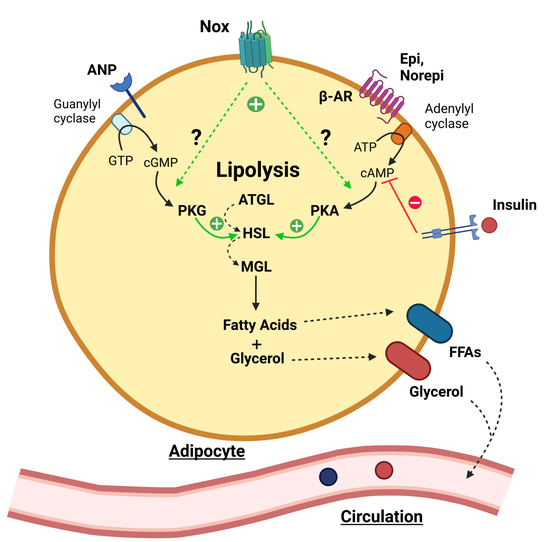
- According to the CDC (2023), cardiovascular diseases are the leading cause of death in the United States and type 2 diabetes is the eight-leading cause of death.
- Previous research has found a link between a high concentration of reactive oxygen species (ROS) and cardiometabolic diseases (Mahmoud et al. 2017; Sedeek et al. 2012).
- Evidence in cell culture studies suggest that high ROS production contributes to dysregulated lipolysis, which is a common feature of cardiometabolic diseases (Pizzino et al. 2017).
- Lipolysis is the breakdown and release of triglyceride stores in adipose tissue cells (Meza et al. 2019).
- NADPH Oxidase (NOX) are enzymes that are major sources of ROS in adipose tissue and have been shown to regulate lipolysis (Pizzino et al. 2017). However, the mechanisms through which this is done remain to be fully determined.

PURPOSE

The purpose of this study is to examine a major source of ROS production, NADPH Oxidase (NOX), as a regulating factor of lipolysis and how the effects of Nox on lipolysis impact vascular function and glucose metabolism.

LIPOLYSIS SIGNALING PATHWAYS

Figure 1. Lipolysis Signaling Pathways in Adipocytes



METHODS

Participant Characteristics:

- N= 8 males and females; age= 22.3 ± 5.0; Body mass index: 25.0 ± 9.5 kg/m^2

Preliminary Health Assessments:

- Participants took part in three health screening assessments prior to microdialysis procedures: VO_2max, brachial artery flow-mediated dilation (FMD) and DXA (body composition).

Microdialysis Procedures:

- Three microdialysis probes were inserted into subcutaneous abdominal adipose tissue.
- The procedures began with the participant in a fasted state followed by procedures during a "fed" state.

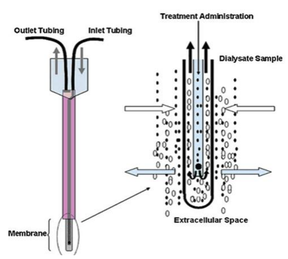


Figure 2. A diagram of the semi-permeable microdialysis probe membrane.

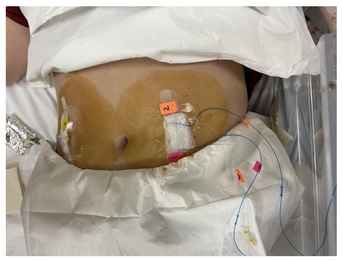
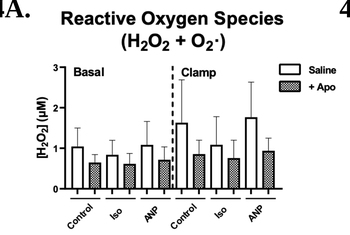


Figure 3. A representative image of microdialysis probes inserted into subcutaneous abdominal adipose tissue.

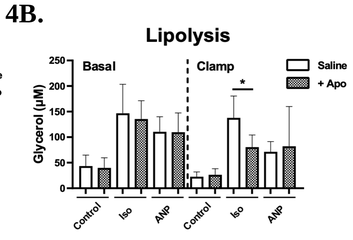
- Lipolysis was stimulated through two independent signaling pathways by perfusing isoproterenol (a β-adrenergic stimulus) or atrial natriuretic peptide (ANP) through separate probes. A third probe containing saline (no lipolytic agonists) was used as a control. Glycerol concentrations in the dialysate fluid were used as an indicator of lipolysis.
- To determine whether NOX alters lipolysis through β-adrenergic- or ANP-dependent pathways (Figure 1), a NOX inhibitor (apocynin) was perfused into each probe following lipolytic stimulation.
- ROS levels (expressed as the combination of hydrogen peroxide and superoxide concentrations) were measured from the dialysate samples using a fluorometer.
- A hyperinsulinemic-euglycemic clamp (clamp) was utilized to mimic a "fed" state, where plasma insulin levels are elevated. The clamp procedure involves infusion of glucose at a variable rate together with insulin infusion at a fixed rate of 12mU/m^2/min. A higher glucose infusion rate indicates greater responsiveness/sensitivity to insulin.

RESULTS

4A.



4B.



- ROS levels decreased in each probe when the local Nox-inhibitor, apocynin (Apo), was introduced (**Figure 4A**).
- Nox-derived ROS levels were augmented during hyperinsulinemic conditions compared to fasted conditions (**Figure 4B**).
- Glycerol concentrations (as an indicator of lipolysis) were decreased in the isoproterenol (Iso) probe compared to the ANP probe upon NOX inhibition during the hyperinsulinemic-euglycemic clamp.

CONCLUSIONS

- We provide evidence for the first time in human participants that NOX contributes to increases in adipose tissue lipolysis.
- NOX are a significant generator of adipose tissue ROS production *in vivo*, which lead to increased lipolysis through the β-adrenergic cellular pathway. This effect of Nox on lipolysis is augmented during a post-meal condition.
- Our data suggest that an increased NOX activity can lead to dysregulated lipolysis, which is a common feature of obesity-related conditions that contributes to vascular complications and high blood glucose.

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REFERENCES

Meza, La Favor, J. D., Kim, D.-H., & Hickner, R. C. (2019). Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? International Journal of Molecular Sciences, 20(15), 3775.

Centers for Disease Control and Prevention. (2023). *FASTSTATS - Leading causes of death*. Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm

Pizzino G., Irrera N., Cucinotta M., Pallio G., Mannino F., Arcoraci V., Squadrito F., Altavilla D., Bitto A. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Med. Cell. Longev.* 2017;2017:8416763.

Mahmoud, Ali, M. M., Miranda, E. R., Mey, J. T., Blackburn, B. K., Haus, J. M., & Phillips, S. A. (2017). Nox2 contributes to hyperinsulinemia-induced redox imbalance and impaired vascular function. Redox Biology, 13(C), 288–300.

Sedeek, Montezano, A. C., Hebert, R. L., Gray, S. P., Di Marco, E., Jha, J. C., Cooper, M. E., Jandeleit-Dahm, K., Schiffrin, E. L., Wilkinson-Berka, J. L., & Touyz, R. M. (2012). Oxidative Stress, Nox Isoforms and Complications of Diabetes—Potential Targets for novel Therapies. Journal of Cardiovascular Translational Research, 5(4), 509–518.