HIV prevention by inducing immune quiescence using low-dose aspirin: potential involvement of the lipoxygenase pathway?

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"Conflict of Interest Disclosure: I have no conflicts of interest"

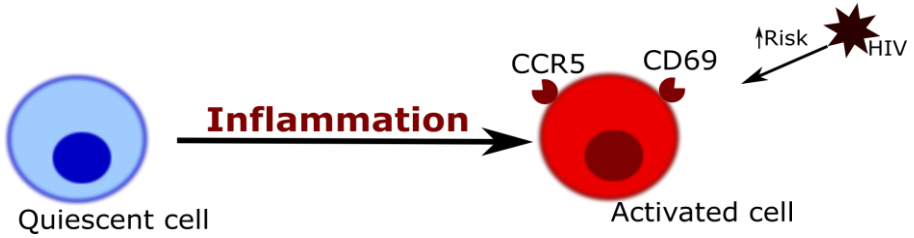
Introduction/Background

HIV prevention

- Globally 1.5 million new HIV infections occurred in 2020
  - New prevention strategies are needed
- Inflammation recruits HIV target cells to the genital tract (Lajoie, Mwangi, & Fowke, 2017)

Increased inflammation = **increased** risk for HIV acquisition

- STIs: Cause localized genital inflammation
- Microbicides: CAPRISA-004 (1% tenofovir gel) (Abdool et al 2010)
  - Increased inflammation resulted in increased risk of HIV acquisition



Decreased inflammation = **decreased** risk for HIV acquisition

- HESN (HIV-exposed seronegative) from Majengo cohort
  - Remain HIV uninfected despite intense exposure to HIV
  - Associated with a resting immune state (Immune Quiescence) (Lajoie, Mwangi, & Fowke, 2017)

Aspirin (ASA)

- Non-steroidal anti-inflammatory drug
- Used daily (81mg) for treatment of autoimmune diseases
- Safe, affordable, globally accessible, community accepted

Aspirin and HIV target cells

- Pilot study conducted by our lab found: (Lajoie *et al.*, 2018)
  - Decrease in **expression** of CCR5 on CD4+ T cells in the **blood**
  - 35% decrease in **proportion** CCR5CD4+ T cells at the **genital track**

Cyclooxygenase (COX) and Aspirin

- Aspirin inhibits COX enzyme function preventing pro- and anti-inflammatory prostaglandin and pro-inflammatory thromboxane synthesis (Gabbs et al 2015)

Lipoxygenase (LOX) and Aspirin

- Lipoxygenases (LOX) responsible for the synthesis of oxylipins from polysaturated fatty acids
  - 5-LOX: present in mature **leukocytes** (granulocytes, monocytes, mast cells, B-cells, DC) (Zappavigna et al 2020)
  - 12-LOX: present in epithelial and myeloid cells (esp. **platelets**) (Mashima et al 2015)
  - 15-LOX: present in epithelial cells and **leukocytes** (Mashima et al 2015)
- LOX Effects on Inflammation
  - **Both Pro- and Anti-inflammatory effects**
  - LOX expression increased following inflammation (IL-4 or IL-13) (Kühn et al 2006)
- Very little known about ASAs interaction with lipoxygenase pathway
- Observational studies in preeclampsia and aspirin sensitive respiratory diseases
  - **No effect of ASA on select lipoxygenase metabolites** (Gray et al 2002, Walsh 2021)

Participants (N=38)

Participants from Pumwani and Baba Dogo communities

General Characteristics	
Age (mean [SD])	32 [8.0]
Douching (n(%))	21 (55.3)
Contraception	
No Hormonal Contraception (n(%))	12 (31.6)
Depot/DMPA (n(%))	21 (55.3)
Oral HC (n(%))	2 (5.2)
Other (n(%))	2 (5.2)
No information given (n(%))	1 (2.6)
Regular Partner	
Yes (n(%))	30 (78.9)
No (n(%))	4 (10.5)
Not Disclosed (n(%))	4 (10.5)
Times sexual intercourse with regular partner in last 7 days (mean [SD])	1.19 [1.1]
Used condom with regular partner in the last 7 days (n(%))	5 (13.2)

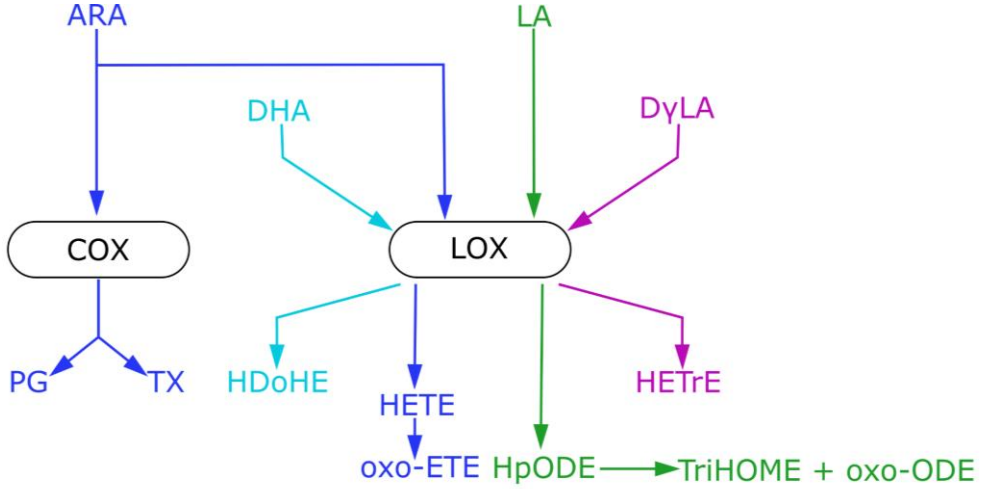
Methods

- Plasma samples from pre-ASA (visit 1) and last day of 6-weeks ASA (visit 3) were stabilized using an antioxidant cocktail and spiked with an internal standard
- Oxylipin analytes were selected using Strata-X SPE column and quantified using liquid chromatography tandem mass spectroscopy

Gaps in Knowledge/Hypothesis

Gaps in Knowledge

- Mechanism of ASA induced immune quiescence remains unknown
- Oxylipins are known to modulate the immune system
- Effect of daily low-dose ASA on inflammatory oxylipins from non-COX pathways in otherwise healthy adult women is understudied



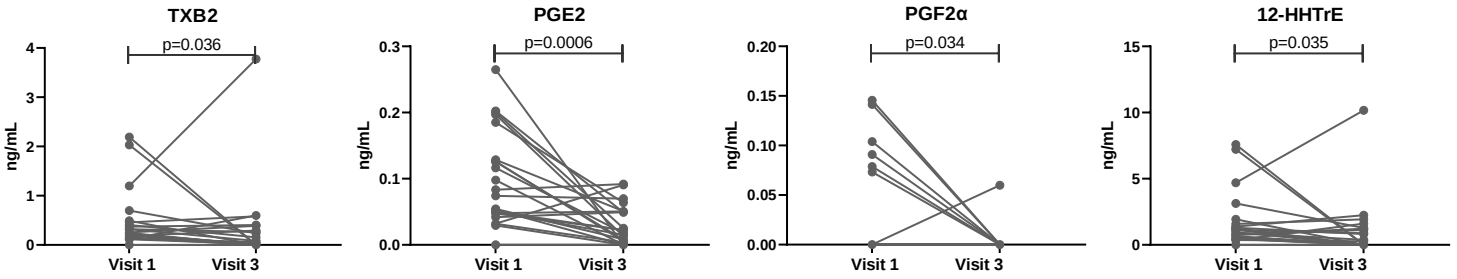
Summary of oxylipin pathways assessed using LCMS. COX; cyclooxygenase, LOX; lipoxygenase, ARA; arachidonic acid, DHA; docosahexaenoic acid, LA; linoleic acid, DγLA; dihomo-γ-linoleic acid, PG; prostaglandin, TX; thromboxane, HDoHE; hydroxydocosahexaenoic acid, oxo-ETE; oxo-eicosatetraenoic acid, HETE; Hydroxyeicosatetraenoic acid, HpODE; hydroperoxyoctadeca-dienoic acid, TriHOME; trihydroxy-octadecenoic acid, oxo-ODE: oxo-octadecadienoic acid, HETrE; Hydroxyheptadecatrienoic acid.

Goal: To assess if ASA use reduces mediators of inflammatory pathways such as the lipoxygenase pathway.

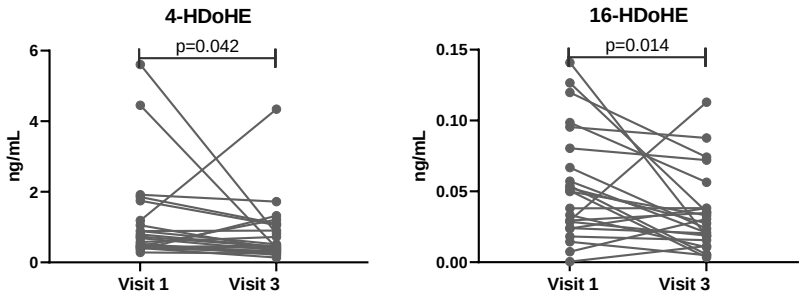
Hypothesis: daily low dose ASA will result in the reduction in oxylipin mediators of inflammation

Results: The Cyclooxygenase and Lipoxygenase pathways

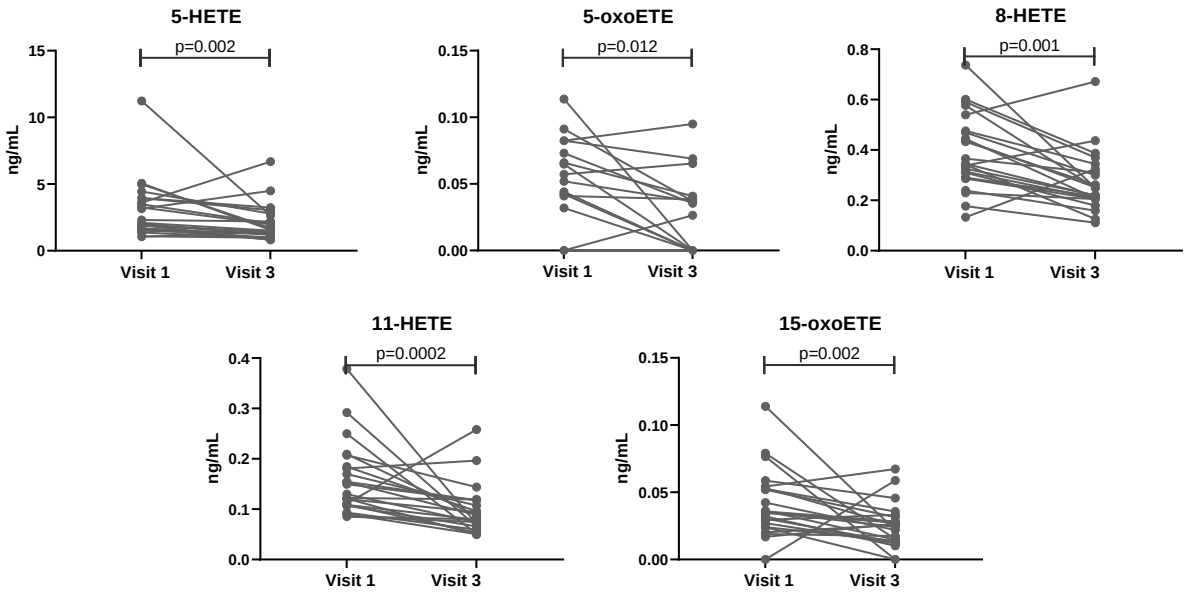
Aspirin downregulated COX metabolites from the arachidonic acid pathway



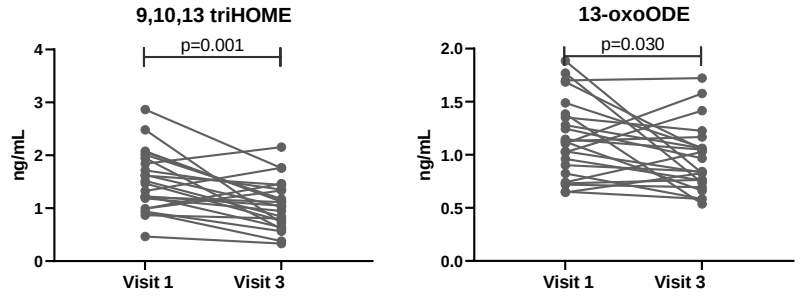
Aspirin downregulated LOX metabolites from the docosahexaenoic acid pathway



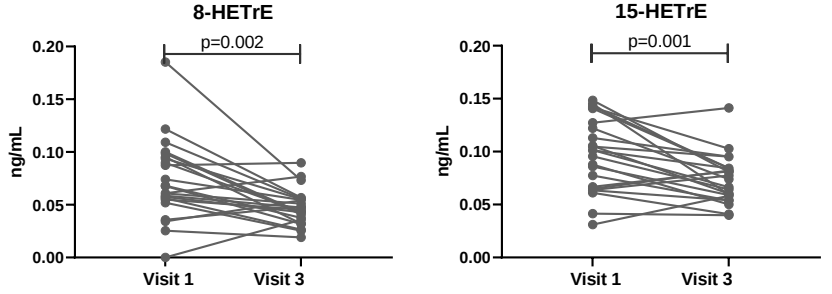
Aspirin downregulated LOX metabolites from the arachidonic acid pathway



Aspirin downregulated LOX metabolites from the linoleic acid pathway

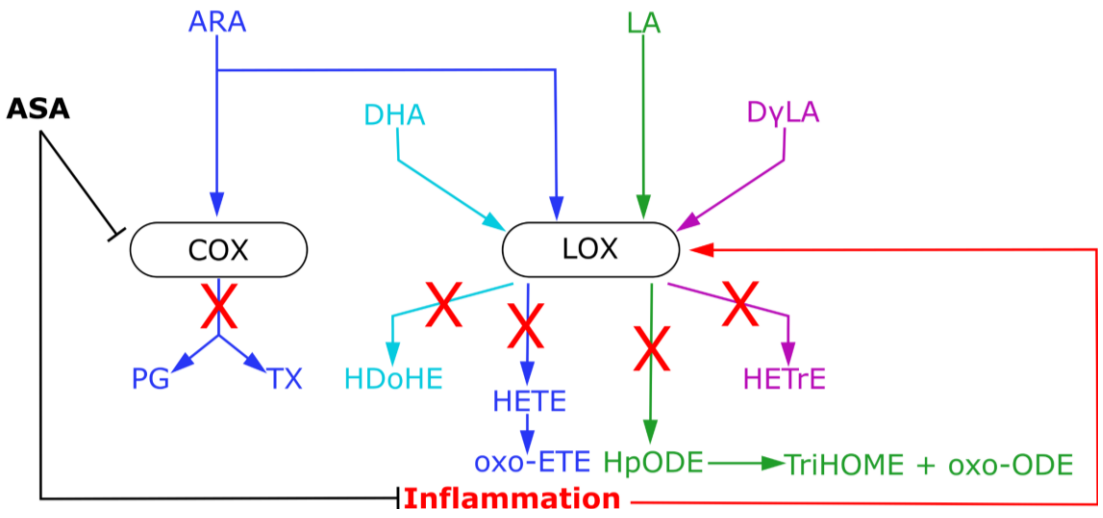


Aspirin downregulated LOX metabolites from the dihomo-γ-linoleic acid pathway



Visit 1 is before drug, visit 3 is last day of drug. Wilcoxon signed-rank paired test was run on all samples due to normality, $p<0.05$ were considered significant.

Summary of Findings



Summary of findings from mechanism of aspirins effect on oxylipin production, red X denotes reduced production following 6-weeks daily ASA. COX; cyclooxygenase, LOX; lipoxygenase, ARA; arachidonic acid, DHA; docosahexaenoic acid, LA; linoleic acid, DγLA; dihomo-γ-linoleic acid, PG; prostaglandin, TX; thromboxane, HDoHE; hydroxydocosahexaenoic acid, oxo-ETE; oxo-eicosatetraenoic acid, HETE; Hydroxyeicosatetraenoic acid, HpODE; hydroperoxyoctadeca-dienoic acid, TriHOME; trihydroxy-octadecenoic acid, oxo-ODE: oxo-octadecadienoic acid, HETrE; Hydroxyheptadecatrienoic acid.

Thank you!

- Fowke lab members
- Baba Dogo and Pumwani Staff
- Dr. Yoav Keynan
- UNITID Lab Staff
- **Participants**
- Funding Sources

Significance/Conclusion

- Following 6 weeks of ASA treatment, metabolites from both the lipoxygenase and cyclooxygenase pathways were down regulated.
- ASA is known to directly inhibit cyclooxygenase function however this is not the case with lipoxygenase.
- Inflammation has been found to increase lipoxygenase expression.
- Additional studies on the effect of 6-weeks ASA on the immune system in this cohort found ASA reduced systemic inflammation.
- Therefore, We speculate that ASA-associated reduction in inflammation, decreased lipoxygenase expression resulting in decreased lipoxygenase metabolites.

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