Gender-Based Violence, Hair Cortisol Levels and Immune Activation in HIV-Negative Female Sex Workers in Kenya

Alanna Mozena, MD, MPH

Project PI and Mentor: Susan Graham, MD, PhD

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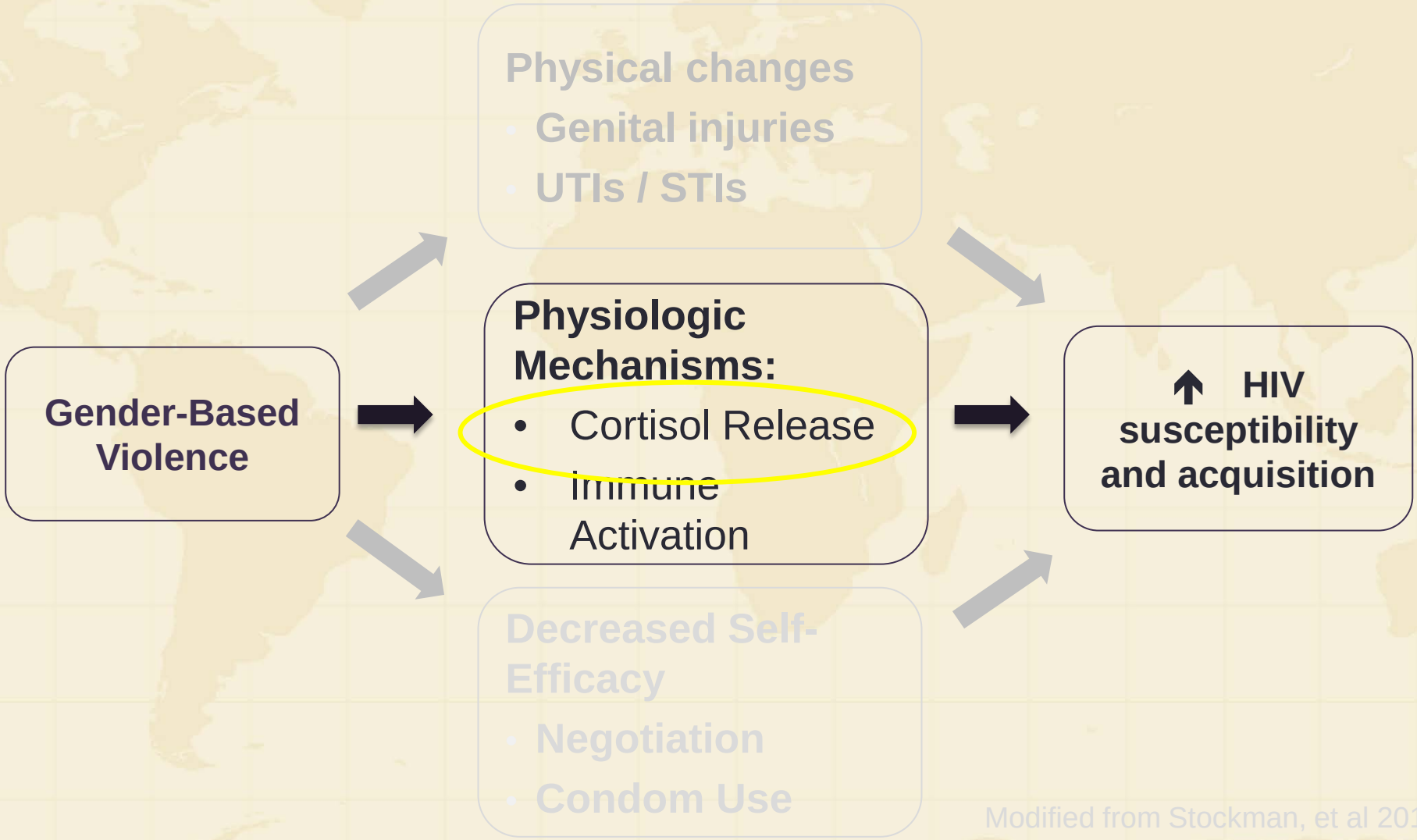
Background

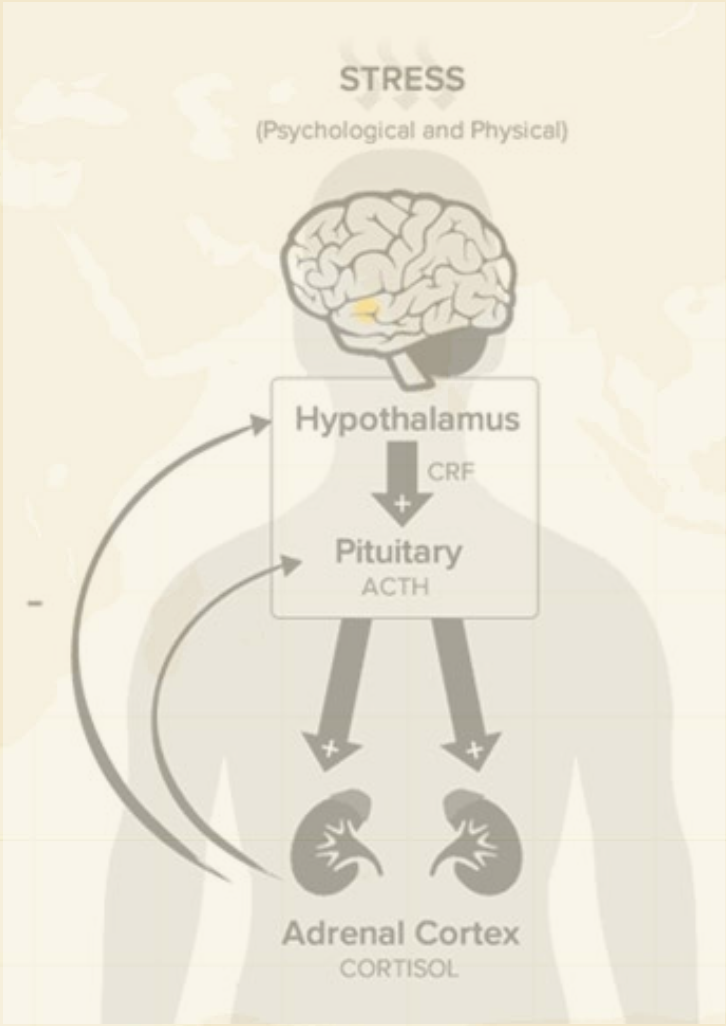
- **Violence against women is prevalent**
  - WHO estimates 1 in 3 women experience gender-based violence (GBV) worldwide
  - Women in Africa more likely to experience GBV (~46%)
  - Female sex workers especially vulnerable
- **GBV has serious mental and physical health consequences**
  - Exposure to GBV (from intimate partners or others) increases risk for HIV acquisition



Modified from Stockman, et al 2013

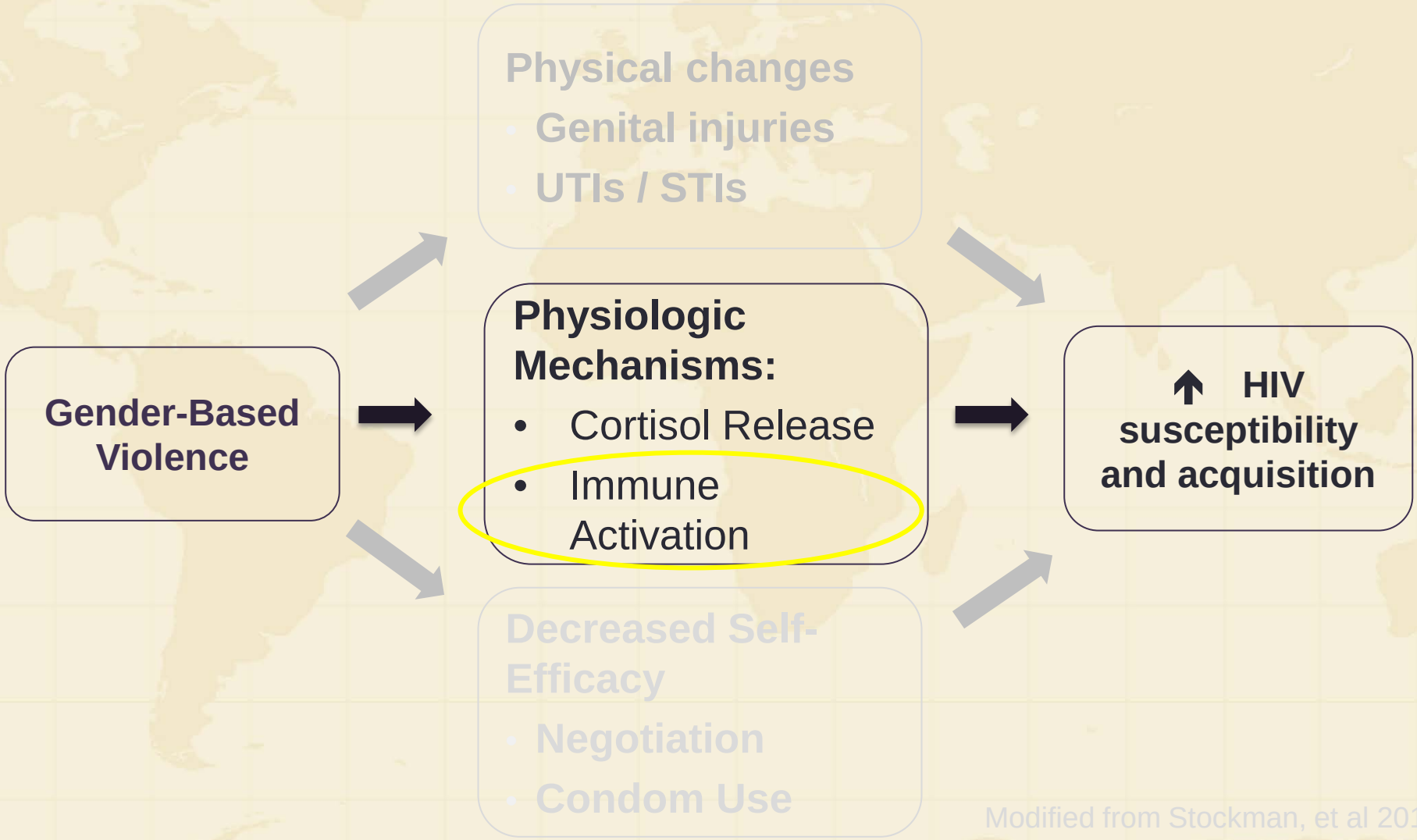
Does GBV influence cortisol secretion?

- Existing literature demonstrates conflicting results
- The HPA axis is difficult to study
  - Timing of cortisol measurement is critical otherwise the results can be difficult to interpret
  - Severity and timing of violent event(s) / exposure



http://www.integrativepro.com/Resources/Integrative-Blog/2016/The-HPA-Axis

Pinto et al 2016
Kim et al 2015
Seedat et al 2013



Modified from Stockman, et al 2013

Is GBV associated with immune activation?

- Lifetime IPV associated with immune activation in Atlanta (Kalohke, et al 2016)
  - Increase in markers of CD4+ activation
  - Transition from naïve to effector memory CD4+ phenotypes: which are thought to be more vulnerable to HIV infection
- Immune activation is associated with differences in HIV serostatus (Koning, et al; Begard et al)
- High-risk seronegative individuals were more likely to have:
  - Decreased frequency of activated and proliferating T cells
  - Suggests that a “quiet” immune system may be protective against HIV infection

Sexual Violence Study

- Cross-sectional study of HIV-negative women participating in the Mombasa Cohort
- Study Population: n = 283
  - Women aged 16-60 years
  - Residence within one day commuting distance to clinic
  - Self-report exchanging sex for payment in cash or kind
  - Not currently menstruating, pregnant or <6 weeks post-partum
- Exposure: Gender-Based Violence (GBV)
  - Physical or sexual violence from a partner or sexual violence from a non-partner
  - Measured using a modified version of the WHO Violence Against Women Instrument

Biomarker Testing

- Hair samples for cortisol levels:
  - 150 strands were collected from the vertex
  - Cortisol was extracted from hair and quantified by a competitive microtiter plate enzyme immunoassay using a purified polyclonal anti-cortisol antibody
- Peripheral blood samples for flow cytometry:
  - Plasma and PBMCs separated soon after collection
  - Specimens cryopreserved and shipped in liquid nitrogen to Fred Hutchinson Cancer Research Center
  - Prepared according to standard protocol, viability assessed using AViD staining
  - Surface antibodies against 46 T cell markers and 16 markers of innate immunity

Participant Characteristics

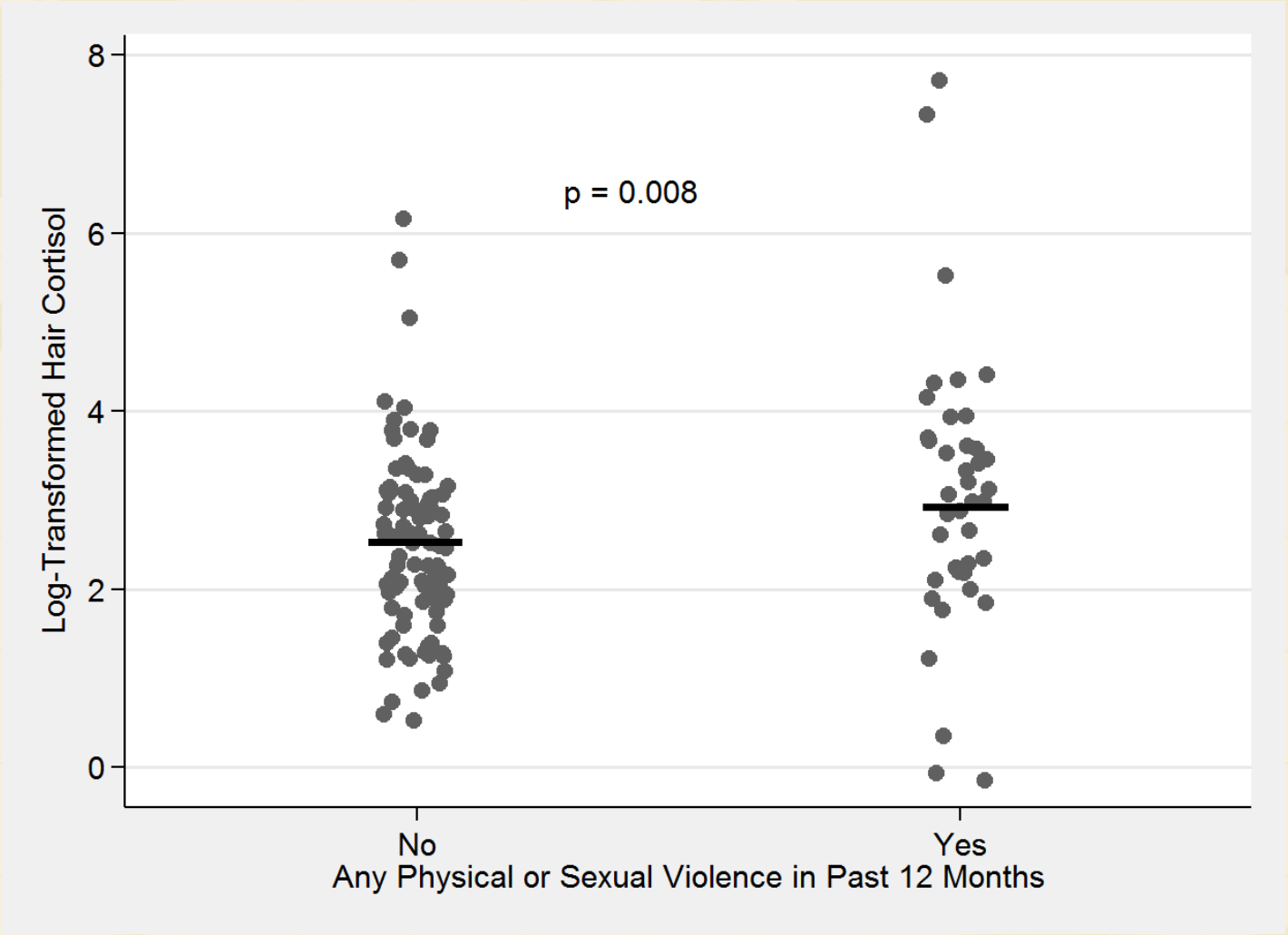
- Prevalence of physical or sexual GBV in the past 12 months: 32.0%
- Prevalence of lifetime physical or sexual GBV: 76.7%
- Women who reported physical or sexual violence in the past 12 months were younger than those who did not (p=0.0013)

	Overall (n = 283)	**Exposed (n = 90)**	**Unexposed (n = 193)**
Age (years)	33.5 (27.2 – 40.6)	31.0 (24.5 – 38.4)	34.4 (28.7 – 42.7)

Health-Related Characteristics

	Overall (n = 276)	Exposed (n = 90)	Unexposed (n = 193)
Mental Health Disorders			
Depression (PHQ-9)	10.6%	13.3%	9.3%
PTSD (PLC-C)*	16.2%	25.6%	11.9%
Substance use disorders			
Current tobacco use	12.4%	15.6%	10.9%
Harmful alcohol use (AUDIT)*	11.7%	21.1%	7.2%
Harmful use of substances (DAST)*	6.7%	12.2%	4.2%
Sexually Transmitted Disease	9.5%	12.2%	8.3%
HSV-2 seroprevalence (n = 226)	78.8%	73.9%	80.8%
Hormonal Contraceptive Therapy	25.1%	31.1%	22.3%

Hair Cortisol Concentrations



GBV and CD4 Cell Markers: Preliminary Results

Immune Function	CD4 Cell Marker	H	GBV exposed§	GBV unexposed§	p-value*
Markers of Immune Stimulation	HLA-DR+	↑	3.1 (2.0 – 4.5)	3.5 (2.3 – 4.8)	0.08
	Ki-67+BcL2Lo+	↑	1.2 (0.8 – 1.6)	1.2 (0.9 – 1.8)	0.63
	CD38brHLA-DR+	↑	1.4 (0.9 – 2.6)	1.8 (1.1 – 2.7)	0.18
Co-receptor for HIV	CCR5+	↑	15.2 (11.7 – 20.3)	15.1 (9.8 – 23.4)	0.99
Naïve	CD27+CD45RA+	↓	18.3 (10.6 – 26.6)	19.5 (9.5 – 26.7)	0.91
Central Memory	CD27+CD45RA-	↑	55.2 (48.7 – 61.5)	52.4 (43.8 – 60.5)	0.08

*Wilcoxon Rank Sum Results
§Sample size was variable depending on viability for flow analysis
NOTE: Analysis excluded 7 participants with acute illness (TB treatment, malaria, HSV)

Additional Analyses Planned

- Markers of innate immunity
  - Has not previously been investigated
- Associations with lifetime physical and sexual violence
  - Predictor used in Kalohke et al study
- Correlations between hair cortisol levels and immune markers

Limitations

- High rate of refusal for hair sample collection
  - Most cited technical difficulties as the cause (related to hair style)
  - Those that experienced violence were less likely to provide hair sample
- Multiple comparisons for analysis of immune markers (exploratory)
- Some PBMC samples had poor viability

Conclusions

- Significant difference in hair cortisol concentrations in those who were exposed to physical or sexual GBV in the past 12 months
- No significant differences in CD4+ activation among women exposed to recent physical or sexual GBV
- Additional results pending

Questions?

Acknowledgements

- Study participants
- SV Study Team
  - University of Washington
    - Susan Graham – Principal Investigator
    - Vrasha Chohan
    - Madeleine Heller
    - Sarah T. Roberts
  - Fred Hutchinson Cancer Research Center
    - Steve De Rosa
    - Erica Lovelace
- Funding from the UW CFAR (NIH P30AI027757)
- Inter-CFAR Collaborative Symposium on HIV Research in Women (NIH R13 AI127307)

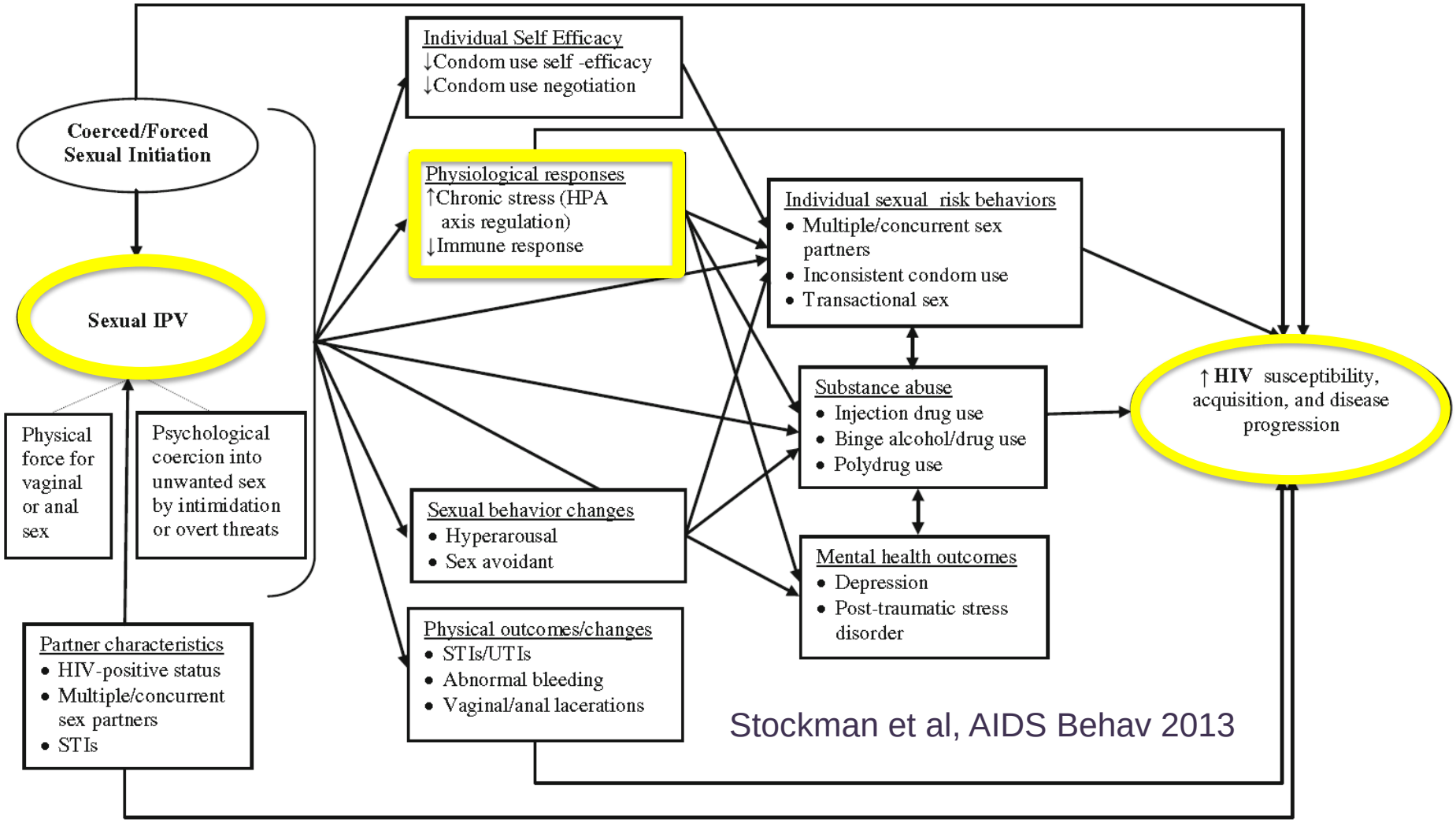


Fig. 1 Behavioral and physiological mechanisms for the relationship between coerced/forced sexual initiation, sexual IPV, and HIV/AIDS. *HPA* hypothalamic–pituitary–adrenal, *STI* sexually transmitted infection, *UTI* urinary tract infection

Does GBV Influence Cortisol Secretion?

Author	Measurement	Summary
Pinto et al, 2016	Salivary cortisol (wakening – 30 min later)	Chronic severe violence was significantly associated with *variance in cortisol awakening response*
Kim et al, 2015	Salivary cortisol (wakening, midday and afternoon)	Women with higher levels of victimization exhibited *flatter patterns of diurnal cortisol*: higher midday levels and attenuated decreases in cortisol over the course of the day
Seedat et al, 2003	Plasma cortisol (0900-1200)	Women who were victims of physical IPV had *lower mean cortisol levels* compared to women who are not victims.

Physical/Sexual GBV and CD4 Cell Markers, Hypotheses

Immune Function	Cell Marker	Hypothesis	Comment
Immune stimulation	HLA DR+	↑	Previously found to be low in highly exposed seronegative women
Co-receptor for HIV entry	CCR5+	↑	Up-regulated with T – cell activation
Surrogate for cellular activation	Ki-67+ BcL2Lo+	↑	
Activation	CD38br HLA-DR+	↑	
Naïve	CD27+ CD45RA+	↓	
Central Memory	CD27+ CD45RA-	↑	
Effector Cells	CD27- CD45RA-	↑	

Adaptive Immune System

Immune Function	CD4+ Cell Marker	Function	Hypothesis
Marker of Immune Stimulation	HLA-DR+	Found to be low in highly exposed but HIV-negative	↑
Surrogate for Cellular Activation	Ki-67+	Nuclear protein associated with activation	↑
Activated CD4+	CD38+	Associated with HIV progression	↑
Co-receptor for HIV entry	CCR5+	Preferentially support HIV replication	↑
Naïve	CD27+CD45RA+	Less susceptible to HIV infection	↓
Central Memory	CD27+CD45RA-	More susceptible to HIV infection	↑