STATE OF THE SCIENCE CONCEPTS IN REHABILOMICS: HORMONE RELEVANT BIOMARKERS IN REHABILITATION RESEARCH

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Gender and TBI Epidemiology

- Overall incidence: 200 per 100,000 per year
  - Males Under 25: 700 per 100,000 per year
- Men have a higher incidence of TBI than women at all ages, with reported incidence ratios ranging from 2.0:1-2.8:1 and mortality ratios of 3.5:1
- The increased incidence in men may be secondary to greater prevalence of men involved higher risk activities and occupations
  - Contact sports
  - Military
- **Heterogeneity with injury types:** mixture of diffuse and focal injury
- **Heterogeneity with injury mechanisms:** blunt, penetrating, blast, varied extra-cerebral trauma
Translational and Experimental Research approaches to answering Rehabilitation Relevant questions targeting disease/injury heterogeneity and recovery:

- What is the underlying acute and chronic physiology linking TBI to a heterogeneous range of impairments?
- How can practitioners determine who is at risk for complications or poor outcomes?
- How do both acute and chronic treatments effect brain recovery?
- How can treatment approaches be tailored meet the needs of each individual with TBI?
- How to traditional individual factors like age and gender contribute to molecular mechanisms of injury, recovery, and treatment?
The WHO-ICF Model is clinically intended for:

- Understanding disease beyond the traditional medical model
- Allows for needs assessment across domains of impairment, functioning, participation
- matching interventions to specific health conditions and complications
- rehabilitation and outcome evaluation

The Rehabilomics adaption of the WHO-ICF model is a framework to integrate a biomarkers based understanding of secondary injury with REHABILITATION RELEVANT and multi-dimensional view of recovery.

- Biomarkers provide unique information about disease pathology and risk for complications.
- Framework suggests that biomarkers carry some capacity to discriminate multidimensional outcomes
- Biomarkers may provide the ability to personalize TBI care and management

TBI hormone physiology and neuroendocrine dysfunction contextualized
- Genetic Biomarkers
- Steroidogenesis biomarkers
- Global outcomes, Cognition, QOL
Myth 1: Ovarian Hormones are neuroprotective, so women should have better outcomes than men.

Myth 2: Endogenous estradiol for men & testosterone for women do not impact TBI physiology.

Myth 3: Hormone profiles & aging in men should not really affect TBI physiology or outcome.

Myth 4: Serum hormone physiology is a likely reflection of CNS hormone physiology.

Myth 5: Hypogonadism is a transient syndrome and probably does not influence outcome.

Myth 6: Pre-clinical study models capture the necessary information for adequate clinical trial designs that include

Myth 7: Women would not benefit from hormone therapy after TBI since they already have protective hormones.
What are the Research Questions?

- How are hormones interrelated?
- What mechanisms of injury can hormones target?
  - Generated from the experimental literature
**Hormone Synthesis and Metabolism**

*Adrenal and Gonadal Synthesis Possible
Peripheral aromatization*
Secondary Injury Cascades

- Demyelination
- Inflammatory Cytokines
- Receptor activation
- Pro-survival Cascade
- Apoptosis
- Pro-apoptotic cascade
- Cerebral Edema
- Ischemia
- Mitochondrial Uncoupling
- Free radicals & lipid peroxidation
- Excitotoxic cascade
- Adenosine Response
**NeuroActive Mechanisms of Sex Hormones After TBI**

- **Estrogen**
  - Maintaining cerebral blood flow
  - Prevents apoptosis
  - Serves as a growth factor
  - In the presence of toxic levels of glutamate, estrogens attenuate NMDA mediated Ca++ activity
  - Scavenging of glutamate driven free radical formation
  - Reactive maintenance of lactate production and neuronal lactate utilization
  - Dossett: Known to increase with stress of critical illness and trauma
    - ?? Adrenal synthesis and/or peripheral aromatization
    - Peripheral aromatase not present in rodents
    - Likely cause due to inflammatory surge associated with trauma or critical illness

- **Testosterone**
  - Yang SH 2002: Exacerbate cerebral ischemia-reperfusion injury
  - Yang SH 2005: Testosterone reduction decreases lesion volume in MCAO model
  - Roselli CF 2007 aromatization in cortical astrocytes is associated with cell survival and may be important for neuroprotection
  - Aromatization in peripheral adipocytes via TNF-alpha mediated transcription.
Hormone Synthesis and Influences on Neurological Function after TBI

**Progesterone**
- Decreases cerebral edema
- Influences brain aquaporin levels
- Decreases lipid peroxidation
- Attenuates BBB disruption
- Affects neuro-inflammation
  - Decreases in IL-1B levels
- Improves behavioral outcome in male rats after acute injury
- Major metabolite Allopregnanolone is also neuroprotective

**Cortisol**
- Virgin CE: glucocorticoids might impair the ability of astrocytes to remove damaging glutamate from the synapse
- Llompart-pou JA 2008: Adrenal insufficiency noted in a significant portion of people with severe TBI.
- Cortisol synthesized in the periphery only in humans.
- Progesterone is the primary substrate for adrenal cortisol synthesis.
How are hormones interrelated?

- Steroidogenesis pathways include the major reproductive hormones and can be generated in the gonad, the adrenal, and in peripheral sites like adipose tissue for humans.
- ***Rodents do not have peripheral aromatase activity in adipocytes, which may diminish the ability to translate animal studies to humans.

What mechanisms of injury can hormones target?

- Generated from the experimental literature
- Estrogen and Progesterone can be neuroprotective when manipulated or administered as pharmacotherapeutics
  - Hormones like progesterone and estrogen are pleiotropic compounds that can target many of the same targets implicated in secondary injury.
  - Testosterone and Cortisol have a mix of potentially protective vs. deleterious effects that are situationally dependent
Menopause Hormones & Acute Outcomes

- **Farin (2003)**
  - Tirilizad cohort (957 patients) with severe TBI
  - Women less than 51 years had more episodes of intracranial hypertension, brain swelling independent of GCS and a trend for worse outcomes.

- **Davis (2006)**
  - 13,437 patients with severe TBI
  - Outcome significantly better in postmenopausal females versus males
  - Post menopausal females do better than age matched males

- **Ottochian (2009)**
  - 1,807 TBI patients severe (isolated) TBI
  - Women greater than 55 years had higher acute mortality rates.

- **Berry (2009)**
  - National Trauma Database version 6.2 (2000-2005) 72,294 patients with isolated moderate to severe TBI.
  - Females showed a lower risk in both mortality and in developing any type of complications than males (multivariate analysis)
  - Perimenopausal and postmenopausal women showed a lower mortality risk than younger women.
  - No difference in mortality in premenopausal women compared with their male age-matched counterparts
What are the Research Questions

• **What happens to endogenous hormone levels after TBI?**
  - Can early endogenous hormone levels be linked to outcome?
  - How does aging influence hormone physiology?
Hypogonadism in TBI

- **Acute Hypogonadism**
  - Agha A (2004): Measurements median 12 days (range 7-20 days) post injury in Mod/severe TBI
    - Hypogonadism in 80% of males and 90% females
    - Low gonadotrophins in concert with low estrogen/testosterone.

- **Chronic hypogonadism:**
  - Schneider HJ (2006): Affects 21-32% of patients 3-12 months after TBI
    - hypogonadism is largely secondary to pituitary dysfunction.
  - Ripley DM 2008: Menstrual cycle function can be impaired for women up to several months after severe TBI
    - can have a negative impact on outcome.
Wagner (2011): Temporal characterization of hormones over an acute time course post TBI

- Identify potential sources of endogenous hormone production after severe TBI
- 117 men and women with severe TBI
- Daily serum samples for 1 week post-injury
- Determine if serum hormone profiles can be linked at all to later outcome
Pituitary Hormones and TBI

**Serum FSH (IU/L)**

- Post-menopausal: 51.6 IU/L
- Post-menopausal: 26.2 IU/L

**Serum LH (IU/L)**

- Post-menopausal: 51.6 IU/L
- Post-menopausal: 26.2 IU/L
Serum Hormones and TBI

A. Serum Cortisol (ng/mL)

- Mean Cortisol levels measured over several days from injury.
- Graph shows cortisol levels for males + females.

B. Serum Progesterone (ng/mL)

- Mean Progesterone levels measured over several days from injury.
- Graph shows progesterone levels for different groups:
  - Female
  - Luteal females
  - Follicular females + males

C. Serum Testosterone (nmol/L)

- Mean Testosterone levels measured over several days from injury.
- Graph shows testosterone levels for males + females.

D. Serum Estradiol (pg/mL)

- Mean Estradiol levels measured over several days from injury.
- Graph shows estradiol levels for different groups:
  - Luteal females
  - Males
Group based trajectory analysis (TRAJ)

- A specialized application of finite mixture modeling that allows for the assessment of patterns of change over time (Nagin D 2005).
  - Determines trends in longitudinally collected data by identifying trajectory groups on a likelihood basis
  - Does not rely on mean or peak measures for analysis.
  - Group-based trajectory analyses are designed to identify clusters of individuals following similar progressions of some behavior or element over age or time.
  - Used in social sciences: e.g. cohort crime rates over time

This study applies this novel method to examine clusters of patients following similar hormone trajectories across time.

- **Traditional:** Look at overall concentrations (single point estimate) across time
- **TRAJ:** Identify populations substructures that may have similar clinical/demographic characteristics

- Rank hormone data for analysis
• 75% of females in higher PROG TRAJ (*p=0.01); No gender exclusive groups
• Older subjects in TRAJ groups with higher hormone levels
• Injury severity (ISS, GCS) and hypothermia treatment not predictive of TRAJ group membership
• High group PROG: GOS-6 and Acute Mortality
• Serum Cortisol not associated with outcome.
• Older subjects in TRAJ groups with higher hormone levels; no gender exclusive groups
• Injury severity (ISS, GCS) not predictive of TRAJ group membership
• High E2 associated with high serum E2:T ratios
• High group EST and TEST TRAJ membership associated with worse outcome: GOS-6 and Acute Mortality
Bivariate Analysis: Age vs. Gender

<table>
<thead>
<tr>
<th>Hormone Trajectories</th>
<th>Gender % Female</th>
<th>Age Mean ± SE</th>
<th>GCS Median</th>
<th>ISS Mean ± SE</th>
<th>Acute Care Mortality % Dead</th>
<th>Six Months GOS, %</th>
<th>Post Menopause %</th>
<th>Male Age greater than 40%</th>
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<td>31</td>
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<td>35.0 ± 1.0</td>
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<td>Group 2</td>
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<td>36 ± 1.8</td>
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<td>35.8 ± 1.3</td>
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<td>38</td>
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<tr>
<td>Group 3</td>
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<td>40 ± 3.0</td>
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<td>33.2 ± 1.5</td>
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- No gender exclusive groups among hormone TRAJ
- Stronger age differences than gender differences among hormone TRAJ
• CORT levels are strongly correlated to residual PROG as pituitary hormones shut down.
• The resultant TRAJ PROG group differentiation likely reflects adrenal fraction of this hormone.
• AI status is linked to all other TRAJ group profiles, indicating that a large part of the residual hormone profile for each of these hormones is synthesized in the adrenal or generated from hormones synthesized in the adrenal.
Theoretical Hormone Prognostic Model

Figure 1B:

PROG -> *TEST -> OUTCOME
PROG -> *CORT
PROG -> *EST

Direct Effects

*Mediating Effects
Acute Hormone Profiles: Outcome Prediction

*Significant mediation effect of Serum PROG on outcome through Its association with EST
What happens to endogenous hormones after TBI and are they neuroprotective?

- Endogenous pituitary gonadotrophins plummet over first few days post-TBI.
- Unique TRAJ profiles for gonadal/adrenal hormones
- Peripheral aromatization and/or adrenal synthesis involved in residual testosterone and estrogen levels in response to stress for both men & women
- Elevated estrogen and testosterone associated with WORSE outcomes.
  - Estrogen is the strongest predictor of acute mortality and poor global outcome
  - Progesterone is the adrenal metabolic precursor to other hormones associated with outcome
  - Progesterone associations with outcome mediated through relationship with other hormone synthesis
  - Serum cortisol levels not associated with outcome, despite a significant portion of the population with AI
- Aging (rather than sex) is a primary effector of peripheral hormone physiology
What are the Research Questions?

- How are chronic hormone levels affected by injury?
- Are chronic hormones and hypogonadism related to TBI outcomes.
Chronic Hypogonadism: Male Survivors

- **Wagner 2012**
  - 38 males with severe TBI who survived their injuries
    - Day 0 for survivors
    - Week 1 averaged
    - Every two week sampling
    - LH levels examined in the setting of EST, TEST, and EST/TEST ratios.
  - 6 month outcomes
    - FIM-COG
    - Cognitive Composite
    - Global Outcome
A. Testosterone by hypogonadism

B. LH by hypogonadism

C. Estradiol by hypogonadism

- Clinical Lab Normal Range
- Mean Healthy Control Values
- ERHH
- PHH
6 Month Outcomes

**Graph A:**
- Hypogonadism by 6m GOS group proportions
- ERHH vs. PHH
- Percentages for GOS 4-5 and GOS 2-3
- Significant difference indicated by asterisk

**Graph B:**
- Hypogonadism by 6m DRS
- ERHH vs. PHH
- Significant difference indicated by asterisk

**Graph C:**
- Hypogonadism by 6m FIM-COG
- ERHH vs. PHH
- Significant difference indicated by asterisk

**Graph D:**
- Hypogonadism by 6m Composite Score
- ERHH vs. PHH
- Significant difference indicated by asterisk
Chronic Hypogonadism Combined Cohort
- N=78 survivors severe TBI
- Hormone assessments wk. 1, 2-26, 52
- 6 month outcomes
Autoimmunity and Persistent Hypogonadism

**Figure 2.** IHC staining of human pituitary section with human chronic TBI and control serum. Human serum dilution at 1:30; Olympus BX60 microscope at 400x. TBI #894 serum has a strong immunoreaction with the hormone-releasing granule-bearing cells (indicated by arrows). TBI #799 has only a weak reaction, while control sera were negative.
What happens to chronic endogenous hormones after TBI and how do they relate to TBI outcomes?

- Testosterone profiles for male survivors show that, over the long term, low testosterone levels associated with hypogonadism are also associated with poor outcome.
- Low estradiol levels noted for PHH group.
- Hypogonadism can be considered a chronic condition for some, and may be linked to persistent autoimmune dysfunction or other pathology.
- More work on chronic hormone profiles for females is needed.
What are the Questions?

• How does aromatase genetics influence physiological hormone levels and Long Term Functional Outcomes after TBI?

• What are CNS hormones profiles like after TBI and how do they relate to outcome and to serum hormone profiles?
Aromatase Gene Variability after TBI

- Wagner (2013)
- 110 subjects with severe TBI
- 18 tagging SNPs and 4 functional SNPs
  - **Rs2470152**: locus is a potential binding site for a transcription protein cAMP response element (CRE) (Koudu et al., 2011; Sofi et al., 2003).
  - **Rs4646**: a tSNP located on the 3’ untranslated region (UTR) of the gene and is in close proximity to the functional SNP **rs700519**, a non-synonymous coding SNP.
    - Several studies report genetic influences at this locus with regard to hormone levels.
  - **rs2470144** is a tSNP located in intron 1 on the 5’ region of the gene (Guo et al., 2006).
    - May affect RNA folding and other transcriptional effects.
Single Nucleotide Polymorphisms (SNPs) are genetic variations in DNA in which a single nucleotide is replaced by another e.g.: AGGTGA to ATGGTTA

A Tagging SNP (tSNP) is a representative SNP in a region of the genome with high linkage disequilibrium

Linkage disequilibrium: represents an area of gene that is unlikely to undergo variation due to recombination.

A functional SNP is a SNP that alters gene function or expression.

Since each individual has two copies of each gene, SNP changes at a locus are represented in varying genotypes e.g. GG vs. AG vs. AA

Grouped Genotype (GG+AG vs. AA) OR (GG vs. AG+AA)
**CSF Estradiol:** Compared to controls. CSF estradiol are lower after TBI and not affected by sex.
- Mean serum and CSF estradiol correlation
  - (R²=0.4127, n=240 samples, N=86 patients)

**CSF Testosterone:** Levels are initially higher than controls but decline over time such that they are similar (and even lower than controls by day 6 post injury).
- Mean CSF and serum testosterone
  - (R²=0.0202, n=245 samples, N=88 patients)

**CSF E2:T Ratio:** Levels are lower than controls early after injury, but then increase over time.
- Increased E2:T ratios associated with better outcome in multivariate analysis when paired with rs4646
Genotype Associations with CNS Hormones

A rs2470152: E2:T Levels

B rs2470144:E2:T Levels

C rs4646:E2:T Levels

D rs2470152: E2:T Levels

E rs2470144: E2:T Levels

F rs4646: E2:T Levels

Legend:
- C/C
- T/T + T/C
- A/A
- G/G + G/A
- C/C
- A/A + A/C
- C
- T*
- G*
- A
- A*
- C

Control
SEM Control
Genetic variation in the aromatase gene is linked to serum hormone E2:T ratios, CSF E2:T hormone ratios, and global outcomes with GOS scores at 6 months post injury.

- Statistically different aromatase SNPs have unique effects on CNS hormones vs. outcomes.

Serum and CSF testosterone levels are not at all correlated early after injury.

Serum and CSF E2 levels moderately correlated early after TBI.

Unique E2 and T hormone physiology in CNS vs. Periphery after TBI.

- Higher CSF E2:T ratios are tend to be associated with better outcome

CNS-periphery links with chronic hormone physiology after TBI not known.

CSF progesterone and cortisol analysis upcoming.
What are the Research Questions?

- Can hormones be used as pharmacotherapies for neuroprotection after TBI
**Answers: Hormones as Therapies**

- **Progesterone Therapy**
  - Wright: *ProTECT phase II clinical trial: safe and potentially neuroprotective
  - *Xiao: Protective in Severe TBI
  - Wright/BHR: *ProTECT phase III clinical trial—ongoing

- **Estrogen Therapy**
  - Wigginton: RESCUE – TBI Single dose IV Premarin---phase II clinical trial

- **Glucocorticoids**
  - Bracken (2005): CRASH trial--Acute methylprednisolone increases risk of death after TBI

- **Testosterone:**
  - Supplementation done clinically in post acute phase.??

- Therapeutic benefit may be a function of Dose, Duration, Timing
- Unknown how hormone profiles, hormone synthesis, and hormone metabolism affected by hormones used as a pharmacotherapy
- Therapeutic targets in RCT require biomarker assessments
Collaborators: Yvette Conley, PhD, C. Edward Dixon, PhD, Tony Fabio, PhD, Joseph Ricker, PhD, Sarah Berga, MD., Tammy Loucks, PhD., Kevin Wang, PhD

Students: Megan Miller, MS., Chris Brett, BS, Julie Dobos, BS, PA.,

Staff: Sandra Deslouches, BS, Christian Niyonkuru, MS, Emily McCullough MPH, Joelle Scanlon, PhD, Martina Santarsieri, BS

Centers/Institutes: UPMC Trauma Registry, Rehabilitation Institute, Safar Center

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