Dietary supplementation with branched chain amino acids in Veterans with TBI

Jonathan Elliott, PhD
Sleep Health & Applied Research Program
December 13, 2019
Oregon Health & Science University
VA Portland Health Care System
TBI and sleep disturbances
*Insomnia, hypersomnia, circadian rhythm disorders, etc.*

Prevalence of ~50-70%

Mathias *et al*, *Sleep Medicine*, 2012

Lack mechanistic-based therapies

- Sedative-hypnotics or stimulants
- Cognitive behavioral therapy
- Acupuncture
- Bright light therapy
- Sleep hygiene education

Sandsmark, Elliott, & Lim. *SLEEP*, 2017
Mild TBI

Cortical impairment

Decreased glutamate input to hypothalamus

Orexin/Hypocretin Dysfunction & Sleep-Wake Disturbances

Negative neurologic outcomes (e.g., cognitive dysfunction)

Branched chain amino acids (Leucine, Isoleucine, Valine)
- Substrate for 50% of de novo glutamate synthesis in the brain

Sakai et al, J Neurochem, 2004
Yudkoff, Glia, 1997

Decreased plasma BCAA levels in TBI
Elliott et al, SLEEP, 2018
Jeter et al, J Neurotrauma, 2013
Willie and Lim et al, J Neurotrauma, 2012

Restored with BCAA administration and improves outcomes
Aquilani et al, Arch Phys Med Rehab, 2005
Ozgultekin et al, e-SPEN, 2008

Cole et al, PNAS, 2010
Paterno et al, J Neurotrauma
BCAA: metabolism

Plasma levels rise rapidly in proportion to their ingested dose

Matthews, J. Nutri, 2005

Substrate for ~50% of de novo CNS glutamate synthesis

Sakai et al, J Neurochem, 2004
Yudkoff, Glia, 1997
BCAA: Pleiotropic benefits

1. Improved cognitive function in hepatic cirrhosis and extended exercise
   Horst et al, Hepatology, 1984
   Blomstrand, Amino Acids, 2001

2. Reduction in manic episodes in bipolar disorder
   Scarna et al, Br J Psychiatry, 2003

3. Improvement tardive dyskinesia severity
   Richardson et al, Psychopharmacology, 1999

4. Reduced cachexia in cancer
   Cerra et al, Crit Care Med, 1983

5. Restored cortical excitation in ALS and SCA
   Tandan et al, Neurology, 1996
   Mori et al, J Neurol Sci, 2002
Randomized, double-blind, placebo-controlled trial of dietary BCAA supplementation in Veterans with TBI

Sleep Health & Applied Research Program
research coordinators, technicians, and graduate students

VA Research Pharmacy
# Experimental design and timeline

<table>
<thead>
<tr>
<th>Pre</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial visit</td>
<td>Baseline</td>
<td>BCAA or placebo intervention</td>
<td>Post-study visit</td>
</tr>
</tbody>
</table>

- **Informed consent**
- **TBI Interview**
- **Surveys**
- **Neuropsych testing**
- **Blood draw**
- **Actigraphy**
- **Study diary**

*Start BCAA or placebo*

- **Surveys**
- **Neuropsych testing**
- **Blood draw**
- **End actigraphy**
- **End study diary**

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- **BCAA**
  - 30 g + flavoring (B.I.D.)
- **Placebo (Rice Protein)**
  - 30 g + flavoring (B.I.D.)
- Consumed after waking and ~6 hrs later, on an empty stomach
- Block randomized controlling for age by VA Research Pharmacy
BCAA Dosing/Duration

• **5-90 grams, acute**
  *well tolerated without any adverse events*
  
  De Palo *et al*, *Amino Acids*, 2001
  Gijsman *et al*, *Psychopharmacology*, 2002
  Hasseman *et al*, *Nutrition*, 1994
  Kirvela *et al*, *Pharmacol Biochem Behav*, 1998
  Struder *et al*, *Horm Metab Res*, 1998
  Van Hall *et al*, *J Physiol*, 1995

• **15-60 grams/day for 7 days to 12 months**
  *mild gastrointestinal discomfort noted in one study*
  
  For Review:
  Fernstrom, *J Nutri*, 2005
  Fernstrom, *Amino Acids*, 2013

• **5 days to see improvements in mice**
  100 mMol = 0.36 g/kg/day = ~25 g/day for a 70 kg human
  
  Elkind and Lim *et al*, *Frontiers in Neurology*, 2015
## Demographics

<table>
<thead>
<tr>
<th>Summary: Demographics</th>
<th>BCAA</th>
<th>Rice Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>48 ± 9</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>Sex, n=female</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>35 ± 5</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>PCL, n&gt;33</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OSA, n=yes</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Exercise, n&gt;30 min</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Retired/disability</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
Feasibility and acceptability metrics

- **Dropout rate:**
  - 13% withdrawal rate (mainly from a lack of time)

- **Adherence:**
  - 100% self-reported adherence to supplement
  - 98% days with actigraphy worn

- **Summary of BCAA side effects:**
  - 2 of 7 reported transient nausea
  - 3 of 7 reported weight loss/appetite suppression
  - 1 of 7 noted increased muscle bulk in leg post-injury
<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Purpose</th>
<th>Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Hygiene Index (SHI)</td>
<td>Assess sleep habits, scores a participant’s sleep hygiene</td>
<td>13</td>
</tr>
<tr>
<td>Functional Outcomes of Sleep (FOSQ)</td>
<td>Assess the impact of excessive sleepiness on functional outcomes</td>
<td>11</td>
</tr>
<tr>
<td>Insomnia Severity Survey (ISI)</td>
<td>Measures insomnia from none/mild to severe</td>
<td>7</td>
</tr>
<tr>
<td>Morningness-Eveningness Questionaire (MEQ)</td>
<td>Measures chronotype, determines if participant is a “morning” or “evening person”</td>
<td>5</td>
</tr>
<tr>
<td>Patient Health Questionnaire (PHQ-9)</td>
<td>Assist the clinician in making the diagnosis of depression from mild to severe</td>
<td>10</td>
</tr>
<tr>
<td>PTSD Checklist (PCL-5)</td>
<td>Assess the presence and severity of PTSD symptoms from mild to severe</td>
<td>20</td>
</tr>
<tr>
<td>Neurobehavioral Symptoms Inventory (NSI)</td>
<td>Evaluation of post-concussive symptoms following TBI</td>
<td>22</td>
</tr>
<tr>
<td>NIH PROMIS Emotional-distress-anxiety</td>
<td>Assess self-reported fear, hyperarousal and somatic symptoms related to arousal</td>
<td>4</td>
</tr>
<tr>
<td>World Health Organization Disability Assessment Scale (WHODAS 2.0)</td>
<td>Measuring health and disability across cultures, gives as a percentage</td>
<td>15</td>
</tr>
<tr>
<td>NIH PROMIS Global Health</td>
<td>Assesses an individual’s physical, mental, and social health</td>
<td>10</td>
</tr>
<tr>
<td>NIH PROMIS Pain Interference</td>
<td>Assess self-reported consequences of pain on relevant aspects of one’s life</td>
<td>4</td>
</tr>
<tr>
<td>NIH PROMIS Pain Intensity</td>
<td>Assess how much a person hurts</td>
<td>3</td>
</tr>
<tr>
<td>NIH PROMIS Cognitive Function</td>
<td>Assess patient-perceived cognitive deficits</td>
<td>4</td>
</tr>
<tr>
<td>NeuroQoL Participation</td>
<td>Adult ability to Participate in social roles</td>
<td>8</td>
</tr>
<tr>
<td>NeuroQoL Satisfaction</td>
<td>Adult satisfaction with social roles and activities</td>
<td>8</td>
</tr>
</tbody>
</table>
Improved insomnia with BCAA supplementation

*Functional Outcomes of Sleep Questionnaire: ~15% in BCAA vs ~4% in Rice

*World Health Organization Disability Scale: ~7% in BCAA vs ~0% in Rice
Actigraphy

* Decreased sleep onset latency
Summary of BCAA Pilot Study in Veterans

- Short-term BCAA supplementation is feasible and acceptable in Veteran subjects
- BCAA significantly improve self-reported insomnia
- VA Merit Proposal: Limited efficacy, longer duration, 3 doses (20, 40, 60g), PSG/EEG
Portland VA/OHSU: Miranda Lim, MD, PhD
Jonathan Elliott, PhD
Carolyn Jones, PhD
Kris Weymann, PhD, RN
Peyton Wickham, BSc
Alisha McBride, BSc
Randall Olson, BA
Nadir Balba, MS
Cadence Michel, BA
Kate Gutowsky, BA

Collaborators:
Mo Modarres, PhD
Risa Richardson, PhD
Megan Callahan, PsyD
Mary Heinricher, PhD
Matt Butler, PhD

Current support:
VA BLRD & RRD Career Development Award
VA RRD SPIRE and Merit Award
Department of Defense PH-TBI Award
USUHS/CNRM/DoD Award
NIH NIA
NIH BUILD EXITO Institutional Core
Oregon Institute of Occupational Health Sciences
National Science Foundation
Portland VA Research Foundation
OHSU Medical Research Foundation
Neuropsych: WAIS-IV, HVLT, DKEFS, COWAT
Neuropsych trends?

DKEFS Color-Word Interference Test

Test 2 (T2): **Write the color**

- Pre
- Post

$P = 0.09$
Actigraphy

- **Sleep onset latency**
- **Total sleep time (TST)**
- **Sleep efficiency** (time in bed ÷ TST)
- **Sleep onset**
- **Wake-up**
- **Wake after sleep onset (WASO)**

12 PM | 6 PM | 12 AM | 6 AM | 12 PM
Prior studies:
- BCAA for PKU for 2y
- Safe upper limit of leucine in rats is 8.9 g/kg (~600 g/d in human)

Berry et al., 1982, *Pediatr Res*
Knapik et al., 2016, *Sport Med*
Pencharz et al., 2008, *J Nutr*
Mild TBI causes orexin dysfunction in mice

Willie and Lim *et al.*, *J Neurotrauma*, 2012

Chemelli *et al.*, *Cell*, 1999
BCAAs increase glutamate within nerve terminals contacting orexin neurons

Elliott et al, SLEEP, 2018
BCAAs promote wakefulness and restore orexin function after TBI

BCAA therapy restores hippocampal physiology and memory

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Sham (nmol/mg protein)</th>
<th>FPI (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>16.33 ± 1.64</td>
<td>8.04 ± 1.35*</td>
</tr>
<tr>
<td>Leucine</td>
<td>15.03 ± 1.92</td>
<td>7.17 ± 1.48*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>10.19 ± 0.51</td>
<td>8.04 ± 0.96*†</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.58 ± 1.30</td>
<td>3.91 ± 0.95</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.18 ± 0.12</td>
<td>0.41 ± 0.91</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.87 ± 0.26</td>
<td>1.07 ± 0.79</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.08 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

Contextual fear conditioning:

Slice electrophysiology:

Cole et al, PNAS, 2010
BCAA Dosing Experiments

Elkind and Lim et al, Frontiers Neurology, 2015
mTBI as a multi-scale, complex system
# Daily Study Diary

**Key**

- **Asleep:**
  - Option 1: Light blue
  - Option 2: Red
  - Option 3: Black

- **In bed but awake:** White

- Leave all other boxes empty:

**Instructions:**
Indicate the time of day that you are in bed and asleep and the time of day that you are in bed but awake. Each box represents 30 minute increments, there is no need to divide them into smaller increments. Please be consistent in your labeling, see the above key for examples.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Type of Day</th>
<th>Time of Day</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tue</td>
<td>7.17.18</td>
<td>Work</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Wed</td>
<td>7.18.18</td>
<td>Work</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Thu</td>
<td>7.19.18</td>
<td>Vacation</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Fri</td>
<td>7.20.18</td>
<td>Vacation</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Sat</td>
<td>7.21.18</td>
<td>Vacation</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Sun</td>
<td>7.22.18</td>
<td>Off</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
<tr>
<td>Mon</td>
<td>7.23.18</td>
<td>Work</td>
<td>08 09 10 11</td>
<td>D</td>
</tr>
</tbody>
</table>
Objective adherence: Serum BCAA levels
Blood-based Brain Biomarkers

A

$F_{(1,59)} = 10.50; P < 0.05$

Exosome Total Tau, pg/mL

No TBI | mTBI

B

$F_{(1,59)} = 5.31; P < 0.05$

Exosome Total Amyloid Beta-42, pg/mL

No TBI | mTBI

Courtesy of Jessica Gill
Sleep Disturbances in Traumatic Brain Injury: A Meta-Analysis

Natalie Grima, DPsysch\textsuperscript{1,4}; Jennie Ponsford, PhD\textsuperscript{2,4}; Shantha M. Rajaratnam, PhD\textsuperscript{4,5,6}; Darren Mansfield, MD, PhD\textsuperscript{3,4}; Matthew P. Pase, PhD\textsuperscript{7,8} 2016

Search terms: \textit{TBI} and \textit{Sleep}

Primary outcomes: \textit{Polysomnography metrics}

Secondary outcomes: \textit{Self-report metrics}

- 7,068 articles screened
- 228 potentially relevant

16 studies included
- 637 TBI patients
  - 32 ± 7 years of age, 71% male
- 567 controls
  - 32 ± 10 years of age, 66% male

TBI Patients experienced
- Increased wake after sleep onset
- Reduced total sleep time
- Poorer sleep efficiency
- Increased sleep onset latency
- No differences in sleep architecture (trend for reduced REM sleep)

- Higher Epworth Sleepiness Scale scores
- Higher Pittsburgh Sleep Quality Index scores
Electron microscopy visualization of glutamate in orexin synapses
Measuring sleep in a mouse model of TBI
Mild TBI causes sleep-wake impairments in mice

Lim et al, Sci Transl Med, 2013
How might mild TBI affect deep orexin neurons?

↓ Orexin activity
↓ Sleep-wake disturbances
CSF orexin is acutely suppressed after moderate/severe TBI

Baumann et al, Ann Neurol, 2009

Baumann et al, Brain, 2007
Could BCAA directly activate orexin neurons?

**Activation of Central Orexin/Hypocretin Neurons by Dietary Amino Acids**

Mahesh M. Kamani,1 John Apergis-Schoute,1 Antoine Adamantidis,2 Lise T. Jensen,3 Luis de Lecea,4 Lars Fugger,5 and Denis Burdakov1,6

1Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, UK
2Department of Psychiatry, McGill University, Montreal (Quebec) H4H 1R3, Canada
3Aarhus University Hospital, Aarhus N 8200, Denmark
4School of Medicine, Stanford University, Stanford, CA 94305, USA
5Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK

*Correspondence: dib22@cam.ac.uk
DOI 10.1016/j.neuron.2011.08.027

Orexin sensing of macronutrient intake?
Loss of orexin neurons in post-mortem TBI

Baumann et al., 2009, *Ann Neurol*
CSF orexin mostly normalizes after 6 months

Baumann et al., 2007, *Brain*