Effects of ASEA beverage intake on endurance performance in mice

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Background

- ASEA® is a saline-based beverage that undergoes a proprietary process and contains reactive redox-signaling molecules.
- 1H-NMR and EPR experiments utilizing spin trap molecules (DIPPMPO) were used to explore the bioavailability of ASEA for free radicals. An additional experimental 13C NMR DIPPMPO with and without superoxide dismutase was conducted. Results supported the presence of stable peroxyl and/or superoxide radicals in ASEA.
- The theory of hormesis involves repeated exposure to a mild physical, chemical, or biological stress resulting in increased resistance to subsequent exposures to otherwise harmful doses of the same stressors. The exposures to mild stressors are thought to induce beneficial cellular responses leading to increased whole organism resistance to the stress. Common examples of this beneficial response include, exercise, ischemic preconditioning, and caloric restriction (Mattson, 2008). ASEA may increase exercise performance through a hormesis effect, but this has not yet been established.

- PURPOSE: To determine if mice given ASEA® have increased endurance treadmill run times compared to placebo and investigate potential mechanisms.

Methods

Animals: Six-week old male specific pathogen-free C57BL/6J mice (n=80) were purchased from Jackson Laboratory. Mice were randomly assigned to each of the four treatment groups (n = 15 each). Mice were housed group (5-4 cages) and provided standard rodent chow and water ad libitum. All animal procedures were reviewed and approved by the North Carolina Research Campus IACUC.

Treatment and Design: ASEA or placebo (same ingredients as ASEA beverage without undergoing the proprietary processing) was administered via gavage once per day for 1 week. The average body mass of all the mice at the start of the study determined the volume of ASEA used for the gavaging, but the volume did not exceed 0.1 ml. Following the 1-week treatment period (7 days) mice were euthanized and tissues harvested for further analysis of outcome measures. Mice from the endurance testing treatment groups were randomized to the treadmill in the following fashion. During the three day period preceding the maximal endurance test, mice were oriented (trained) to the treadmill for 15 minutes. Speeds for the training days were 10 m/min, 15 m/min, and 18 m/min respectively. Then, on the final day of treatment mice underwent the maximal endurance capacity test on the treadmill (Table 1). For the treadmill orientation and endurance protocols, mice were run on a multi-lane rodent treadmill (Columbus Instruments, Columbus OH) equipped with a first treadmill orientation (basal speed 10 m/min and 18 m/min for the first 2 minutes). After the orientation period, mice were run on a multi-lane rodent treadmill (Columbus Instruments, Columbus OH) equipped with a second experimental treadmill. The mice were placed on a treadmill with a speed of 18 m/min (increased by 1 m/min every 2 minutes) and allowed to run until exhaustion. 1H-NMR spectrum of a mixture of DIPPMPO and ASEA beverage.

Table 1: Treadmill Endurance Protocol

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Treadmill Endurance Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASEA Run Group</td>
<td>No Treadmill</td>
</tr>
<tr>
<td>Placebo Run</td>
<td>No Treadmill</td>
</tr>
<tr>
<td>ASEA Sedentary</td>
<td>Treadmill</td>
</tr>
<tr>
<td>Placebo Sedentary</td>
<td>Treadmill</td>
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Figure 1: NMR spectra analysis of ASEA beverage

Enzyme Assays: 1H- and 31P-NMR spectra were obtained in 100 mmol/L phosphate buffer, pH 7.4. Membranes were collected and the peroxide released was measured by spectrophotometer.

Western Blotting: Western blotting was performed as previously described (Lane et al., 2001). The following antibodies were used: Carnitine Palmitoyltransferase I (CPT1) (Santa Cruz Biotechnology, Santa Cruz, CA). ASEA-Carnitine Palmitoyltransferase (ACC), and phospho-ACC (Ser 79) (Cell Signaling, Danvers, MA). Whole gastrocnemius homogenates were separated by SDS-PAGE, transferred to polyvinylidene fluoride membranes. Membranes were exposed to the appropriate primary antibodies and bands were visualized by chemiluminescence (Pierce SuperSignal, Rockford, IL). Band density was detected using a Chemidoc XRIS Molecular Imager and Image Lab Software (BioRad, Hercules, CA). Phosphorylated ACC (Ser 79) protein was normalized to total ACC protein.

Statistical Analysis: Two-way ANOVA was performed. Following a significant F-test, Student’s t-test was performed to determine differences between treatment. Significance was established at P ≤ 0.05.

Conclusions

- When adjusted to runway time, the estimated rate of muscle glycogen depletion was different between ASEA Run and Placebo Run groups.
- Skeletal muscle phosphorylated acetyl-CoA carboxylase (p-ACC) was significantly increased in ASEA Run compared to ASEA Sedentary (p=0.020) and Placebo Run groups (p=0.045). Fatty acyl CoA transport (CPT1), and beta-oxidation (beta-HAD) were not different between ASEA Run and Placebo Run groups.
- ASEA increased runway time to exhaustion by 29% in mice, potentially through less inhibition of fatty acid oxidation via increased P-ACC, and muscle glycogen sparing (30%).
- The data support increased endurance capacity and altered substrate utilization in mice after one week of ASEA intake. Further research is warranted to determine if these findings are due to hormesis influences from the ASEA beverage.