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.

► ^{31}P NMR and EPR experiments utilizing spin trap molecules (DIPPMPO) were used to explore the ASEA beverage for free radicals. An additional experiment using ^{31}P 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-month old male specific pathogen-free C57BL/6 mice (n =60) were purchased from Jackson Laboratory. Mice were randomly assigned to each of the four treatment groups (n = 15 each). Mice were group housed (3-4/cage) 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 0.3mL. 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 oriented 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 min/day. 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 shock grid at the back. When the mouse could either no longer run (as assessed by sitting on the shock grid with all 4 paws off of the belt for more than 5 seconds), the mouse was removed from the shock grid immediately and placed back into the home cage. The mice were monitored for recovery for a period of at least 20 minutes following the orientation bouts. Mice were euthanized within 30 minutes of the final endurance test.

Glycogen: Post-exercise and end point liver and muscle glycogen levels were assayed using the Glycogen Assay Kit (700480, Cayman Chemical Company, Ann Arbor MI). Rate of muscle glycogen usage was estimated for both ASEA Run and Placebo Run groups. Example Calculation: Average muscle glycogen (Placebo Sedentary) – Average muscle glycogen (Placebo Run) / Average Placebo Run Time.

Enzyme Assays: β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities were determined in whole gastrocnemius homogenates using methods previously described (Laye, 2009). Briefly, powdered frozen muscle was homogenized in buffer containing HEPES, Na pyrophosphate, Na^+, EDTA, Triton, and protease and phosphatase inhibitors. CS activity was measured in homogenate incubated in buffer containing oxaloacetate and dithiobis(2-nitrobenzoic acid) (DTNB). Acetyl-CoA was added to the buffer and CS activity was determined by the appearance of reduced DTNB at a wavelength of 405nm. β-HAD activity was measured in homogenate incubated in buffer containing triethanolamine, EDTA, and nicotinamide adenine dinucleotide (NADH). Acetyl-CoA was added to the buffer and β-HAD activity was determined by the disappearance of NADH at a wavelength of 340nm. All assays were performed at 37°C.

Western Blotting: Western blotting was performed as previously described (Laye et al. 2009). The following antibodies were used: Carnitine Palmitoyltransferase-1 (CPT1) (Santa Cruz Biotechnology, Santa Cruz, CA), Acetyl-CoA Carboxylase (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 and secondary antibodies and bands were visualized by chemiluminescence (Pierce SuperSignal, Fisher Scientific, Rockford, IL). Band density was determined using a ChemiDoc XRTS Molecular Imager and Image Lab Software (BioRad, Hercules, CA). Phosphorylated-ACC (Ser^{79}) protein was normalized to total ACC protein.

Statistical Analyses: Two-way ANOVA was performed. Following a significant F-ratio, Student 's t-test were performed to determine differences between treatments. Significance was established at $P \leq 0.05$

Results

Table 1: Treadmill Endurance Protocol

Time (min)	Speed (m/min)	Details
1	0	adjustment to treadmill
5	10	"warm up"
2	12	
2	14	
2	16	
2	18	
2	20	
2	22	Speeds between 20-24 correspond to roughly 80% VO2max for mice
2- end	24	Mice will stay at this speed until they reach exhaustion (sit on shock grid for 5 full seconds)

Study Design

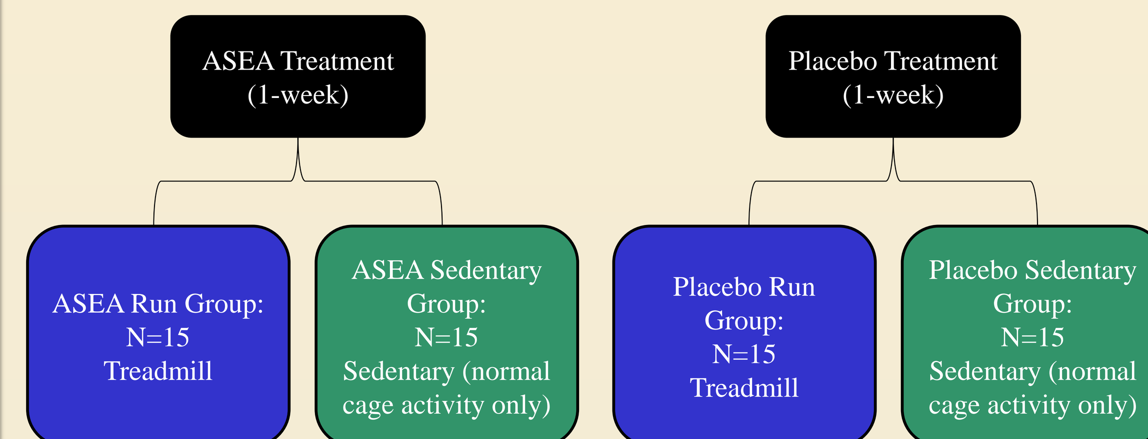
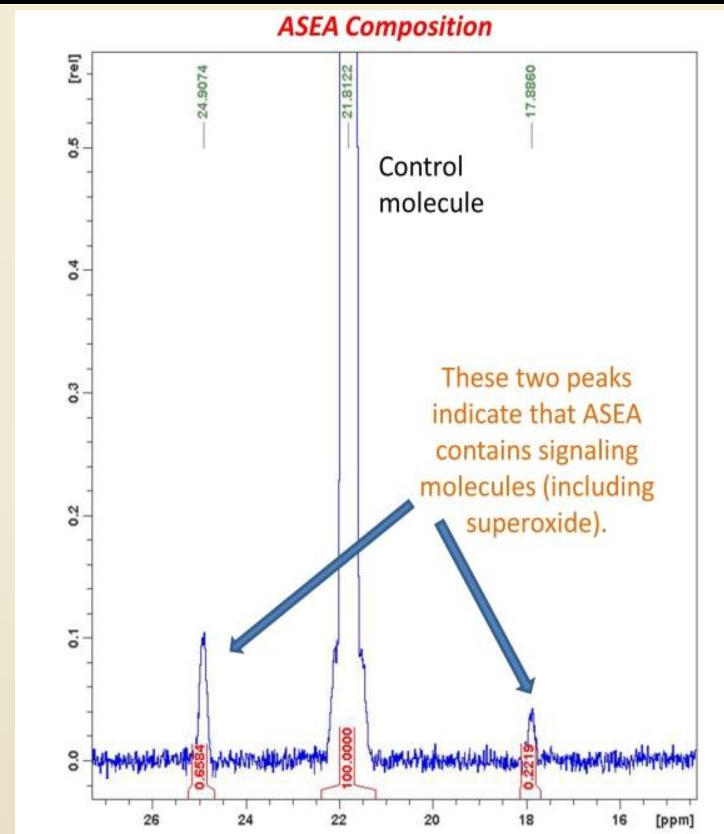
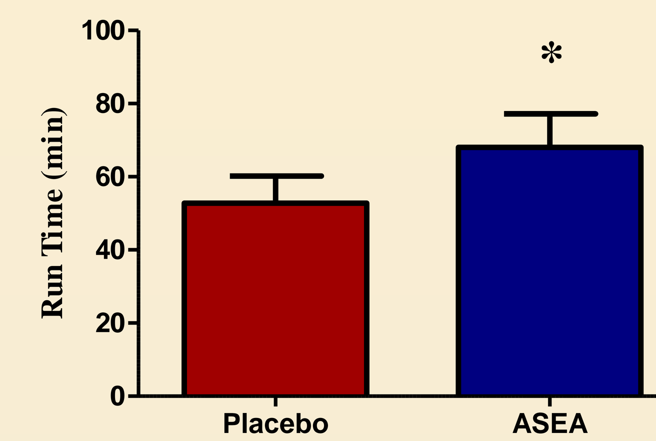


Figure 1: NMR Spectra analysis of ASEA beverage



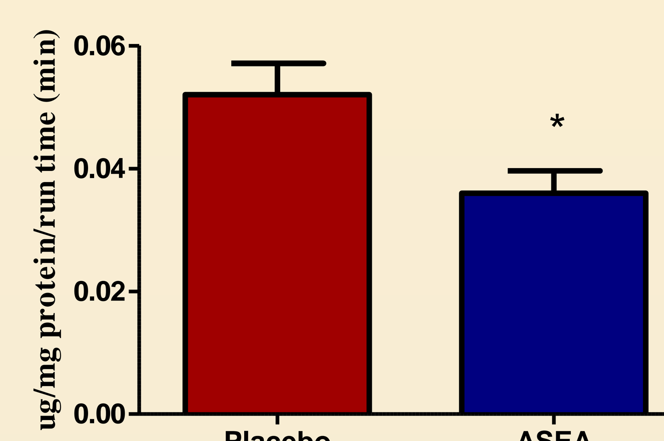
^{31}P NMR spectrum of a mixture of DIPPMPO and ASEA beverage. Green numbers are peak chemical shifts, red numbers are integral values of corresponding peaks.

Figure 2: Endurance Run Time



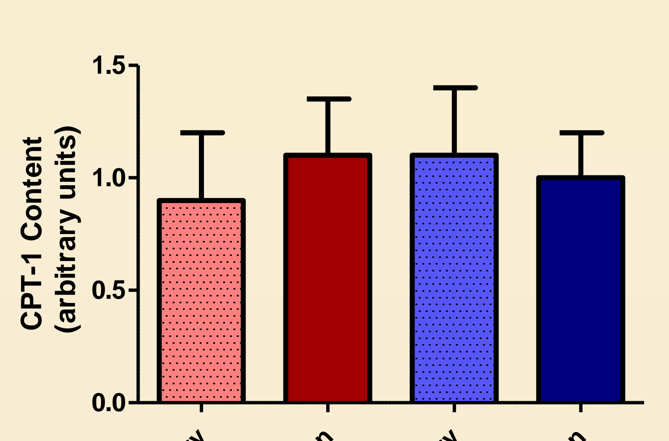
Endurance Run Time (minutes) ASEA Run group significantly different than Placebo Run group (p<0.001).

Figure 3: Rate of Muscle Glycogen Depletion



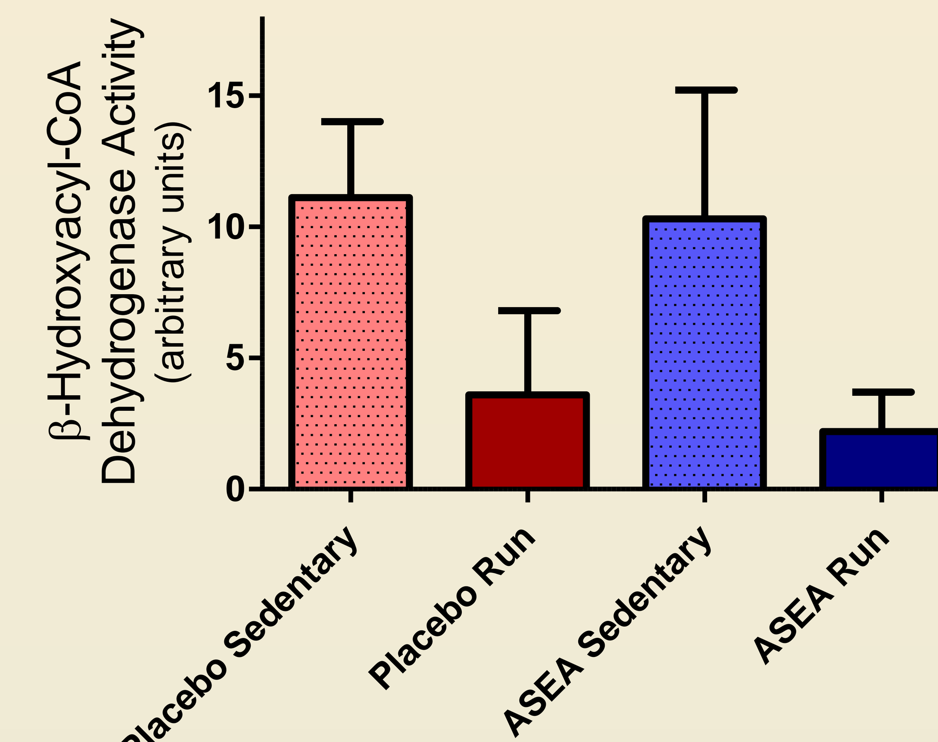
Estimated rate of muscle glycogen depletion. ASEA Run group significantly different than Placebo Run group (p=0.017).

Figure 4: Carnitine Palmitoyltransferase I



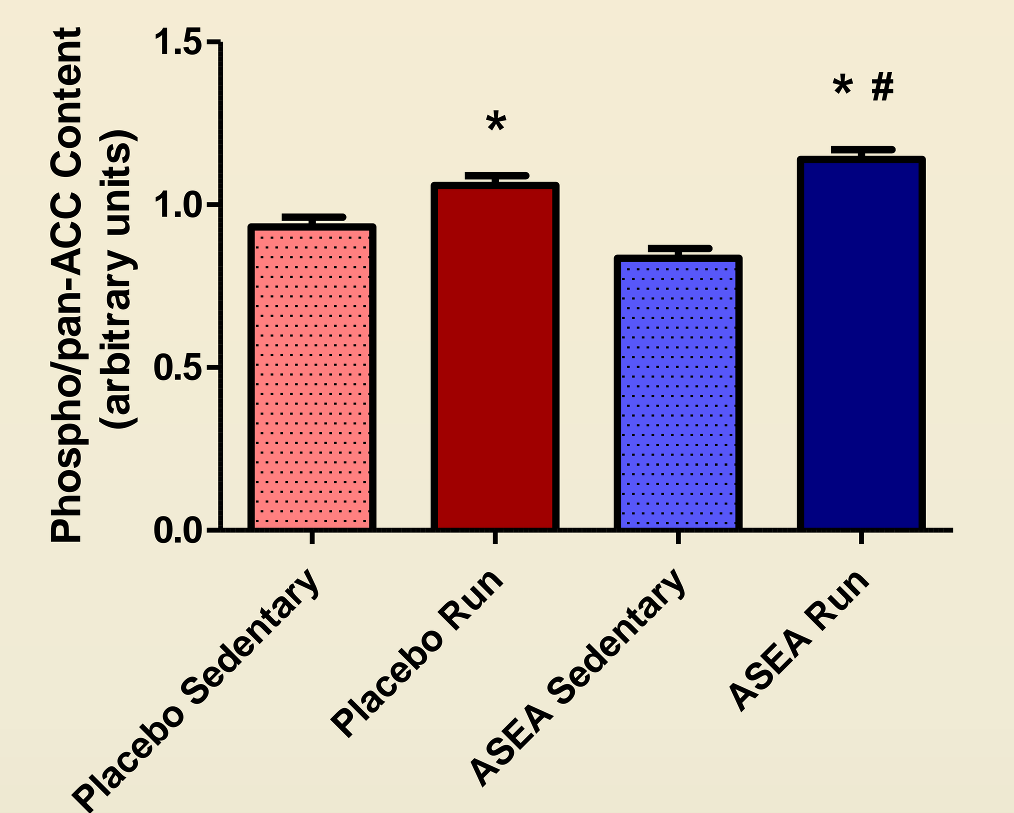
CPT-I as measured by Western Blot in muscle tissue. No significant differences between groups (p>0.05).

Figure 5: β-Hydroxyacyl-CoA Dehydrogenase



β-HAD enzyme activity was measured in muscle tissue. No significant differences between sedentary groups or run groups (p>0.05).

Figure 6: Phospho/pan-ACC



Phosphorylated ACC was normalized to ACC content. Both were measured via Western Blot analysis in muscle tissue.
* - Significantly different from Sedentary within same treatment (p=0.02)
# - Significantly different than Placebo Run (p=0.045)

Conclusions

► When adjusted to run 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 run 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.

Mark P. Mattson. Hormesis Defined. Ageing Res Rev. 2008 January; 7(1): 1-7

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