The Safety of Pre-Workout Supplementation in Recreationally Active Adult Females for 28 Days

Roxanne M. Vogel, Jordan M. Joy, Paul H. Falcone, Matt M. Mosman, Michael P. Kim, and Jordan R. Moon

ABSTRACT

Pre-workout supplements (PWS) have increased in popularity among athletic populations for their purported ergogenic benefits. Currently, little research exists on the safety and potential side effects of chronic consumption of PWS, especially among females. **Purpose:** To examine the clinical safety of consuming a PWS daily in healthy adult females for 28 days. **Methods:** 34 recreationally active adult females (27.1 ± 5.4 years, 165.2 ± 5.7 cm, 68.2 ± 16.0 kg) participated in this study. Participants were randomly assigned to consume either 1 (G1) or 2 (G2) servings daily of a commercially available PWS, or remain unsupplemented (CRL) for a period of 28 days. Fasting blood samples, as well as resting blood pressure and heart rate, were taken before and after the supplementation period. Blood samples were analyzed for CBC, CMP and lipid panels. **Results:** Significant ($p < 0.05$) group by time interactions were present for absolute monocytes, MCH, creatinine, eGFR, and total cholesterol. All remained within accepted clinical reference ranges. There were no significant interactions for any other variables. Means ± standard deviation for significant interactions are presented in Table 1.

Conclusion: This study confirms our hypothesis that a PWS containing caffeine, beta-alanine, and nitrates will not cause abnormal changes in hematological markers or resting vital signs among adult females. Although there were statistically significant ($p < 0.05$) group by time interactions for absolute monocytes, MCH, creatinine, eGFR, and total cholesterol, all values remained well within accepted physiological ranges and were not clinically significant nor cause for concern. In sum, daily supplementation with up to 2 servings per day of a PWS is apparently safe for consumption among active adult females for a 28 day period.

INTRODUCTION

Nutrient timing refers to the methodical, timed ingestion of carbohydrate, protein, fat and other dietary supplements either before, during, or after physical activity. Supplementation during the period immediately preceding physical activity has become an increasingly popular strategy among competitive and recreational athletes alike as a means of improving performance. In response to this trend, manufacturers have developed pre-workout supplements (PWS), which typically combine caffeine with any number of purported ergogenic substances, such as beta-alanine, nitrate, and amino acids. As the number of PWS available on the market grows, each containing their own “proprietary blend” of active ingredients, it must be determined which, if any, are safe for chronic consumption. This becomes particularly important as concerns have arisen over the concept of proprietary blends, namely the fact that the Food and Drug Administration does not monitor the amounts of ingredients used in these blends or the accuracy of product labeling by manufacturers.

Despite the existing literature pertaining to individual ingredients contained in PWS and the growing number of studies that address multi-ingredient PWS specifically, we are unaware of any published reports examining the safety of PWS in a solely female population. Therefore, the purpose of the present study was to examine the safety of chronic consumption of a PWS over a 28 day period among active adult females. We hypothesized that daily PWS supplementation would not produce abnormal changes in hematological or metabolic safety markers or resting vital signs.

METHODS

Participants

34 recreationally active adult females (27.1 ± 5.4 years, 165.2 ± 5.7 cm, 68.2 ± 16.0 kg) participated in this study. The study was approved by an Institutional Review Board for use of human subjects, and all subjects signed an informed consent prior to starting the study. Inclusion and exclusion criteria for each subject was determined through a health history and exercise status questionnaire. Subjects were required to be apparently healthy and free from disease, have no physical condition that was considered a contraindication to cardiovascular training, and abstain from smoking, alcohol, and anti-inflammatories during the 24 hours prior to blood draws. In addition, all subjects were required to abstain from taking any pre-workout supplements for one month before their first visit. Subjects indicated participation in one or more of the following physical activities at least 3 days per week for three months or more preceding the study: resistance training, elliptical, running, walking, volleyball, soccer, and yoga.

Study Design

Prior to the supplementation period, subjects were instructed to report to a local blood testing facility (Laboratory Corporation of America, Denver, CO, USA) in an 8 hour fasted state and not to exercise the morning of testing. Each subject completed an informed consent, health history, and exercise questionnaire. Resting heart rate and blood pressure was taken using an automated blood pressure cuff. The average of two tests with 2 minutes between tests was recorded and used for analysis. Height and weight was measured using a SECA 703 high capacity column scale. Subjects then provided a baseline blood sample for full safety panel analyses.

After baseline measurements were completed, subjects were provided with the supplement (Fitmiss Ignite™, MusclePharm Corp., Denver, CO) and instructed to consume 1 (G1) or 2 (G2) servings, or remain unsupplemented (CRL), and to record supplementation and adverse events logs for 28 days. They were also instructed to maintain and record their current diet and exercise routine as it had been for at least two months prior to the start of the study. Maintenance of diet was monitored using 3 day food logs for each week. At the conclusion of the 28 day supplementation period, subjects were instructed to return to the blood testing facility in an identical state to baseline testing to provide a post blood sample, weight, blood pressure, and heart rate. Supplementation, food, exercise, and adverse event logs were also collected at this time.

Supplement Facts

Serving Size: 1 Scoop (7g)
Servings Per Container: 30

	Amount Per Serving	%DV
Calories	5	
Calories from fat	0	
Total Carbohydrates	1 g	<1%
Sugars	0 g	
Calcium (as Calcium Silicate)	20 mg	2%
Proprietary FitMiss Ignite Blend	5,700 mg	†

Carnevo® Patented Beta Alanine, Choline Bitartrate, L-Tyrosine, L-Glycine, Taurine, L-Carnitine Base, Beet Root Extract (Beta Vulgaris)(High in Nitrates), Hawthorn Berry Powder (Crataegus Pinnatifida)(Fruit), Agmatine Sulfate, Caffeine Anhydrous, Huperzine A 1% (Huperzia Serrata)

* Percent Daily Values (% DV) are based on a 2,000 calorie diet.
† Daily Value (DV) not established.

Blood Draws

All blood samples were taken via venipuncture by a trained phlebotomist at a local diagnostic laboratory (Laboratory Corporation of America, Denver, CO, USA). Pre-supplementation samples were taken with the subject in an 8 hour fasted state while post samples were taken in an identical state one day after ceasing supplementation and at the same time of day as pre-supplementation blood samples. Variables recorded from the blood and urine analysis consisted of white blood cell count (WBC), red blood cell count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (percent and absolute), neutrophils (percent and absolute), lymphocytes (percent and absolute), monocytes (percent and absolute), eosinophils (percent and absolute), basophils (percent and absolute), serum glucose, blood urea nitrogen (BUN), creatinine, eGFR, BUN:creatinine, sodium, potassium, chloride, carbon dioxide, calcium, protein, albumin, globulin, albumin:globulin ratio (A/G ratio), bilirubin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT)., total cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL).

RESULTS

Variable	Treatment	Pre	Post	Delta	Reference Interval
Resting Systolic Blood Pressure (mmHg)	CRL	111.7 ± 7.6	115.4 ± 10.0	3.8 ± 6.5	
	1	105.0 ± 8.2	106.3 ± 10.9	1.3 ± 8.6	< 120
	2	108.9 ± 7.3	105.8 ± 6.9	-3.1 ± 6.9	
Resting Diastolic Blood Pressure (mmHg)	CRL	72.2 ± 7.9	73.7 ± 8.7	1.5 ± 6.7	
	1	71.4 ± 7.9	77.2 ± 9.2	5.8 ± 7.7	< 80
	2	75.9 ± 9.3	73.9 ± 9.3	-2.0 ± 8.8	
Resting Heart Rate (b/min)	CRL	65.2 ± 10.7	66.8 ± 7.4	1.7 ± 7.1	
	1	69.1 ± 8.6	70.9 ± 9.3	1.8 ± 7.9	< 100
	2	68.4 ± 9.2	68.2 ± 6.9	-0.2 ± 6.4	
WBC (x10E3/uL)	CRL	6.50 ± 1.94	5.91 ± 1.56	-0.59 ± 0.96	
	1	6.18 ± 1.97	6.18 ± 1.81	0.00 ± 0.85	3.4 - 10.8
	2	5.78 ± 1.75	6.06 ± 2.09	0.29 ± 1.02	
RBC (x10E6/uL)	CRL	4.78 ± 0.22	4.74 ± 0.28	-0.04 ± 0.14	
	1	4.85 ± 0.38	4.83 ± 0.46	-0.02 ± 0.24	3.77 - 5.28
	2	4.81 ± 0.33	4.79 ± 0.35	-0.03 ± 0.15	
Hemoglobin (g/dL)	CRL	14.66 ± 0.92	14.47 ± 1.01	-0.19 ± 0.45	
	1	14.60 ± 1.08	14.70 ± 1.18	0.10 ± 0.78	11.1 - 15.9
	2	14.64 ± 0.82	14.46 ± 0.62	-0.18 ± 0.52	
Hematocrit (%)	CRL	43.75 ± 2.64	43.24 ± 2.53	-0.51 ± 1.38	
	1	44.27 ± 2.92	44.31 ± 3.22	0.04 ± 2.18	34.0 - 46.6
	2	43.63 ± 2.64	43.15 ± 1.76	-0.48 ± 1.56	
MCV (fL)	CRL	91.38 ± 2.92	91.38 ± 2.75	0.00 ± 1.93	
	1	91.50 ± 5.60	92.30 ± 5.10	0.80 ± 1.48	79 - 97
	2	90.75 ± 3.88	90.38 ± 4.47	-0.38 ± 2.45	
MCH (pg)	CRL	30.66 ± 1.08	30.53 ± 1.03	-0.13 ± 0.46	
	1	30.18 ± 1.92	30.54 ± 1.61*†	0.36 ± 0.52	26.6 - 33.0
	2	30.46 ± 1.41	30.28 ± 1.16	-0.19 ± 0.39	
MCHC (g/dL)	CRL	33.49 ± 0.68	33.45 ± 0.81	-0.04 ± 0.79	
	1	32.97 ± 0.70	33.15 ± 0.63	0.18 ± 0.59	31.5 - 35.7
	2	33.55 ± 0.63	33.50 ± 0.48	-0.05 ± 0.91	
RDW (%)	CRL	13.35 ± 0.63	13.31 ± 0.40	-0.04 ± 0.53	
	1	13.34 ± 0.65	13.30 ± 0.48	-0.04 ± 0.48	12.3 - 15.4
	2	13.24 ± 0.58	13.23 ± 0.45	-0.01 ± 0.34	
Platelets (x10E3/uL)	CRL	271.0 ± 53.2	262.3 ± 37.6	-8.8 ± 29.3	
	1	250.9 ± 47.4	267.5 ± 51.0	16.6 ± 25.2	150 - 379
	2	272.0 ± 84.7	268.0 ± 70.7	-4.0 ± 34.3	
Neutrophils (%)	CRL	55.2 ± 9.6	53.4 ± 9.9	-1.8 ± 5.5	
	1	55.9 ± 10.2	52.8 ± 8.3	-3.1 ± 5.0	40 -74
	2	50.4 ± 6.9	50.3 ± 9.0	-0.1 ± 8.0	
Lymphocytes (%)	CRL	33.5 ± 8.3	36.1 ± 9.1	2.6 ± 5.4	
	1	34.0 ± 9.5	36.9 ± 7.6	2.9 ± 5.4	14 - 46
	2	38.3 ± 6.5	38.9 ± 9.3	0.6 ± 6.7	
Monocytes (%)	CRL	8.6 ± 2.0	7.9 ± 1.7	-0.8 ± 1.3	
	1	7.7 ± 1.8	7.9 ± 1.9	0.2 ± 1.1	4 - 12
	2	7.9 ± 2.0	7.6 ± 2.0	-0.3 ± 1.3	
Eosinophils (%)	CRL	2.0 ± 1.4	2.1 ± 1.1	0.1 ± 0.9	
	1	2.0 ± 1.8	2.0 ± 0.8	0.0 ± 1.4	0 - 5
	2	2.8 ± 1.2	2.5 ± 0.9	-0.3 ± 1.6	
Basophils (%)	CRL	0.6 ± 0.8	0.5 ± 0.6	-0.1 ± 0.7	
	1	0.4 ± 0.7	0.4 ± 0.7	0.0 ± 0.0	0 - 3
	2	0.8 ± 0.5	0.8 ± 0.5	0.0 ± 0.5	
Neutrophils (Absolute) (x10E3/uL)	CRL	3.67 ± 1.51	3.20 ± 1.21	-0.47 ± 0.80	
	1	3.48 ± 1.50	3.33 ± 1.43	-0.15 ± 0.56	1.4 - 7.0
	2	2.91 ± 0.90	3.16 ± 1.56	0.25 ± 0.88	
Lymphocytes (Absolute) (x10E3/uL)	CRL	2.13 ± 0.62	2.11 ± 0.59	-0.02 ± 0.32	
	1	2.08 ± 0.74	2.21 ± 0.52	0.13 ± 0.36	0.7 - 3.1
	2	2.20 ± 0.67	2.25 ± 0.40	0.05 ± 0.52	
Monocytes (Absolute) (x10E3/uL)	CRL	0.56 ± 0.16	0.46 ± 0.14*‡	-0.10 ± 0.10	
	1	0.44 ± 0.08	0.47 ± 0.14	0.03 ± 0.13	0.1 - 0.9
	2	0.48 ± 0.29	0.49 ± 0.31	0.01 ± 0.12	
Eosinophils (Absolute) (x10E3/uL)	CRL	0.13 ± 0.08	0.14 ± 0.07	0.01 ± 0.07	
	1	0.12 ± 0.13	0.15 ± 0.07	0.03 ± 0.11	0.0 - 0.4
	2	0.19 ± 0.08	0.15 ± 0.05	-0.04 ± 0.09	
Basophils (Absolute) (x10E3/uL)	CRL	0.02 ± 0.05	0.02 ± 0.04	0.00 ± 0.04	
	1	0.01 ± 0.03	0.01 ± 0.03	0.00 ± 0.00	0.0 - 0.2
	2	0.01 ± 0.04	0.03 ± 0.05	0.01 ± 0.04	

Table 1. Variables collected pre and post supplementation period, including clinical reference ranges for each.

Variable	Treatment	Pre	Post	Delta	Reference Interval
Glucose (mg/dL)	CRL	79.9 ± 6.7	81.9 ± 6.1	1.9 ± 7.4	
	1	85.4 ± 6.5	83.0 ± 5.7	-2.4 ± 4.3	65 - 99
	2	87.5 ± 6.5	84.4 ± 6.1	-3.1 ± 6.1	
BUN (mg/dL)	CRL	16.5 ± 4.5	16.5 ± 3.8	0.0 ± 3.1	
	1	15.3 ± 6.8	15.5 ± 4.8	0.2 ± 4.8	6 - 20
	2	15.8 ± 5.1	15.8 ± 4.1	0.0 ± 4.0	
Creatinine (mg/dL)	CRL	0.90 ± 0.10	0.90 ± 0.11	0.00 ± 0.05	
	1	0.96 ± 0.30	0.90 ± 0.25	-0.06 ± 0.13	0.57 - 1.00
	2	0.91 ± 0.17	0.77 ± 0.12*	-0.14 ± 0.08	
eGFR (mL/min/1.73)	CRL	90.93 ± 13.79	90.14 ± 14.27	-0.69 ± 5.97	
	1	89.80 ± 27.23	95.90 ± 22.95	6.10 ± 15.89	> 59
	2	88.25 ± 18.84	102.88 ± 14.02*	14.63 ± 7.11	
BUN/Creatinine Ratio	CRL	18.6 ± 5.6	18.6 ± 5.1	0.1 ± 3.4	
	1	15.5 ± 2.1	17.6 ± 4.1	2.1 ± 5.0	8 - 20
	2	17.1 ± 3.6	20.4 ± 3.7	3.3 ± 4.5	
Sodium (mmol/L)	CRL	137.1 ± 2.8	136.7 ± 1.8	-0.4 ± 2.9	
	1	138.0 ± 2.2	137.9 ± 1.7	-0.1 ± 1.3	134 - 144
	2	139.3 ± 1.6	138.1 ± 2.5	-1.1 ± 2.1	
Potassium (mmol/L)	CRL	3.98 ± 0.26	4.13 ± 0.27	0.16 ± 0.28	
	1	4.15 ± 0.66	4.15 ± 0.46	0.00 ± 0.66	3.5 - 5.2
	2	4.31 ± 0.50	4.05 ± 0.39	-0.26 ± 0.53	
Chloride (mmol/L)	CRL	102.3 ± 3.0	102.6 ± 2.0	0.3 ± 3.0	
	1	102.7 ± 2.7	101.4 ± 3.5	-1.3 ± 2.4	97 - 108
	2	103.6 ± 1.1	102.6 ± 1.6	-1.0 ± 1.7	
Carbon Dioxide (mmol/L)	CRL	21.7 ± 1.9	20.8 ± 2.1	-0.9 ± 2.1	
	1	21.8 ± 2.5	21.4 ± 3.4	-0.4 ± 3.0	18 - 28
	2	22.3 ± 2.1	21.8 ± 1.4	-0.5 ± 1.9	
Calcium (mg/dL)	CRL	9.21 ± 0.35	9.11 ± 0.24	-0.10 ± 0.32	
	1	9.50 ± 0.55	9.34 ± 0.65	-0.16 ± 0.32	8.7 - 10.2
	2	9.51 ± 0.20	9.25 ± 0.26	-0.26 ± 0.33	
Protein (g/dL)	CRL	6.88 ± 0.48	6.72 ± 0.40	-0.16 ± 0.28	
	1	7.04 ± 0.36	7.06 ± 0.37	0.02 ± 0.33	6.0 - 8.5
	2	7.14 ± 0.33	6.88 ± 0.33	-0.26 ± 0.34	
Albumin (g/dL)	CRL	4.33 ± 0.25	4.28 ± 0.30	-0.06 ± 0.27	
	1	4.33 ± 0.37	4.48 ± 0.41	0.15 ± 0.23	3.5 - 5.5
	2	4.53 ± 0.24	4.49 ± 0.18	-0.04 ± 0.23	
Globulin (g/dL)	CRL	2.55 ± 0.42	2.44 ± 0.37	-0.11 ± 0.29	
	1	2.71 ± 0.47	2.58 ± 0.25	-0.13 ± 0.30	1.5 - 4.5
	2	2.61 ± 0.41	2.39 ± 0.36	-0.23 ± 0.28	
Albumin/Globulin Ratio	CRL	1.73 ± 0.29	1.79 ± 0.34	0.06 ± 0.30	
	1	1.67 ± 0.44	1.76 ± 0.29	0.09 ± 0.25	1.1 - 2.5
	2	1.76 ± 0.34	1.91 ± 0.32	0.15 ± 0.21	
Bilirubin (mg/dl)	CRL	0.53 ± 0.25	0.46 ± 0.18	-0.08 ± 0.26	
	1	0.50 ± 0.15	0.46 ± 0.24	-0.04 ± 0.15	0.0 - 1.2
	2	0.56 ± 0.32	0.64 ± 0.32	0.08 ± 0.17	
Alkaline Phosphatase (IU/L)	CRL	50.6 ± 12.8	51.3 ± 12.3	0.6 ± 4.3	
	1	59.2 ± 17.8	59.9 ± 15.8	0.7 ± 5.5	39 - 117
	2	56.8 ± 13.8	55.9 ± 12.9	-0.9 ± 3.9	
AST (IU/L)	CRL	24.1 ± 8.5	23.6 ± 5.2	-0.6 ± 7.0	
	1	27.1 ± 19.2	24.5 ± 8.3	-2.6 ± 13.7	0 - 40
	2	20.1 ± 5.3	18.4 ± 4.0	-1.8 ± 4.4	
ALT (IU/L)	CRL	20.3 ± 6.8	21.2 ± 5.6	0.9 ± 5.3	
	1	21.3 ± 12.1	22.3 ± 7.3	1.0 ± 10.2	0 - 32
	2	18.9 ± 3.2	15.8 ± 4.2	-3.1 ± 3.1	
Total Cholesterol (mg/dL)	CRL	160.75 ± 27.81	158.31 ± 24.39	-2.44 ± 13.63	
	1	155.10 ± 9.00	169.50 ± 24.37*†	14.40 ± 27.32	100 - 189
	2	165.75 ± 21.86	155.38 ± 21.55	-10.38 ± 15.39	
Triglycerides (mg/dL)	CRL	81.5 ± 30.7	83.4 ± 48.8	1.9 ± 30.8	
	1	96.4 ± 46.1	98.5 ± 35.0	2.1 ± 30.2	0 - 114
	2	80.0 ± 38.2	75.5 ± 24.7	-4.5 ± 45.2	
HDL Cholesterol (mg/dL)	CRL	63.8 ± 14.2	64.5 ± 16.0	0.8 ± 6.6	
	1	59.2 ± 13.6	64.4 ± 17.7	5.2 ± 10.5	> 39
	2	61.5 ± 15.0	59.9 ± 13.2	-1.6 ± 5.4	
LDL Cholesterol (mg/dL)	CRL	79.4 ± 22.4	77.1 ± 14.5	-2.3 ± 16.6	
	1	76.7 ± 16.8	85.4 ± 21.9	8.7 ± 18.8	0 - 119
	2	88.1 ± 20.4	80.1 ± 13.2	-8.0 ± 14.0	

Legend:

CRL = Control (n=16); 1 = Group 1 (n=10); 2 = Group 2 (n=8); Data are reported as means ± SD.

*Different from CRL ($p < 0.05$)
†Different from G1 ($p < 0.05$)
‡Different from G2 ($p < 0.05$)

CONCLUSIONS

This study supports the hypothesis that a PWS containing caffeine, beta-alanine, and nitrate will not cause abnormal changes in hematological or clinical chemistry/metabolic markers, or resting vital signs among recreationally active females. Although there were statistically significant ($p < 0.05$) group by time interactions for absolute monocytes, MCH, creatinine, eGFR, and total cholesterol, all of the results remained well within accepted physiological ranges and were not clinically significant. In sum, it appears as though daily supplementation with up to 2 servings of the PWS used in this investigation, over a period of 28 days, had no adverse impact on markers of clinical safety among active adult females. To our knowledge, this is the first study assessing the clinical safety of a PWS in an all-female population. Our results agree with previously reported studies that chronic consumption of a PWS containing similar ingredients is safe in healthy individuals.

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