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A Randomized, Single-Blind, Crossover Study to Evaluate the Efficacy of a Novel Dietary Supplement Blend with L-Citrulline on Biomarkers of Hydration, Muscle Size, Affect, Inflammation, and Muscular Endurance

Original Research

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Abstract

Introduction: Citrulline is a non-essential amino acid that has previously been shown to improve athletic performance, reduce fatigue, and increase blood flow. The purpose of this study was to examine the efficacy of a novel dietary supplement blend (3D PUMP BREAKTHROUGH®, 3DPump) vs. citrulline alone on changes in muscle volume, fluid shifts, markers of hydration, inflammation, recovery, affect, and muscular endurance.

Methods: Twenty-eight recreationally active subjects (6 women, 22 men) participated in a randomized, positive-controlled, single-blind, crossover study which involved 4 testing visits (2 workout visits each with a 24-hr follow-up visit). Participants ingested 3DPump (containing 3g L-citrulline, 1.2g glycerol, 165mg Amla fruit extract) or 8g of L-citrulline alone 45min before an aerobic and resistance training workout. Assessments of body fluid shifts (via BIA), markers of hydration (urine and serum osmolality, USG, hemoglobin), and appendicular girth were taken before and after exercise while markers of damage and inflammation (neutrophils, CK, ESR, MCP-1, CRP) were taken before and 24 hours after exercise, muscular endurance (reps to failure) was assessed during resistance exercises, and subjective measures of affect and recovery were taken before, after and 24 hours after exercise. Mixed factorial ANOVAs with dependent t-tests were used to compare treatments.

Results: A significant interaction occurred for right thigh circumference; however post hoc testing indicated both groups increased similarly in post workout girth ($p<0.05$). A significant interaction occurred for ECF/TBW indicating post workout was greater than pre workout in 3DPump only ($p=0.002$). An interaction trend ($p\leq0.010$) occurred for SBP and DBP indicating that 3DPump was uniquely able to significantly lower blood pressure (SBP and DBP) post workout compared to baseline and 24 hours after exercise ($p<0.001$).

Conclusions: 3DPump, which contains 3g L-citrulline was able to provide similar effects in muscular endurance, muscle pump and other subjective feelings of affect, hydration, damage and inflammation markers, and body fluid shifts as an 8g dose of L-citrulline alone. 3DPump may have unique benefits on improving post exercise vascular tone and ECF/TBW.

Key Words: muscle girth, repetitions to failure, muscle pump, citrulline.

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Introduction

Cellular swelling or the 'pump' has been highly sought after by avid weightlifters of all levels. Many pre-workout products have capitalized on this desire by including ingredients purported to enhance the muscle pump. One of the most commonly used ingredients to induce a muscle pump while also providing potential ergogenic effects on aerobic and anaerobic exercise performance is L-citrulline. Mechanistically, L-citrulline acts to enhance the muscle pump by promoting arginine production and subsequently nitric oxide (NO) availability leading to smooth muscle relaxation, vasodilation, and possibly enhanced blood flow [1–3]. In lieu of the observed physiological enhancements, L-citrulline has been deemed a popular 'muscle pump' ingredient and is sold as a stand-alone ingredient as well as a key component in various multi-ingredient pre-workout supplements. For recent reviews, readers are referred to [4–6].

L-citrulline is sometimes combined with malate, and at oral doses of 6-8g administered 60 min before exercise, has been shown to acutely improve muscular endurance (i.e., repetitions to failure) and reduce perceived exertion and subjective muscle soreness in men [7–9] and in women [10], albeit with some inconsistent findings [11–13]. As a result, L-citrulline has been the topic of several recent reviews in an attempt to develop a consensus of the literature [4–6,14–16]. Despite the somewhat equivocal performance outcomes in the literature L-citrulline still appears to confer a small, but significant benefit to resistance exercise performance [5,15,16]. However, investigations examining L-citrulline on recovery responses from an exercise bout are limited [14].

Previous investigations have thought to combine citrulline with other ingredients to expand upon the physiological benefits to exercise. Specifically, there is a potential synergist relationship between citrulline and antioxidants further enhancing NO synthesis [17]. Although prior investigations have not observed a significant impact on exercise performance by adding an antioxidant source to citrulline [18–20], there may still be a beneficial effect [5]. The prior investigations adding an antioxidant source were not done over an acute whole body resistance training workout and most of them used watermelon juice as the source of antioxidant and citrulline. An antioxidant ingredient much less studied in response to exercise is amla extract. Amla (*Emblica officinalis*) fruit extract is an important medicinal herb native to India composed of a rich source of vitamin C, minerals, amino acids, polyphenols (e.g., gallic acid, ellagic acid) and low molecular weight tannins (Emblicanin A, Emblicanin B, Pedunculagin, and Punigluconin) [21,22]. Traditionally amla has implications as an antioxidant, anti-inflammatory, antiaging, and cytoprotective just to name a few [21]. Amla fruit extract has been shown to enhance mitochondrial function by improving cells' response to various types of stress, stimulating mitochondrial biogenesis and antioxidant capacity, and protecting cells against oxidative damage in conjunction with increased oxygen consumption in myoblasts [23]. Preliminary human studies have also reported improvements in endothelial function and reduced systemic inflammation with Amla consumption [24]. Additionally, glycerol has been shown to have a hyperhydrating effect on athletes within hot and humid environments and may combat the exercise performance decrements associated with dehydration [25,26], however most reports have come out of aerobic exercise bouts lacking in resistance training. With glycerol enhancing fluid retention and thereby increasing fluid compartments within the body it is plausible this effect may facilitate a greater muscle pump. Therefore, combining L-citrulline, glycerol, and amla extract may have potentially synergistic effects compared with the ingestion of L-citrulline alone with regards to hydration, muscle pump, performance, and recovery from resistance exercise.

The purpose of this study was to test the efficacy of 3DPump, a novel product containing standardized amounts of amla extract, glycerol, and low-dose L-citrulline against a previously established effective dose (8 g) of L-citrulline alone on changes in muscle volume, fluid shifts, markers of hydration, inflammation, and recovery, muscular endurance performance, and subjective changes in muscle pump, fatigue, satisfaction, alertness, focus, mood, and energy.

Scientific Methods

Experimental Design

This was a randomized, positive-controlled, single-blind, crossover study consisting of five study visits. This study employed a positive control group (i.e., 8g of L-citrulline) rather than a traditional placebo group to ascertain the effectiveness of 3DPump. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the WCG IRB on 4/22/22 (#3DPump-001-2022). Written informed consent was obtained from all subjects. This study was conducted at a contract research organization (CRO) in Northeast Ohio. During the screening visit (visit 1), each participant's medical history and routine blood work [Complete Blood Count (CBC), Comprehensive Metabolic Panel (CMP), and Lipid Panel] were collected, and a 24-hr dietary recall was recorded. On the second and fourth visits (i.e., exercise visits) subjects performed a moderate intensity walk/jog on a treadmill followed by upper and lower body resistance exercises, underwent a fluid shift assessment [via bioimpedance analysis (BIA)] before and after the entire workout, provided a urine sample for osmolality (Urine OsM) before and after the workout and urine specific gravity (USG) before, after the walk/jog (mid), and after the resistance exercises, provided subjective assessments {markers of recovery [Perceived Recovery Scale (PRS)] and visual analog scales (VAS) for changes in muscle pump, fatigue, product satisfaction, alertness, focus, mood, and energy}, core temperature, and body weight before and after the entire workout. Participants bilateral arm and thigh circumferences were measured before and three minutes after the conclusion of their upper body resistances exercises (dumbbell arm curls and triceps pushdown) and lower body resistances exercises (bilateral leg extension and Smith machine squats), respectively. Participants also underwent a blood draw for markers of hydration [osmolality (Serum OsM) and hematocrit (Hct)], and markers of damage and inflammation [neutrophils (absolute and relative), creatine kinase (CK), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and monocyte chemoattractant protein-1 (MCP-1)] before the workout. After the workout a second blood draw for serum OsM, Hct, neutrophils (absolute and relative), and MCP-1took place. On the third and fifth visits (24-hr follow-up) participants underwent a blood draw for (CK, ESR, and CRP) and provided subjective assessments [Perceived Recovery Scale and VAS for changes in muscle pump, fatigue, product satisfaction, alertness, focus, mood, and energy]. After completion of visit 1 (screening), participants were randomized in a parallel, single-blind, cross-over design to ingest either 6g of 3DPump dissolved in 16 fluid ounces of water or 8g of L-citrulline dissolved in 16 fluid ounces of water 45 minutes before exercise commenced. The purity and potency of all experimental products was confirmed by an independent third-party lab.

Prior to all study visits, participants were asked to replicate their initial dietary intake for the 24hr prior to their visit (including all fluids), refrain from caffeine and alcohol for 24hr, refrain from exercise for 72hr, and fast for 10hr (including no water), and have a good night's rest. The first participant was enrolled on 4/27/22, and data collection concluded on 11/8/22. Comprehensive side effect profile/adverse event monitoring took place throughout the study.

Participants

Twenty-eight (6 women and 22 men) (29.4 ± 10.1yr, 174.3 ± 9.0cm, 78.7 ± 13.2kg, 25.7 ± 2.8kg/m^2) participants were randomized (allocation ratio 1:1 via research randomizer, https://www.randomizer.org/) into the study by the researchers so that half of the subjects started with citrulline first while the other half started with 3D PUMP BREAKTHROUGH® first. Table 1 presents the baseline demographics of the study cohort. Review of health/medical history documents and a physical exam showed that all study participants were free of chronic health issues. Inclusion criteria were established so that all participants were required to be between 18 - 55 years old, have a minimum body mass of 120 pounds (54.5 kg), have a body mass index (BMI) between 20.0 - 34.99 kg/m2, and be recreationally resistance trained (minimum of 2-3d/wk for 1 year). Participants were also required to be normotensive (<150 / <90 mm Hg) with a normal resting heart rate (<100 beats/min), a core body temperature <99.6°F, and an oxygen saturation ≥95%. Female participants were not eligible if they were determined to be pregnant, nursing, or trying to become pregnant. Exclusion criteria included any history of: unstable or new-onset cardiovascular or cardiorespiratory disease; stroke, diabetes, or other endocrine disorder; use of any nutritional supplement promoting hydration or muscle pump (i.e., sports drinks, recovery beverages, pre-workouts, beetroot supplements, muscle pump products) within the past 7 days and throughout the study duration; use of any PED's that are listed as "banned" in the current WADA handbook; any changes in diet within 4 weeks of study start date or throughout study duration; use of any medication that could adversely affect the measured outcomes (i.e., testosterone or other anabolic steroids, diuretics, OTC or prescription nitrate drugs); individuals who have ADHD or other psychiatric conditions requiring pharmacotherapeutic intervention; individuals with erectile dysfunction and/or who use Phosphodiesterase-5 inhibitors; smokers; concomitant use of corticosteroids or testosterone replacement therapy (ingestion, injection, or transdermal); individuals who are cognitively impaired and/or who are unable to give informed consent; individuals with excessive caffeine intake (600mg/day) or alcohol consumption (more than 2 standard alcoholic drinks per day or more than 10 drinks per week) or drug abuse or dependence; clinically significant abnormal laboratory results at screening; malignancy in the previous five years except for non-melanoma skin cancer (basal cell cancer or squamous cell cancer of the skin); prior gastrointestinal bypass surgery (i.e., Lapband); any known gastrointestinal or metabolic diseases that might impact nutrient absorption or metabolism [e.g. short bowel syndrome, diarrheal illnesses, history of colon resection, gastroparesis, Inborn-Errors-of-Metabolism (such as PKU)]; prior gastrointestinal bypass surgery (i.e., Lapband, etc.); any chronic inflammatory condition or disease (e.g. rheumatoid arthritis, Crohn's disease, ulcerative colitis, Lupus, HIV/AIDS, etc.); known sensitivity to any ingredient in the test formulations as listed in the certificates of analysis. Participants were excluded if they were currently participating in another research study with an investigational product or had participated in another research study in the past 30 days, or if they had any other diseases or conditions that, in the opinion of the medical staff, could confound the primary endpoints or place the subject at increased risk of harm if they were to participate.

Table 1. Anthropometrics and vitals of study participants.

	Total (N=28)	
Variable	Mean	SD
Age (years)	29.4 ± 10.1	10.1
Men	22 (78.6%)	
Women	6 (21.4%)	
Height (cm)	174.3 ± 9.0	9.0
Weight (kg)	78.7 ± 13.2	13.2
Body Mass Index (kg/m²)	25.7 ± 2.8	2.8
Systolic Blood Pressure (mm Hg)	119.4 ± 10.1	10.1
Diastolic Blood Pressure (mm Hg)	72.9 ± 11.5	11.5
Resting Heart Rate (bpm)	66.1 ± 8.4	8.4

Protocol

Height, Body Mass, Heart Rate, Blood Pressure, Mid-Thigh and Mid-Arm girth, Bioelectrical Impedance Analysis (BIA)

Standing height was determined using a stadiometer with participants in socks or bare feet with heels together. Body mass was measured using a Seca 767™ Medical Scale. Resting heart rate and blood pressure were measured using an automated blood pressure cuff (Omron HEM-780) after participants had remained seated for a minimum of five minutes. Mid-thigh and mid-arm girth were measured with a standard tape measure (Blue Jay) with the left and right limbs averaged together for arms and thighs respectively. Mid-thigh circumference was measured half-way between the superior aspect of the patella and the inguinal grease whereas mid-arm circumference was measured half-way between the acromion and olecranon processes.

Body fluid components [total body water (TBW), extracellular fluid (ECF), and intracellular fluid (ICF)] were assessed via bioimpedance analysis (BIA) (570, Inbody USA, Cerritos, CA, USA). Both absolute (in L) and relative (%) ECF and ICF were used for statistical analysis along with ECF/TBW. Participants followed the preparatory procedures outlined by the BIA device as their study visit preparation. In addition, subjects removed all jewelry, shoes, and socks and wiped their hands and feet with an InBody specific prep pad before stepping onto the InBody device. Body mass, blood pressure, heart rate, mid-thigh, mid-arm, and body fluid components were measured before ingestion of the investigational product (which was administered before the exercise bout) (PRE) and after the exercise bout (POST).

Visual Analog Scales (VAS) and Perceived Recovery Scale (PRS)

VAS was used to rate muscle pump, fatigue, product satisfaction, alertness, focus, mood, and energy. VAS questions were constructed using a 10 cm line anchored by "Lowest Possible" and "Highest Possible" except for fatigue which was anchored by "High Fatigue" and "No Fatigue" and product satisfaction which was anchored by "Highly Disappointed" and "Highly Satisfied". The validity and reliability of VAS to assess fatigue and energy have been previously established [27] and reported [28,29]. Muscle pump, focus, and alertness were administered before and after the workout. The level of satisfaction was administered POST and 24hr POST, whereas fatigue, mood, and energy were administered PRE, POST, and 24hr POST. A perceived recovery scale using a numerical scale from 0 to 10 was administered to assess participants' recovery PRE, POST, and 24hr POST.

The Investigational Product

After qualifying for the study, subjects were assigned to receive two different treatments in a randomized, counterbalanced order such that half the participants ingested 3D PUMP BREAKTHROUGH® first and then citrulline while the other half of the participants ingested citrulline first and then 3D PUMP BREAKTHROUGH®. Treatments (beverages) were mixed by the research staff during study visits and were consumed 45 min prior to exercise (and after all PRE measurements) in the presence of the medical/research staff during study visits 2 and 4. Treatments consisted of: 1) 6g of 3D PUMP BREAKTHROUGH® dissolved in 16 fl. Oz. of bottled water or 2) 8g of citrulline dissolved in 16 fl. Oz. of bottled water. 3D PUMP BREAKTHROUGH® consists of 3g L-citrulline, 1.2g glycerol, and 165mg Amla (Phyllanthus emblica) Fruit extract. Following consumption of the test product participants sat quietly for 35 minutes until they moved into a heated room to begin preparations (and acclimation) for the exercise bout.

Exercise bouts

Participants underwent 30 min of walking/jogging on a treadmill in a heated room (80°F-90°F/26.7°C-32.2°C) at a self-selected pace that would elicit 60-70%HRmax. Maximal heart rate was measured via the equation 220 – age for each participant prior to entering the heated room. The researcher instructed each participant to maintain a pace within their respective heart rate range while being constantly monitored for compliance to that pace. The 30 min timer did not start until participants established a pace within their target 60-70%HRmax range. Participants entered the heated room 35 minutes after consuming the investigational product so that they would have a 10 min acclimation period where they were fitted for a heart rate monitor and provided instructions for the upcoming bout. Therefore, participants did not begin exercise until 45 min post ingestion of the investigational products. Immediately before the walk/jog participants were monitored for heart rate and core body temperature while the room was assessed for ambient temperature. During the walk/jog core body temperature via infrared forehead thermometer, heart rate via Polar heart rate strap, and ambient temperature via weather station (Davis Vantage VUE) were constantly monitored by the researcher and recorded every 10 minutes. Following the aerobic portion of the workout participants had 5 minutes to provide a urine sample to measure USG via pocket refractometer (Atago, PAL-10S). After a 5-minute rest, participants performed bilateral leg extensions and Smith machine squats for 2 sets of 12 repetitions with 60s of rest in between sets. Participants were instructed to choose a load they could do for at least 12, but no more than 15 repetitions on each exercise. On the third set of each exercise participants were instructed to perform as many repetitions as possible [Reps to Failure (RTF)]. Failure was defined as the inability to complete a full repetition. The load for each set was kept the same between each treatment (Citrulline and 3D PUMP) for all participants. Participants were allowed to choose their own cadence, but they were instructed to go slow and controlled. Following the 3rd set of Smith machine squats participants were given 3 min of rest before bilateral mid-thigh gith was measured. Following the mid-thigh measurement participants performed dumbbell arm curls and triceps rope pushdowns under the same parameters as the lower body exercises. Following 3 min of rest bilateral mid-arm gith was measured. All training sessions were monitored by a strength and conditioning specialist. The time of day for each participant's workout was kept consistent between treatments.

Blood and Urine collection

Prior to supplementation, venous blood was obtained by a research nurse in two 8.5 mL serum separator tubes (BD Vacutainer, Franklin Lakes, NJ, USA), and one 4 mL K2 EDTA treated tube (BD Vacutainer, Franklin Lakes, NJ, USA). Upon collection, each whole blood and serum sample was slowly inverted ~8 times. The serum tubes were set to clot for approximately 30 min at room temperature prior to being centrifuged (Drucker Diagnostics Horizon Model 642E, Phillipsburg, PA) at room temperature for 10 min at 1,600 xg. Serum aliquots of 1.5 mL were obtained from one serum tube and placed in 5 mL polypropylene tubes (Wheaton Science Products, Millville, NJ) and stored at -80◦C until batch-processing for serum analysis for MCP-1. The second 8.5 mL tube of separated serum and the 4 mL whole blood sample were stored for less than 6 hours in refrigerator and sent for analysis of serum osmolality, total creatine kinase, sedimentation rate-modified Westergren, C-reactive protein, quantitative, and CBC with differential for hematocrit and neutrophils (LabCorp, Dublin, OH). Approximately 10 min following exercise testing, venous blood was obtained by the same process for MCP-1, serum osmolality, and CBC with differential for hematocrit and neutrophils. Subjects returned 24 hours after exercise bout for venous sampling of total creatine kinase, sedimentation rate-modified Westergren and C-reactive protein.

Prior to supplementation and ~12 min following exercise testing, mid-stream urine osmolarity samples of ~40 mL were collected in 90 mL sterile urine specimen container (Parter Medical, Carson, CA), refrigerated and sent for analysis (LabCorp, Dublin, OH).

Statistical Analysis

Primary outcome measures included mid-thigh girth, mid arm girth, body fluid shifts (via BIA), hematocrit, serum and urine osmolality, USG, neutrophils, markers of inflammation (MCP-1, CK, ESR, and CRP). Secondary outcome measures included subjective changes in recovery, muscle pump, fatigue, product satisfaction, alertness, focus, mood, and energy, and RTF in each resistance exercise. Tertiary outcome measures included changes in vital signs, core body temperature, side effect profile/adverse events monitoring and adverse events. A priori power analysis was conducted via G*Power for a mixed factorial ANOVA with repeated measures within-between interaction given a small effect of 0.25, with two groups and two time points a sample size of 34 was needed to achieve 80% power or with three time points a sample size of 28 was needed to achieve 80% power. However, the computed effect sizes in the current investigation provide more than adequate statistical power. Normality of each variable was assessed using the Shapiro-Wilk test. Two (Citrulline vs. 3D PUMP) x 2 (PRE, POST or 24hr POST) or 2 (Citrulline vs. 3D Pump) x 3 (PRE, MID, POST, or 24hr POST) mixed factorial ANOVAs were completed to assess group, time, and group x time interaction effects. When sphericity was violated, Greenhouse-Geisser corrected P-values were used for main effects/interactions. In the event of missing data, a mixed-effects model was utilized in GraphPad Prism. Sidak post-hoc procedures were used to assess individual comparisons between time points and/or groups. A significance level of ≤ 0.05 was accepted as statistical significance, whereas a significance level of >0.051 to ≤ 0.10 was accepted as a statistical trend. Trend and significant interactions were followed up with dependent t-tests to identify potential differences. Dependent t tests were used for RTF in the resistance exercises. For primary outcome variables data points less than -3SD or greater than +3SD were deemed outliers and removed before analyses. Effect sizes are expressed as Cohen's d with 95% confidence intervals and interpreted as ≥ 0.2 (small), ≥ 0.5 (moderate), and ≥ 0.8 (large). All analyses were completed with GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA).

Results

Primary Outcomes

Average thigh circumference indicated a significant interaction ($p=0.017$) and main effect of time ($p<0.001$). Post hoc testing revealed that Citrulline and 3DPump were significantly greater post ($p<0.001$, mean difference: 1.38 cm, 95%CI: 1.08 to 1.67, d = 2.06 and $p<0.001$, mean difference: 1.03 cm, 95%CI: 0.78 to 1.29, d = 1.80, respectively). Left thigh and right thigh circumference indicated a significant main effect of time ($p<0.001$ and $p<0.001$, respectively) indicating that post was greater than pre. The right thigh also had a significant interaction ($p=0.010$). Regarding the right thigh, post hoc testing for Citrulline and 3DPump revealed that post was significantly greater than pre ($p<0.001$, mean difference: 1.55 cm, 95%CI: 1.10 to 2.00, d = 1.84 and $p<0.001$, mean difference: 0.96 cm, 95%CI: 0.575 to 1.35, d = 1.33, respectively). However, there were no post hoc differences between groups for the right thigh. Average arm circumference indicated a significant main effect of time ($p<0.001$) indicating that post was greater than pre. Left arm and right arm circumference indicated a significant main effect of time ($p<0.001$ and $p<0.001$, respectively) indicating that post was greater than pre.

Neutrophils (%) indicated a significant time effect ($p=0.003$) indicating that post was greater than pre. Neutrophils (absolute) indicated a significant time effect ($p<0.001$) indicating that post was greater than pre. CK indicated a significant time effect ($p<0.001$) indicating that post was greater than pre. ESR indicated a significant time effect ($p<0.001$) indicating that post was less than pre. There were no main effects or interaction for CRP. MCP-1 indicated a significant time effect ($p<0.001$) indicating that post was greater than pre.

TBW, ICF, and ECF (in L) indicated a significant time effect ($p<0.001$) indicating that post was greater than pre. ICF and ECF (%) indicated a significant time effect ($p=0.020$) indicating that post was greater than pre. ECF/TBW indicated a significant interaction ($p=0.021$) and a time effect ($p=0.001$) . Post hoc testing for 3D Pump revealed that post was significantly greater than pre ($p=0.002$, mean difference: 0.0012 au, 95%CI: 0.000438 to 0.00201, d = 0.71), however there were no post hoc differences between groups.

Serum osmolality indicated a significant time effect ($p<0.001$) indicating that post was greater than pre. Urine osmolality indicated a significant time effect ($p<0.001$) indicating that post was less than pre. USG indicated a significant time effect ($p<0.001$). There were no main effects or interaction for hematocrit.

Secondary Outcomes

Muscle Pump indicated a significant time effect ($p<0.001$) indicating that post was greater than pre. Fatigue indicated a significant time effect ($p<0.001$). Focus indicated a significant time ($p=0.023$) and group effect ($p=0.29$). Energy indicated a significant time effect ($p<0.001$) indicating that post was less than pre and 24 hours post. Mood indicated a time effect trend ($p=0.075$). There were no main effects or interactions in subjective changes of alertness, product satisfaction, or RTF for any of the resistance exercises. PRS indicated a significant time effect ($p<0.001$) indicating that post was less than pre.

Table 2. Appendicular girth

	Time	N	Citrulline		N	3D Pump					
Variable			**Mean**	**SD**		**Mean**	**SD**	**Between group differences**	**Main Effect for Time**	**Main Effect for Group**	**Interaction**
Left Thigh (cm)	Pre	28	56	5	28	56.3	4.8		p<0.001	0.226	0.580
	Post	28	57.2	5	28	57.4	5				
Right Thigh (cm)	Pre	28	55.9	4.6	28	56.1	4.8	0.519	p<0.001	0.923	0.010
	Post	28	57.4	4.8	28	57.1	4.9	0.214			
Within group differences			**P<0.001 (d= 1.84, 95%CI: 1.10 to 2.00)**			**P<0.001 (d= 1.33, 95%CI: 0.58 to 1.35)**					
Average Thigh (cm)	Pre	28	55.9	4.8	28	56.2	4.8	0.337	p<0.001	0.582	0.017
	Post	28	57.3	4.9	28	57.2	4.9	0.900			
Within group differences			**P<0.001 (d= 2.06, 95%CI: 1.08 to 1.67)**			**P<0.001 (d= 1.80, 95%CI: 0.78 to 1.29)**					
Left Arm (cm)	Pre	28	32.9	4.1	28	32.9	4.2		p<0.001	0.512	0.113
	Post	28	34.6	4.1	28	34.4	4.3				
Right Arm (cm)	Pre	28	32.9	4.1	28	32.8	4		p<0.001	0.512	0.720
	Post	28	34.5	4.2	28	34.4	4.2				
Average Arm (cm)	Pre	28	32.9	4.1	28	32.9	4.1		p<0.001	0.476	0.214
	Post	28	34.5	4.2	28	34.4	4.2				

Table 3. BIA and markers of hydration.

	Time	N	Citrulline		N	3D Pump					
Variable			**Mean**	**SD**		**Mean**	**SD**	**Between group differences**	**Main Effect for Time**	**Main Effect for Group**	**Interaction**
TBW (L)	Pre	28	47.3	9.5	27	47.1	9.4		<0.001	0.880	0.855
	Post	28	47.8	9.3	27	47.5	9.4				
ICF (L)	Pre	28	47.3	9.5	27	47.1	9.4		<0.001	0.753	0.765
	Post	28	47.8	9.3	27	47.5	9.4				
ICF (%)	Pre	28	63.2	0.8	27	63.2	0.8		0.020	0.928	0.940
	Post	28	63.1	0.8	27	63.1	0.8				
ECF (L)	Pre	28	17.4	3.3	27	17.3	3.3		<0.001	0.973	0.994
	Post	28	17.6	3.2	27	17.4	3.3				
ECF (%)	Pre	28	36.8	0.8	27	36.8	0.8		<0.001	0.928	0.940
	Post	28	36.9	0.8	27	36.9	0.8				
ECF/TBW (au)	Pre	28	0.368	0.008	27	0.367	0.008	P=0.449	<0.001	0.891	0.021
	Post	28	0.369	0.008	27	0.369	0.008	P=0.992			
Within group differences			P=0.183			**P=0.002 (d= 0.71, 95%CI: 0.0004 to 0.0020)**					
Serum Osmolality (mOsm/kg)	Pre	28	288.7	4.4	28	288.1	5.2		<0.001	0.531	0.887
	Post	28	291.6	3.5	28	291.2	4.9				
Urine Osmolality (mOsm/kg)	Pre	28	741.2	195.7	27	741.2	195.9		<0.001	0.966	0.931
	Post	28	579.3	189.7	26	538.4	196.4				
USG (au)	Pre	28	1.0192	0.0053	28	1.0186	0.0054		<0.001	0.982	0.605
	Mid	28	1.0130	0.0065	28	1.0134	0.0069				
	Post	27	1.0167	0.0063	28	1.0168	0.0062				
Hematocrit (%)	Pre	28	45.4	3.6	28	45.3	3.2		0.133	0.952	0.740
	Post	28	45.6	3.3	28	45.7	3.5				

Table 4. Markers of damage and inflammation.

	Time	N	Citrulline		N	3D Pump				
Variable			**Mean**	**SD**		**Mean**	**SD**	**Main Effect for Time**	**Main Effect for Group**	**Interaction**
Neutrophils (x10e3/uL)	Pre	28	3.4	1.4	28	3.3	1.4	<0.001	0.683	0.608
	Post	28	4.4	1.7	28	4.2	2			
Neutrophils (%)	Pre	28	56.0	9.6	28	56.2	10.1	0.003	0.731	0.288
	Post	28	59.2	9.5	28	58.0	11.1			
CK (U/L)	Pre	28	234.6	178.8	28	408.9	1098.0	0.013	0.474	0.281
	24hr Post	28	421.7	329.8	27	486.2	808.2			
ESR (mm/hr)	Pre	27	5.0	3.3	28	5.4	4.2	0.001	0.211	0.885
	24hr Post	27	3.4	1.9	27	4.1	3.9			
CRP (mg/L)	Pre	28	1.0	2.2	28	1.0	2.3	*0.055*	0.604	0.152
	24hr Post	28	1.1	2.0	27	1.7	4.0			
MCP-1 (pg/ml)	Pre	26	223	71	28	233	72	<0.001	0.648	0.998
	Post	26	305	83	28	315	95			

Table 5. Perceived recovery and measures of affect from the VAS.

	Time	N	Citrulline		N	3D Pump				
Variable			**Mean**	**SD**		**Mean**	**SD**	**Main Effect for Time**	**Main Effect for Group**	**Interaction**
PRS (au)	Pre	28	9.2	0.8	28	9.2	0.8	<0.001	0.751	0.491
	Post	28	5.3	2.2	28	5.4	2.0			
	24hr Post	28	7.4	1.7	28	7.1	1.7			
Muscle Pump (cm)	Pre	28	1.2	1.2	28	1.2	1.3	<0.001	0.943	0.986
	Post	28	7.4	1.1	28	7.4	1.3			
Fatigue (cm)	Pre	28	7.2	2.2	28	8.1	2.0	<0.001	0.243	0.157
	Post	28	2.9	2.1	28	3.2	2.5			
	24hr Post	28	6.7	2.3	28	6.4	2.2			
Product Satisfaction (cm)	Pre	28	6.7	1.8	28	7.0	1.8	0.415	0.778	0.208
	24hr Post	28	7.1	1.5	28	6.9	1.6			
Alertness (cm)	Pre	28	6.4	2.4	28	7.1	2.1	0.297	0.170	0.299
	Post	28	6.2	2.2	28	6.4	2.4			
Focus (cm)	Pre	28	7.0	1.8	28	7.5	1.6	0.023	0.029	0.672
	Post	28	6.1	2.2	28	6.7	2.2			
Mood (cm)	Pre	28	7.3	1.5	28	7.6	1.5	*0.075*	0.226	0.946
	Post	28	6.7	2.0	28	6.9	2.5			
	24hr Post	28	7.3	2.2	28	7.5	1.7			
Energy (cm)	Pre	28	6.9	1.6	28	6.8	1.8	<0.001	0.520	0.232
	Post	28	4.6	2.4	28	5.2	2.7			
	24hr Post	28	7.1	2.1	28	6.9	1.9			

Table 6. Resistance exercise repetitions to failure (RTF)

	N	Citrulline		N	3D Pump		
Exercise		**Mean**	**SD**		**Mean**	**SD**	**Between group differences**
Leg Extension	27	18.4	4.9	27	19.0	4.6	P=0.486
Smith Machine Squats	28	10.7	3.3	28	10.7	3.8	P=1.000
Arm Curl	28	12.9	3.6	28	12.9	2.7	P=0.945
Triceps Pushdown	28	13.4	4.0	28	13.3	4.5	P=0.953

Tertiary Outcomes

Body mass indicated a significant time effect ($p<0.001$). SBP indicated a trend for an interaction ($p=0.054$) and a significant time effect ($p<0.001$). Post hoc testing for 3D Pump revealed that post was significantly less than pre and 24 hours post ($p<0.001$, mean difference: -10.4 mmHg, 95%CI: -16.9 to -3.92, $d = 0.77$ and $p<0.001$, mean difference: 9.64 mmHg, 95%CI: 2.46 to 16.8, $d = 0.65$, respectively). However, there were no differences between groups. DBP indicated a trend for an interaction ($p=0.087$) and a significant time effect ($p<0.001$). Post hoc testing for Citrulline revealed that post was possibly less than pre and 24 hours post ($p=0.062$, mean difference: -5.14 mmHg, 95%CI: -10.5 to 0.208, $d = 0.46$ and $p=0.070$, mean difference: 4.96 mmHg, 95%CI: -0.315 to 10.2, $d = 0.45$, respectively). Meanwhile, post hoc testing for 3D Pump revealed that post was significantly less than pre and 24 hours post ($p<0.001$, mean difference: -9.86 mmHg, 95%CI: -13.9 to -5.78, $d = 1.16$ and $p<0.001$, mean difference: 9.25 mmHg, 95%CI: 5.70 to 12.8, $d = 1.25$, respectively). HR indicated a significant time effect ($p<0.001$).

Participants appeared to tolerate the investigational products well, however ~36% of subjects (10 out of 28) during the Citrulline condition felt lightheaded, nauseous, had a headache, and even regurgitated during and/or after the workout, whereas ~21% of subjects (5 out of 28) during the 3D PUMP condition felt lightheaded during and/or after the workout and had diarrhea 2 hours post workout.

Discussion

The current investigation comparing 3DPump (which contains 3 g of L-citrulline) versus 8g of L-citrulline alone at a higher dose displayed similar outcomes and patterns for most variables. Muscle girth via arm and thigh circumference were increased after exercise in both conditions. ECF/TBW was significantly increased from pre to post under the 3DPump condition only. However, both conditions responded similarly in markers of hydration (i.e., TBW, ECF, ICF, serum and urine osmolality, and USG) and in markers of inflammation and damage (i.e., CK, ESR, MCP-1). Absolute neutrophils and relative neutrophils significantly increased from pre to post in both conditions. Both conditions reported similar subjective changes in muscle pump, energy, and perceived recovery score throughout the study. There were no differences between groups in the number of repetitions to failure on any of the resistance exercises. 3DPump may have had the unique capability of lowering blood pressure (SBP and DBP) post workout compared to pre workout and 24 hours after exercise. Both conditions had similar effects on HR and body mass throughout the study. Lastly, fewer adverse events occurred under the 3DPump condition.

Table 7. Vitals and body mass.

	Time	N	Citrulline		N	3D Pump					
Variable			**Mean**	**SD**		**Mean**	**SD**	**Between group differences**	**Main Effect for Time**	**Main Effect for Group**	**Interaction**
SBP (mmHg)	Pre	28	119.8	10.3	28	122.8	9.1	P=0.323			
	Post	28	116.9	11.2	28	112.4	13.9	P=0.417	<0.001	0.884	*0.054*
	24hr Post	28	120.0	8.4	28	122.1	10.4	P=0.652			
Within group differences	Pre vs. Post		P=0.517			**P=0.001 (d= 0.77, 95%CI: -16.9 to -3.92)**					
	Pre vs. 24hr Post		P=0.998			P=0.950					
	Post vs. 24hr Post		P=0.364			**P=0.006 (d= 0.65, 95%CI: 2.46 to 16.8)**					
DBP (mmHg)	Pre	28	75.0	10.7	28	76.3	9.3	P=0.830			
	Post	28	69.9	10.7	28	66.4	11.2	P=0.305	<0.001	0.674	*0.087*
	24hr Post	28	74.8	10.3	28	75.6	9.9	P=0.898			
Within group differences	Pre vs. Post		*P=0.062 (d= 0.46, 95%CI: -10.5 to 0.21)*			**P<0.001 (d= 1.16, 95%CI: -13.9 to -5.78)**					
	Pre vs. 24hr Post		P=1.000			P=0.949					
	Post vs. 24hr Post		*P=0.070 (d= 0.45, 95%CI: -0.315 to 10.2)*			**P<0.001 (d= 1.25, 95%CI: 5.70 to 12.8)**					
HR (bpm)	Pre	28	65.6	9.5	28	68.7	11.9				
	Post	28	99.2	14.1	28	99.7	14.6		<0.001	0.244	0.306
	24hr Post	28	66.1	9.8	28	66.3	12.1				
Body Mass (kg)	Pre	28	77.8	13.5	28	77.7	13.3				
	Post	28	77.3	13.3	28	77.2	13.2		<0.001	0.496	0.700
	24hr Post	28	78.5	13.6	28	78.4	13.3				

Previous investigations commonly examined 8g of citrulline to stimulate vasodilatory and performance effects within the muscle [7–9,13]. Notably both conditions in this study had a similar effect on appendicular girth pre to post workout along with subjective ratings of a muscle pump despite a lower citrulline dose in the 3D PUMP condition. Similarly, Gonzalez et al. [13] found significant improvement in muscle swelling, from pre to post, after an exercise session with both 8g L-citrulline and placebo (water) suggesting that the exercise bout may have been the culprit to muscle swelling. Leg asymmetries in the current investigation may be due to an overemphasis on leg dominance, especially given the stress on the last set of squats and leg extensions which pushed each subject to failure. In other words, when stressed to failure individuals will resort to comfort or their stronger limb to accomplish a physical challenge. Other measures of affect in the current investigation (fatigue, focus, energy, mood) were similar between 3DPump and L-citrulline much like Gonzalez et al. [30] where the authors reported no significant changes between L-citrulline and placebo in subjective measures of energy, focus, fatigue, or muscle pump.

Glycerol has been shown to increase fluid retention by increasing water reabsorption at the kidneys [31]. Glycerol's potential to increase plasma volume [32] may result in body fluid shifts and interestingly enough, ECF/TBW increased from pre to post workout in the 3DPump condition only. Other markers of hydration (serum and urine osmolality, USG, and hematocrit) responded similarly or had no change between conditions over time. This may be due to the lower quantity of water used in this investigation (~473ml) compared to larger volumes (1,500 – 2,000ml) which are needed to provide an osmotic drive to retain water and increase blood osmolality [25]. In addition, the pre-exercise recommendation for glycerol includes ingesting 1.2g/kg of body weight with a fluid amount of 26mL/kg of body weight over a 60-minute period 30 minutes before exercise in order to hyperhydrate [33] which is far greater than the dose used in the current investigation. However, it is possible that glycerol can induce a more optimal state of hydration without any improvements in endurance performance [32]. Nevertheless, the low-intensity bout of aerobic exercise in this study was used to simply induce a greater stress on body fluid dynamics rather than measure a performance marker. Relative to decrements in performance, anaerobic efforts have a lower sensitivity to body water losses compared to aerobic efforts [34]. The lack of experimentally designed perceived exertion assessment during the 30 min aerobic bout along with body fluid measures in between aerobic and anaerobic portions of the workout are a limitation in this study and with participants' heart rate controlled within a range of 60-70% HRmax, the intensity and duration were probably not sufficient to have a detrimental effect on subsequent resistance exercise performance.

Amla extract, which is high in vitamin C, has anti-inflammatory and anti-oxidant properties [35,36], as well has other polyphenols (e.g., gallic acid and ellagic acid) that are responsible for amla's total antioxidant capacity [21,22]. Amla's ability to suppress inflammatory markers [37] may aid in a suppression of inflammatory biomarkers, however that was not observed in the current investigation. Sureda et al. [38] did not observe any increase in CK before, after, or 3 hours after a long duration cycling bout with 6g of citrulline-malate but acknowledged that the cycling session was not strenuous enough to stimulate muscle damage. Martinez-Sanchez et al., [39] analyzed a placebo against watermelon juice enriched with 3.3g of L-citrulline and found a significant positive impact on peak average force during a squat protocol (8x8 with an 8RM load) with all male subjects. In addition, watermelon juice enriched with L-citrulline attenuated post exercise RPE, LDH, muscle damage (i.e., myoglobin), and liver enzymes (AST and ALT) compared to placebo while CK levels were no different between groups [39]. However, the authors measured CK immediately after exercise when CK levels peak 24-96 hours post exercise [40]. Shanely et al. [20] found that MCP-1 went up after a 75km time trial and there were no differences between watermelon juice (which includes carotenoids that exert antioxidant and anti-inflammatory effects) and Gatorade. In this regard, 3DPump may show a greater impact in attenuating the damage occurring from exercise over long-term supplementation use in conjunction with an exercise program.

Although L-citrulline has conflicting reports for improving RTF [4] overall evidence suggests that citrulline offers a modest improvement (6.4%; ~3 repetitions) [16]. Conversely, the current investigation did not observe any differences between 8g L-citrulline and 3g citrulline within 3DPump possibly due to the minimum effective dose of 3g which appears to provide a significant increase in circulating arginine and performance [39,41]. Although subjects were able to select their own resistance for each exercise, the load was kept consistent between conditions in order to alleviate any error in performance. Also, it should be noted that chronic dosing of L-citrulline appears to be more effective than acute doses for enhancing exercise performance [5,42].

There was a possible improvement in vascular tone under the 3DPump condition as denoted by follow-up post hocs from the interaction trends in SBP and DBP. L-citrulline may help attenuate the BP response induced by low intensity exercise [43], however other data has shown L-citrulline to reduce SBP (after 6g/day for 7 days against a placebo) [44] and increase SBP (after 3.4g/day in the form of watermelon juice concentrate for 16 days against a control group) [18]. Since the blood pressure response was more so unique to 3DPump, it may have been partly due to vasoactive effects of Amla extract. Amla extract has been shown to reduce blood pressure and improve endothelial function [24] within longitudinal designs in several animal models (rats) [45–47] and in a human experiment [48]. These possible improvements in vascular tone may be attributed to the ellagitannins (i.e., ellagic acid and gallic acid) in amla extract [49]. To our knowledge, this is the first study using amla extract in response to exercise that observed possible antihypertensive effects after an acute dose.

Conclusions

In conclusion, 3DPump and 8g of L-citrulline have similar effects on acute muscular endurance, muscle damage and inflammation, appendicular muscle girth, and subjective measures of affect. 3D PUMP may have the potential to increase ECF/TBW and improve post workout vascular tone over L-citrulline alone. Future studies should look at chronic use of 3DPump along with an exercise intervention to delineate any potential benefits in recovery and performance.

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