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The Acute Effects of Citrulline Malate and Bonded Arginine Silicate Supplementation on Vasodilation of Young Adults

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Abstract

Background

Clinicians, professional athletes, and recreational athletes are interested in supplementation that up-regulates nitric oxide (NO) production in blood vessel endothelium, increasing arterial vasodilation. Benefits from these supplements include improvements in blood pressure, muscle hyperemia, and exercise performance. Citrulline Malate (CM) is a pre-workout ingredient, popular for its ability to increase exercise performance and blood serum concentrations of L-arginine, resulting in NO production. Recently, Inositol-Stabilized Arginine Silicate (ASI, Nitrosigine) has been added to many of the most popular pre-workout blends, following a group of studies showing ASI increases serum arginine and reduces post-workout muscle damage. Research has yet to compare CM and ASI in-vivo using a flow-mediated dilation (FMD) technique, a validated measure of the vascular endothelium's NO producing ability. Thus, the purpose of this experiment was to determine the effectiveness of ASI, compared to CM and placebo, in up-regulating NO production in blood vessels as measured by acute changes in vasodilation.

Participants

Healthy, normotensive, and at least moderately active male ($n = 16$) and female ($n = 8$) young adults were recruited to participate. All participants reported no pre-workout supplementation within two weeks of their study participation, and all participants abstained from nicotine, caffeine, and alcohol during their participation.

Method

We utilized a double-blind, within-subjects design where participants reported for three trials, each preceded by a 7-day washout period. Upon reporting to the research center,

participants read and signed the informed consent document, gave a brief medical history, and remained in an upright-seated position until their blood pressure and heart rate normalized. The participant then reclined into a comfortable supine position in a phlebotomy chair, and his or her arm was abducted at 70 to 90 degrees and at heart level, depending on the participant's level of comfort. A baseline FMD measurement was obtained followed by consumption of one clinical dose CM (8g), ASI (1.5g), or dextrose placebo (8g); the supplementation order was randomized controlling for potential order effects. Participants completed a brief 24-hour nutrition survey and waited for 60 minutes. After the waiting period, FMD was repeated. We used screen capture software to record the entire FMD procedure and conducted analyses on the videos using Quipu Cardiac Suite software.

Results

Repeated measures analysis of variance yielded a significant supplement x time effect ($p < .001$), such that CM and ASI yielded a greater change in FMD response than placebo. After allometric scaling of the FMD values, supplement x time effect remained significant ($p = .001$).

Discussion

Both CM and ASI increased vascular NO production as measured by a change in FMD and may be particularly beneficial to individuals looking to increase the potential for muscle hyperemia during exercise. Our results support previous findings that CM and ASI increase blood serum concentrations of arginine, and are effective at increasing vascular endothelium nitric oxide producing capacity.

Word count: 459

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Introduction

The use of dietary supplements as ergogenic aids has become increasingly commonplace among both recreational and competitive athletes. Supplements taken to boost the production of nitric oxide (NO) by vascular endothelium comprise a particularly large portion of popular ergogenic aids. Endothelial cells produce NO to increase vasodilation in response to many potential physiologic stimuli, facilitating greater blood flow to tissues. The amino acid L-arginine is the primary amino acid substrate that endothelial cells use to synthesize NO (Green et al., 2004; Hecker et al., 1990). Previously, oral supplementation with L-arginine was the most popular mode of increasing serum concentrations of arginine, which was thought to consequently facilitate an increase in endothelial cell nitric oxide producing capability. In the human cardiovascular system, nitric oxide synthase enzyme uses serum arginine in the production of NO and L-citrulline (Palmer et al., 1988). This NO, also referred to as endothelial relaxing factor, allows arteries to dilate when vascular endothelium is exposed to mechanical stress (Epstein et al., 1993). Both animal model studies and double-blind studies with human participants indicate that L-arginine supplementation can improve outcomes in cardiovascular disease states, especially improving endothelium dependent vessel dilation in people with atherosclerosis (Preli et al., 2002). However, ergogenic and health benefits from large, orally administered doses of L-arginine have not been consistently demonstrated in healthy individuals or athlete populations (Alvares et al., 2014; McConnell, 2007; Tang et al., 2011).

More recently, L-citrulline and bonded arginine silicate (ASI; Nitrosigine) have become widely consumed in place of L-arginine, because they are more effective at increasing serum levels of arginine and may also provide ergogenic benefits (Kalman et al., 2014; Schwedhelm et al., 2008; Wax et al., 2016). L-citrulline is naturally produced in the cardiovascular system when

nitric oxide synthase uses L-arginine to produce NO. Unlike dietary L-arginine, orally consumed L-citrulline is not metabolized by the liver after ingestion, and instead forms an available amino acid pool that can be readily converted into back to arginine via the formation of L-argininosuccinic acid in the urea cycle (Hecker et al., 1990). The cellular recycling process of L-citrulline to L-arginine plays an important role in the production NO, because when arginosuccinate synthase, an enzyme used in the conversion of L-citrulline, is inhibited, NO production also decreases (Flam et al., 2007). ASI, a patented supplement compound, was engineered as a dietary intervention for atherosclerosis and bone mineral density disorders (McCarty & Zielinski, 2002). Dietary silicon may slow the development of atherosclerosis in at-risk individuals, and given the arterial health promoting properties of arginine, the ASI compound has potential to be doubly beneficial (Loeper et al., 1979). Research has demonstrated that dietary ASI increases serum silicate concentrations, and more effectively increases serum arginine than supplementing with unbound L-arginine or arginine hydrochloride (Komorowski & Perez Ojalvo., 2016; Proctor et al., 2007).

In place of L-citrulline, many pre-workout supplements now have included a compound form that is commonly referred to as citrulline malate (CM), and recent research suggests that exercisers may experience different ergogenic benefits when compared to LL-citrulline alone. Similar to supplementing with L-citrulline alone, clinical doses of CM exert some influence on the urea cycle and the production of NO during exercise; blood serum evaluation after intense exercise shows that CM supplementation causes an increase in urea cycle metabolite and nitrite concentrations (Breuillard et al., 2015). Studies examining potential exercise benefits have found the CM can increase total work capacity during high intensity resistance training (Glenn et al. 2015; Perez-Guisado & Jakeman, 2010; Wax et al., 2014). CM ergogenic benefits are not only

limited to anaerobic exercise, because the malate portion of the CM compound may positively

influence aerobic energy production (Bendahan et al., 2002). Similar to CM, ASI also out-

performs L-arginine in terms of increased exercise blood flow and decreased biomarkers of

muscle fatigue following exercise (Rood-Ojalvo et al., 2015). As reported by the manufacturer

and by the current literature, ASI doses range from 0.5 to 2.0 grams, which is a significantly

smaller dose than the typical 6.0 to 8.0 gram dose of CM. A smaller dosage could be beneficial

to individuals who have previously experienced stomach discomfort with pre-workout

supplements (Kalman et al., 2015). Research on the ergogenic effects of ASI is limited because

the supplement compound form used in this study, commonly referred to as Nitrosigine, was

only recently patented in 2002. However, benefits similar to those found with CM

supplementation (increased muscle hyperemia during exercise and decreased muscle damage

biomarkers post-exercise) have been shown in a small sample (Rood-Ojalvo et al. 2015).

Although there is increasing evidence to support the ergogenic benefits of CM and ASI,

little research has examined their effects on NO production as measured with acute changes in

vasodilation. After controlling for confounding variables such as hydration, diet, and exercise, a

change in plasma NO post-supplementation can be inferred by measuring acute changes in flow

mediated dilation using ultrasound, which is a non-invasive and reliable method of measuring

blood vessel diameter and remains a staple in clinical practice (Soga et al., 2007). Additionally,

the current literature contains insight into the effect of CM on flow-mediated dilation both during

and post-exercise, but lacks examination of acute effects of CM supplementation at rest. The

purpose of this study was to determine if an acute 8g dose of CM or a 1.5g dose of ASI causes an

increase in brachial artery diameter over baseline following flow-mediated dilation. The

participants were examined while in a rested state to emulate a pre-exercise condition, because

CM and ASI in many pre-workout supplements are commonly consumed anywhere from 30 to 60 minutes prior to the onset of exercise. This study specifically sought to determine whether acute CM or ASI supplementation effected the NO producing capacity of endothelium dependent vasodilation as measured by change in flow-mediated dilation. We hypothesized that an acute dose of CM would not cause any changes in flow-mediated dilation, because the majority of flow-mediated dilation studies report no significant difference following supplementation. The largest observed changes in blood flow, as a result of CM supplementation, have been immediately post moderate to intense exercise. Studies that reported changes in participant blood pressure have used daily CM doses for at least 2 weeks; neither of those conditions are utilized in the procedure of this study. Thus, there is little evidence to suggest that CM will have any effect when participants are given an acute dose and examined at rest.

Literature Review

Supplementing to Increase Serum Arginine

Interest in LL-arginine supplementation began as an intervention for cardiovascular disease states following research that uncovered the process by which endothelial cells produce a relaxing factor, likely nitric oxide, in response to mechanical stress via the urea cycle (Hecker et al., 1990; Palmer et al., 1988). Increasing the efficiency and production capability of the urea cycle can have a multitude of beneficial effects (Epstein et al., 1993). Increasing urea production capabilities could allow the body to more efficiently reduce harmful ammonia content and better maintain blood pH, an effect that has been substantiated in animal models and in human participants (Callis et al., 1991; Greenstein et al., 1956; Schaefer et al., 2002). With increased serum arginine, the urea cycle has more substrate available for nitric oxide (NO) and LL-citrulline production; L-citrulline can then be easily recycled back to LL-arginine. NO,

previously referred to as endothelial relaxing factor, was initially of greatest interest to clinicians who sought to provide an intervention to individuals with cardiovascular pathology. Studies have found that increasing serum arginine through oral or intravenous administration improves cardiovascular dynamics and serum markers of cardiovascular dysfunction (Bai et al., 2009; Deveaux et al., 2016; Orozoco-Gutierrez et al., 2010; Thorne et al., 1998). However, oral supplementation with LL-arginine may not be the most efficient mechanism of increasing serum arginine. Comparison studies have found that supplementing with L-citrulline, citrulline malate (CM), or inositol stabilized arginine silicate (ASI), more efficiently elevates serum arginine and serum citrulline (Komorowski & Hewlings, 2019; Rood-Ojalvo et al., 2015; Schwedhelm et al., 2008). Nutritional researchers have been particularly interested in L-citrulline because watermelon naturally contains dense quantities. However, studies of watermelon juice and rind have determined that an exerciser seeking a clinical dose would not be able to consume enough watermelon to experience beneficial effects (Cutrufello et al., 2015; Rimando & Perkins-Veazie, 2005).

L-citrulline and Citrulline Malate as Ergogenic Ingredients

Clinical studies of L-citrulline have found mostly similar results to those that administered intravenous arginine to populations with cardiovascular pathologies. Most notable among the clinical benefits from L-citrulline supplementation are its effects on hypertension. Two recent literature reviews and meta-analyses report that in hypertensive or pre-hypertensive participants decreases in systolic and diastolic blood pressure are experienced with long term CM supplementation (Mahboobi et al., 2019; Figueroa et al., 2017). L-citrulline and citrulline malate have both grown increasingly popular in sports nutrition and in strength and conditioning studies. In trained males, CM increases cycling time to exhaustion and repetition-based

performance during high-intensity anaerobic exercise in workouts that target the upper body (Perez-Guisado & Jakeman, 2010; Suzuki et al., 2016). A follow-up study with resistance-trained males, meant to examine potential differences during high-intensity lower body exercise, confirmed the previous finding that a large dose of CM prior to exercise increases the volume of work performed over placebo (Wax et al., 2014). Although most of the positive ergogenic effects have been found in trained male samples, a study with trained females found that CM supplementation increases work capacity during both upper and lower body resistance training (Glenn et al., 2015). The mechanism by which L-citrulline improves exercise work capacity may relate to findings of improved lactate removal following exercise, although this finding has been disputed (da Silva et al., 2017; Kiyici et al., 2017). Consistent with the previously mentioned findings, current CM supplements are oriented toward athletes who perform mostly high-intensity anaerobic exercise, and the majority of positive ergogenic effects have been found in trained populations (Bailey et al., 2015). When comparing the benefits of L-citrulline and citrulline malate, CM may have more influence over aerobic exercise, because of its malate component. Malate may benefit aerobic metabolism via an increase in the efficiency of oxidative ATP production, because of its intermediate role in the tricarboxylic acid cycle (Bendahan et al., 2002). The minimum dose needed to experience a benefit varies with the administration protocol and the outcome variable measured. In the previously mentioned studies that have found increased work capacity, an acute dose of six to eight grams of L-citrulline was administered or a three to six gram dose was administered daily for at least seven days. However, it is worth noting that null results have also been found with doses as large as 12 grams (Cunniffe et al., 2016).

Inositiol-Stabilized Arginine Silicate

Inositol-stabilized arginine silicate (ASI; Nitrosigine) has recently appeared on many pre-workout supplement labels, either in combination with CM or as the exclusive NO booster. As reported by the McCarty and Zielinski (2002), ASI was originally manufactured as a complex meant to increase serum arginine and silicate and provide an effective intervention for atherosclerosis and hypertension. Animal research in particular supports the theory that dietary silicon may prevent atherosclerosis development (Loeper et al., 1979). Similar to oral L-citrulline consumption, ASI appears to lower systolic, and to an even greater degree, diastolic blood pressure (Sylla et al., 2018). ASI has gained a reputation in the pre-workout supplement market as a superior form of L-arginine because of the greater efficiency and length of time for which ASI increases serum arginine levels (Proctor et al., 2007, Komorowski & Perez Ojalvo., 2016). Similar to CM, ASI also out-performs L-arginine in terms of increased exercise blood flow and decreased biomarkers of muscle fatigue following exercise (Rood-Ojalvo et al., 2015). As reported by the manufacturer and by the current literature, ASI doses range from 0.5 to 2.0 grams, which is a significantly smaller dose than the typical 6.0 to 8.0 gram dose of CM. The most common ASI dose used in research thus far follows a 1.5g/day schedule for a period of four or more days. Acute doses are also administered in 1.5g quantities (Komorowski et al., 2015). A smaller dosage could be beneficial to individuals who have previously experienced gastrointestinal discomfort with pre-workout supplements (Kalman et al., 2015). Research on the ergogenic effects of ASI is limited because of its recent rise to popularity, but benefits similar to those found with CM supplementation (increased muscle hyperemia during exercise and decreased muscle damage biomarkers post-exercise) have been shown (Rood-Ojalvo et al. 2015).

Method

Participants

Twenty-four total participants, 16 men and 8 women, were recruited to participate from a large university in the southeastern United States. Participation in the study was open to healthy, regularly exercising young adults between the ages of 18 and 30 years. Exclusionary criteria were as follows: hypertension, metabolic disorders, consumption of any pre-workout type supplements within two weeks of participating in the study, nicotine consumption in any of the previous six months, use of any prescription medications that have the potential to influence cardiovascular response, or use of any prescription drugs that influence the menstrual cycle. Screening for metabolic disease, tobacco use, and prescription drug use was conducted using a standard medical questionnaire adapted to an online survey form. Participants' physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ). All participants were considered to have high levels of physical activity; they reported vigorous exercise on more than three days per week and exceeded 1500 (MET x min / week), or reported physical activity of at least 3000 (MET x min / week). Anthropomorphic, blood pressure, and heart rate data are found in Table 1.

Research Design

This study used a within-subjects, double-blind research design. The order of supplementation was randomized and coded by a researcher who was not involved in data collection or analysis in order to control for order effects and to sufficiently blind the experimenters. Participants were asked to complete the experimental protocol in a fasted state, verified by self-reports, and were asked abstain from caffeine and all other dietary vitamin supplements 24 hours prior to testing. Participants recorded their dietary habits for 24 hours

leading up the first trial, and diet summaries were sent to participants 24 hours prior to

subsequent trials to aid them in replicating their diet as accurately as possible before the second

and third trials. The dietary habits survey was administered through a Qualtrics email link, using

the participants' preferred emails. Each participant's brachial artery flow-mediated dilation was

assesed twice during each trial, once before supplement ingestion, and again 60 minutes post-

ingestion. A second survey was administered following the FMD procedure to screen for

stomach discomfort. Male participants were required to wait for a minimum of seven days

between trials to allow for a supplement washout period, and were tested around the same time

of day i.e. early morning, morning, mid-day, early afternoon, etc. Female participants reported

for supplement trials during the menstrual phase of the menstrual cycle to ensure that results

were not confounded by changes in serum estradiol, which can attenuate flow-mediated dilation

response (Hashimoto et al., 1995). Because female participants allowed for approximately 28

days between trials, no further washout period precautions were taken. Otherwise, the procedure

for female participants did not differ from the male participants.

Supplementation

Participants were randomized into six groups of equal size needed to counterbalance the

order of supplementation and control for potentially confounding order effects. Regardless of the

participant's group designation and location of the placebo trial, all participants completed the

minimum seven-day washout period before the following trial, and the longest time between

trials was 50 days. The supplement doses administered were 8g of CM (Bulk Supplements,

Henderson, Nevada), 1.5g of ASI (Nutrition 21, Purchase, New York), or 8g of dextrose, all of

which were purchased in powder form and mixed with 12 ounces of fruit punch flavored water to

mask the differences in taste between the supplements. Eight grams of dextrose were also added

to the CM and ASI supplement drinks to keep the caloric load identical across trials. Following

baseline FMD and subsequent supplementation, the participants remained seated for 60 minutes

to allow the supplement enough time to pass through the digestive tract, at which point plasma

arginine levels should have been elevated (Kalman et al., 2015). Placebo trials reflected the exact

procedure used during the supplement trials.

Brachial Artery Flow Mediated Dilation

Generally, flow-mediated dilation procedures were determined using recommendations

from the Harris et al. (2010) tutorial on FMD assessment. All FMD measurements and AUC

calculations were completed using Quipu Cardiac Suite software to analyze a 60fps 1040 x 720p

live captured video of the ultrasound monitor during the entire 10-minute FMD procedure. Upon

reporting for trials, participants assumed a supine position in a reclining phlebotomy chair with

his or her left arm abducted from 70 to 90 degrees and placed palm up on an adjustable arm rest.

Blood pressure and heart rate were recorded from the participant's right arm every 5 minutes

until he or she maintained stable cardiovascular values. The experimenter then measured,

marked, and recorded cuff and transducer landmarks on the participant's left arm with a surgical

marker to ensure that the ultrasound transducer and occlusion cuff were placed in the same

location for each trial. The rapid inflation occlusion cuff (E20 and AG101 rapid cuff inflator,

Hokanson, Bellevue, WA) was placed beginning 2 cm distal from the antecubital fold, and a

10MHz ultrasound transducer (LOGIQ e, GE Healthcare, Chicago, IL, USA) was placed

beginning at least 5 cm proximal and no more than 10 cm proximal from the antecubital fold.

Dual mode Doppler ultrasound was used to simultaneously record blood flow velocity, which

would later be used in calculations of maximum shear rate, and shear rate area under the curve to

maximum (AUC). After maintaining a stable image of the brachial artery, in which both arterial

walls were clearly visible, two minutes were allowed to elapse to provide a sufficient amount of

time for the calculation of an average baseline diameter. Following the two-minute baseline

period, the occlusion cuff was rapidly inflated to 250 mmHG for five minutes. After the five-

minute occlusion phase, the cuff was rapidly deflated and changes in artery diameter and blood

flow velocity were monitored for the following three minutes. Identical procedures were used for

the baseline and post-supplementation FMD measurements.

Analysis of Flow-Mediated Dilation

An experimenter who was blinded to the supplement ID codes and the trial numbers of

the videos individually analyzed each FMD procedure video recording using Quipu FMD Studio

software (QUIPU, Pisa, Italy). By identifying the walls of the artery and calculating the average

of several brachial artery diameter measurements in real time, the software was able to provide

an accurate measure of arterial diameter for every second of video. Baseline diameters (D_{Base})

reported in this study are averages of the brachial artery diameter during the entire 120 second

pre-occlusion phase, whereas maximum diameter (D_{peak}) is the largest recorded diameter within

the 180 seconds following the immediate release of the occlusion cuff. The FMD percentage

(FMD%) was calculated using the following equation: (D_{peak} (mm) / D_{Base} (mm)) x 100. Similar

to diameter, the FMD Studio software also calculated an average shear rate for every second of

video. Shear rate was determined from the equation: (blood flow velocity (cm / sec) / diameter

(cm)) for which the values are expressed in units of sec^{-1}, and shear rate AUC to maximum was

calculated by taking the first integral of the shear rate curve from cuff release to maximum

diameter.

Statistical Analyses

A total of ($n = 15$) mean and ($n = 6$) women completed all three supplement trials. Because, a limited number of women were able to complete the study, a 2 (sex) x 2 (time) x 3 (supplement) ANOVA was used to determine sex differences in supplement response. There was no significant interaction with participant sex, so data were compiled and analyzed as one sample for the remainder of the statistical analyses. A 2 (time) x 3 (supplement) repeated measures ANOVA was used to test for significant differences in FMD%, D_{base}, D_{peak}, maximum shear rate, and shear rate AUC to maximum variables.

Examinations of the relationship between FMD baseline diameter (D_{base}) and FMD% have found that there is a significant negative relationship between the two variables, which can unintentionally bias the interpretation of the study (Atkinson & Batterham et al., 2013). In the present study, an examination of the relationship between D_{base} and FMD% yielded a much weaker negative relationship (Figure 5), however corrective analyses were still conducted for comparison purposes. In line with the statistical analysis procedure used by Atkinson (2014), the natural logarithm was calculated for D_{base} and $D_{peak,}$ and the aggregated valued were compiled into a predictive regression to determine the exponent of change (see Figure 6). Corrected FMD % was obtained using the equation $((LnD_{peak} / LnD_{base}^{0.94})-1) \times 100$ for each FMD procedure. Additionally, because some studies have found that habitual protein consumption can effect serum L-arginine concentrations, a 1 x 3 ANOVA was used to check for the potential confound of both protein consumption between trials.

Because the negative correlational relationship between D_{base} and FMD% was not as strong in the present study compared to population scaled FMD studies, further controls were used to ensure that potential changes in the FMD stimulus as measured by maximum shear rate and shear rate AUC did not emerge as confounding variables in the study. Linear regression

models, in which FMD% was the dependent variable and $D_{base,}$ maximum shear rate, and shear

rate AUC were predictor variables for all six FMD groups were used to test for significant

trends. If any of the linear models should be able to significantly predict FMD%, another

ANOVA would be employed using the predicted FMD% values from the regression equation.

Results

A summary of the means, standard deviations, and ANOVA results for the FMD data are

found in Table 2. For the unscaled FMD% values, there was a significant supplement x time

effect, $F = 11.64$, $p < .001$. Post-hoc analysis showed that CM and ASI supplementation resulted

in significantly higher changes in FMD% than placebo, but there was no significant difference

between the two supplements. These findings were unchanged after allometric scaling, $F =$

10.61, $p < .001$. CM increased change in FMD% from baseline by an average of 2.44, and ASI

increased change in FMD% from baseline by 2.48. Graphical plots of FMD% and corrected

FMD% change from pre-supplementation to post-supplementation are found in Figure 3 and

Figure 4, respectively. Statistical analysis found no significant interaction of supplement x time

for baseline diameter, maximum shear rate, and shear rate AUC to maximum. There was no

significant difference in protein consumption in the 24-hour period before each trial $F = 0.485$, p

$= .51$.

Results from the linear regression models and subsequent ANOVA test indicate that

FMD stimulus was not a confounding factor in the previously listed results. The post-supplement

placebo trial was the only linear regression model to significantly predict FMD%, $p = .01$,

although the pre-supplement placebo trial was approaching significance, $p = .07$. An ANOVA of

the regression-predicted values directly reflects the findings from the uncorrected and

allometrically scaled FMD% values; there was a significant supplement x time effect, $F = 11.88$,

$p = .001$, where CM and ASI supplementation resulted in a positive FMD% change compared to placebo, with no significant difference between the two. Graphical regression plots for all six groups and an aggregate plot are found in Figure 7 through Figure 13.

Discussion

The purpose of this study was to determine whether acute CM or ASI supplementation effected the NO producing capacity of endothelium dependent vasodilation as measured by change in flow-mediated dilation. While there are several studies examining the effect of CM on endothelium dependent vasodilation, results have thus far been inconclusive compared to the observed ergogenic effects on high intensity exercise. Meanwhile, the present study may be one of the first to examine the acute effects of ASI supplementation on endothelium dependent vasodilation as measured by FMD. Results from this study indicate that both 8g CM and 1.5g ASI, consumed in a flavored drink 60 minutes prior to testing, increase vascular endothelium relaxing factor production in response to FMD. Although CM and ASI increase serum arginine through slightly different mechanisms, both appear to be equally effective at increasing endothelium response to shear stress. By scaling the data to control for the bias introduced by baseline diameter variation, we can safely conclude that the observed changes were not unduly influenced by statistical flaws. Additionally, using linear regression models to predict FMD% from D_{Base}, shear rate$_{max}$, and shear rate$_{AUC}$ ensures that variance in the FMD stimulus did not predict the changes in FMD% between the supplements. Although, there is some evidence to suggest that correcting for shear rate may not be necessary when using allometrically scaled FMD% values (Atkinson, 2014).

It is difficult to precisely interpret the results from this study with respect to findings from previous research into CM supplement effects on FMD. Research has firmly established

that CM and ASI are both effective at increasing serum L-arginine levels following both acute

and continuous supplementation, and that increase is also associated with other improvements in

markers of cardiovascular function (Bailey et al., 2015; Figueroa et al., 2015; Kalman et al.,

2015; Komorowski et al., 2015). Increasing serum arginine also improves markers of

cardiovascular function and improving cardiovascular dynamics in studies of pathology in both

humans and animals (Giannesini et al., 2009; Orozco-Gutierrez et al., 2010; Proctor et al., 2007).

However, several studies that sought to increase serum arginine and subsequently test for

changes in FMD, have failed to find significant differences (Alvares et al., 2014; Figueroa et al.,

2017; Gates et al., 2007; Schwedhelm et al., 2008). While significant changes in FMD% may not

be the norm in supplement research, a relationship between ADMA, a nitric oxide pathway

inhibitor, arginine and FMD may exist. Specifically, increases in the serum arginine/ADMA

ratio trend toward an increase in FMD% (Alvares et al., 2014; Baum et al., 2016; Gates et al.,

2007; Haberka et al., 2009). Unfortunately, because no blood samples were analyzed in this

study, no further conclusions can be drawn about the arginine/ADMA ratio and its relation to

FMD. Additionally, differences in baseline serum arginine may explain a portion of the

variability in FMD results among L-arginine and L-citrulline studies. When baseline arginine

and FMD% are low, whether because of a lack in dietary protein or cardiovascular pathology,

supplementing to increase serum arginine improves FMD response and other cardiovascular

markers (Bai et al., 2009; Deveaux et al., 2016). Participants with differing cardiovascular

disease risk factors, in which differing serum arginine levels are observed, also respond

differently to increases in serum arginine, and thus experience differing FMD% improvements

(Thorne et al., 1998). Although baseline serum arginine levels were not tested in this study,

baseline arginine levels were likely consistent with population norms for the following reasons:

average protein consumption was above the minimum 0.8 g/kg bodyweight needed to maintain

normal serum levels, all participants reported high levels of physical activity, and none of the

participants met criteria for metabolic or cardiovascular disorders (Zieve, 1986).

Given the large number of variables that have the potential to influence endothelial

function, sample homogeneity is likely an essential factor in determining the presence of an

effect, especially given the small sample sizes in most supplement studies (Preli et al., 2002).

The sample recruited for the present study was almost identical in age, and all reported very high

levels of physical activity, both factors are crucially important in terms of observed FMD

response. Consequently, the supplement effects observed in this study may be limited to highly

trained young adults. Further investigation, specifically a comparison study of younger and older

athletes will be necessary to verify this possibility.

Results from this study support a somewhat novel finding that acute supplementation

with CM and ASI can improve endothelial-dependent vasodilation in trained young adults. Prior

to this study, there was no comparison of CM and ASI effects using FMD. This study supports

previous research findings that ASI may be a beneficial pre-workout supplement, and that a 1.5g

dose of ASI may be equally as effective at increasing endothelial response as a much larger 8g

dose of CM.

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Tables and Figures

Table 1.

Sample characteristics

	N	Height (cm)	Weight (kg)	Systolic BP	Diastolic BP
Male	15	181.7 ± 6.2	78.3 ± 16.5	125.6 ± 11.6	74.9 ± 10.5
Female	6	163.3 ± 3.5	61.8 ± 8.1	111.7 ± 11.4	71.7 ± 9.1

Table 2.

Means, standard deviations, and 2 x 3 repeated measures ANOVA results for all FMD relevant

variables

|	Supplement	Pre-Supplementation	60-minutes Post Supplemetation	ANOVA
Pre-occlusion Diameter (mm)	Citrulline Malate	3.84 ± 0.61	3.79 ± 0.62	$F = 0.834, p = .434$
	Nitrosigine	3.83 ± 0.48	3.78 ± 0.53	
	Placebo	3.74 ± 0.54	3.80 ± 0.52	
Post-occlusion Diameter (mm)	Citrulline Malate	4.12 ± 0.63	4.15 ± 0.64	$F = 8.18, p = .003$
	Nitrosigine	4.14 ± 0.49	4.18 ± 0.56	
	Placebo	4.03 ± 0.55	4.09 ± 0.52	
FMD %	Citrulline Malate	7.16 ± 2.44	9.60 ± 2.72	$F = 11.634, p < .001$
	Nitrosigine	8.00 ± 3.14	10.48 ± 2.64	
	Placebo	8.04 ± 2.90	7.85 ± 2.82	
Corrected FMD %	Citrulline Malate	7.03 ± 1.82	8.79 ± 2.01	$F = 10.61, p < .001$
	Nitrosigine	7.32 ± 2.01	9.18 ± 1.80	
	Placebo	7.72 ± 2.17	7.63 ± 2.07	
Hyperemic Shear Rate to Maximum AUC ($\int$ sec^{-1})	Citrulline Malate	167377.25 ± 179635.56	278367.58 ± 219709.37	$F = 1.33, p = .27$
	Nitrosigine	222580.81 ± 197033.56	264273.34 ± 196650.11	
	Placebo	317124.68 ± 205705.21	308761.67 ± 224332.36	
Maximum Shear Rate (sec^{-1})	Citrulline Malate	761.47 ± 314.66	777.34 ± 256.65	$F = 0.04, p = .91$
	Nitrosigine	793.56 ± 401.52	775.71 ± 287.35	
	Placebo	908.64 ± 490.64	910.30 ± 367.76	

Figure 1.

Box and whisker plot of the uncorrected FMD% change scores for citrulline malate, dextrose,

and Nitrosigine. X indicates the mean, and the horizontal line within the box indicates the
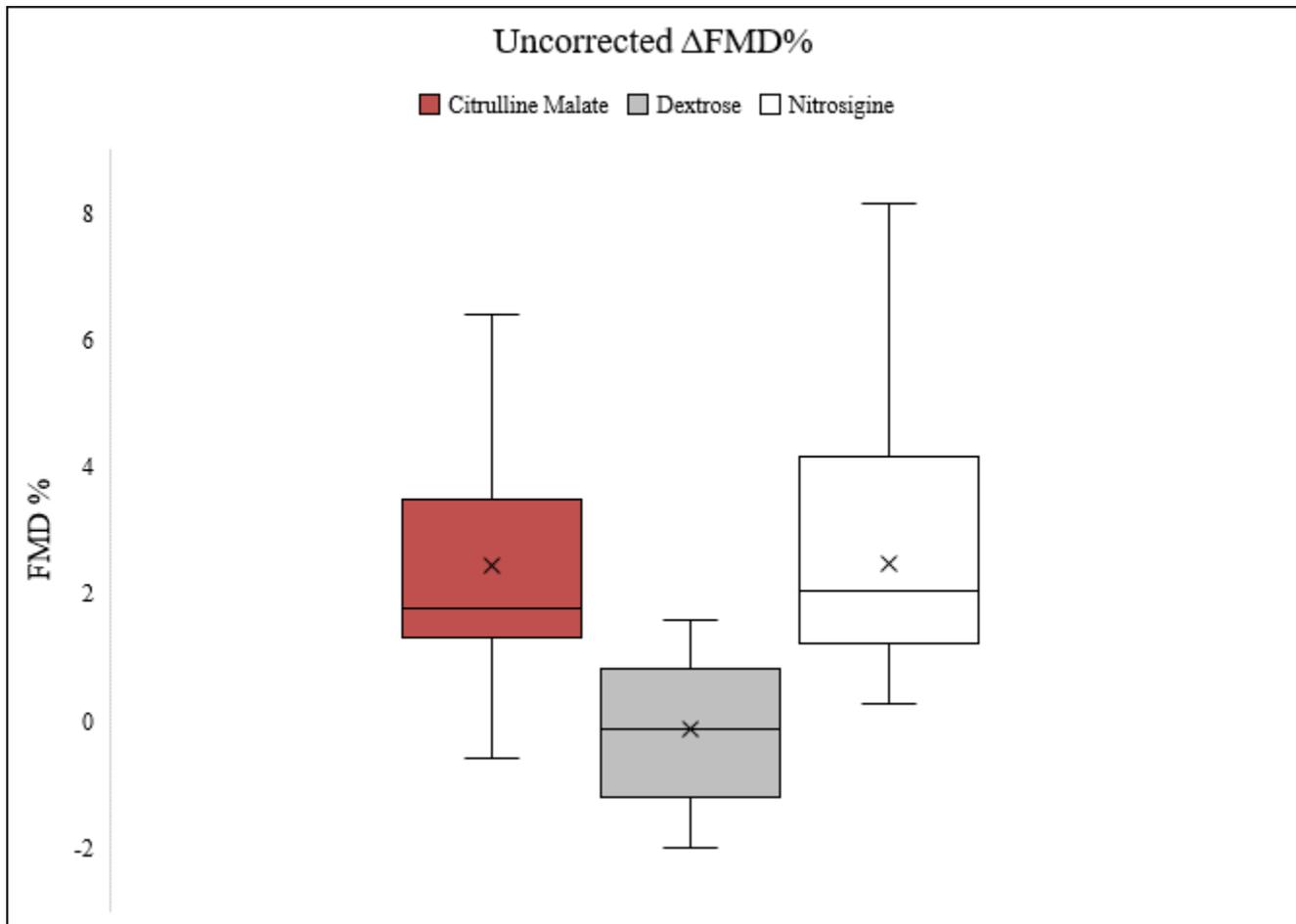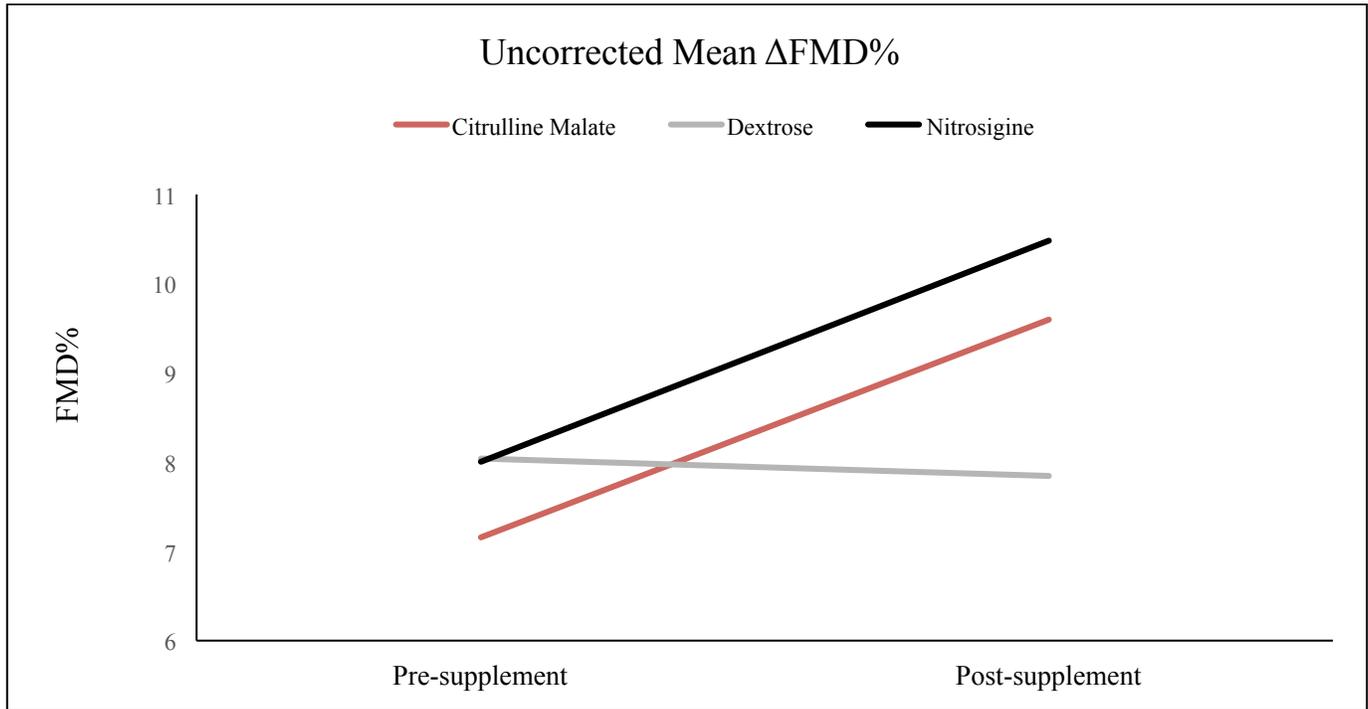
median

Figure 2.

Box and whisker plot of the FMD% change scores for citrulline malate, dextrose, and

Nitrosigine after allometric scaling. X indicates the mean, and the horizontal line within the box
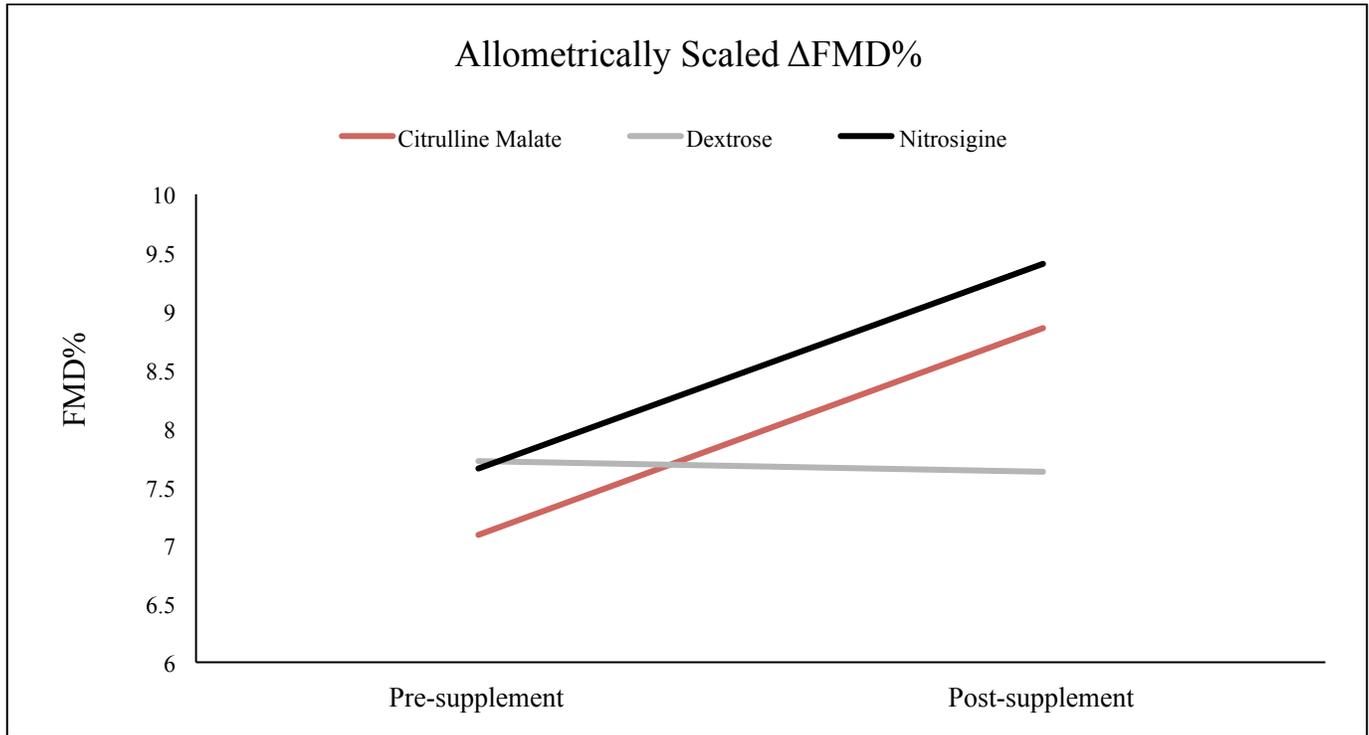
indicates the median
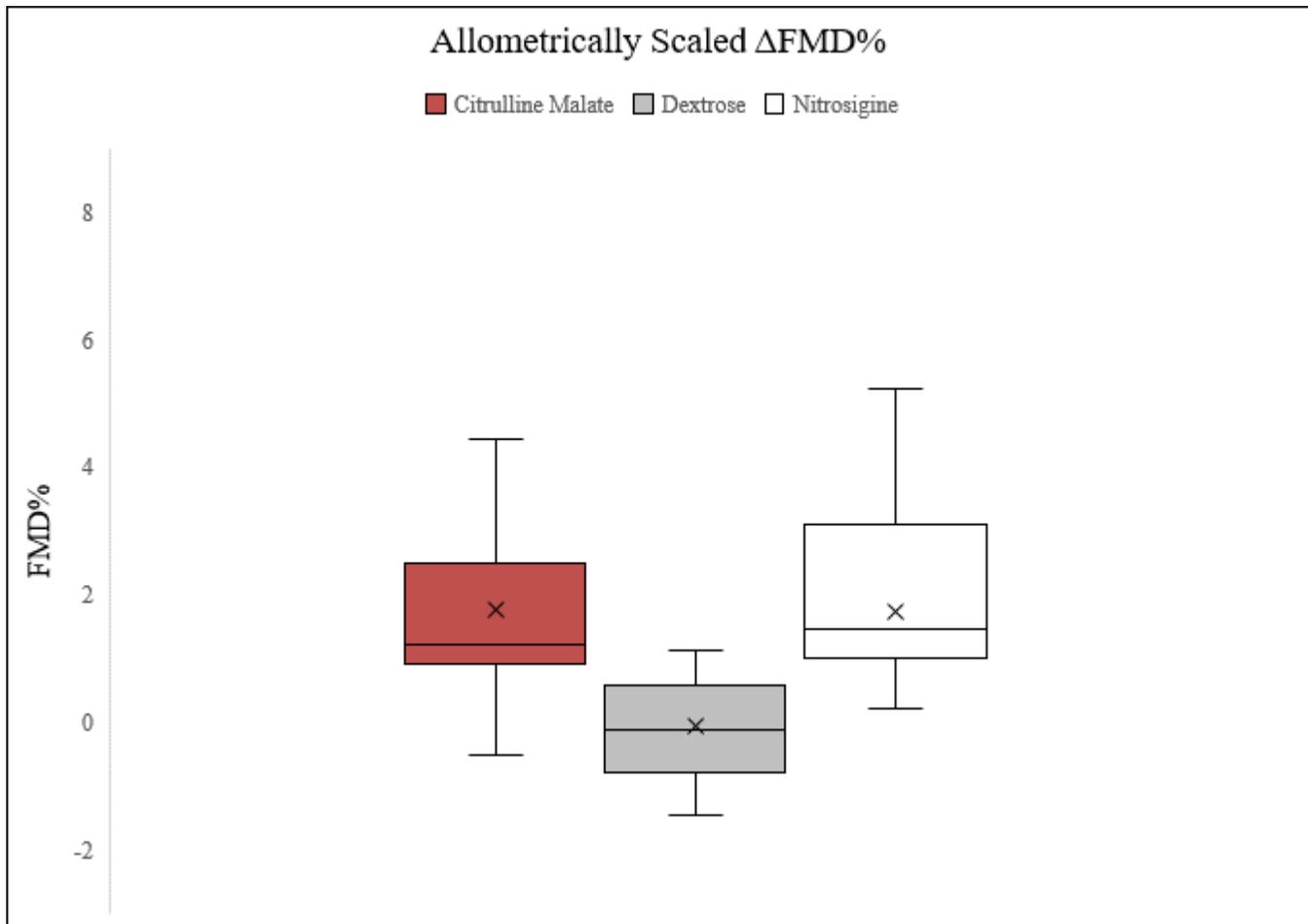
Figure 3.

Line plot indicating the slope of change from pre-supplementation to post-supplementation for

uncorrected FMD%

Uncorrected Mean ΔFMD%

Figure 4.

Line plot indicating the slope of change from pre-supplementation to post-supplementation for
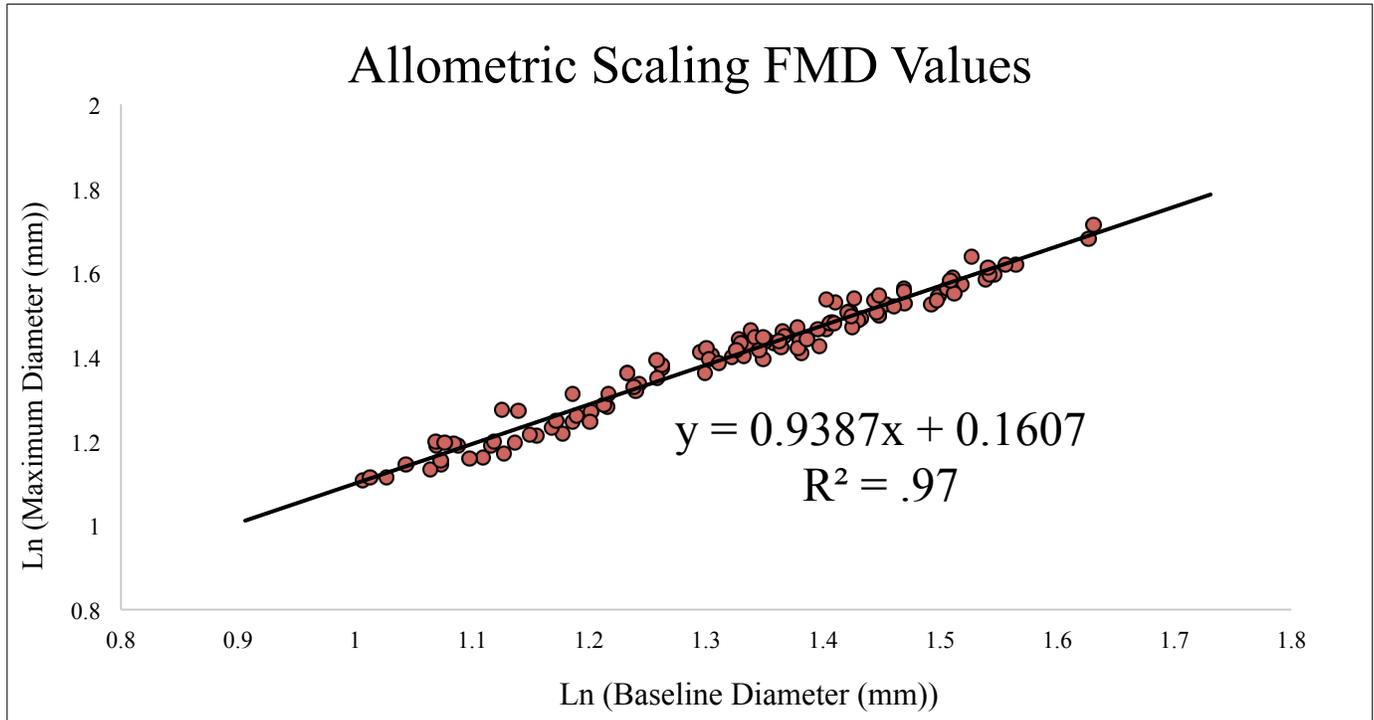
allometrically scaled FMD%

Figure 5.

Negative correlation relationship between baseline diameter and FMD%
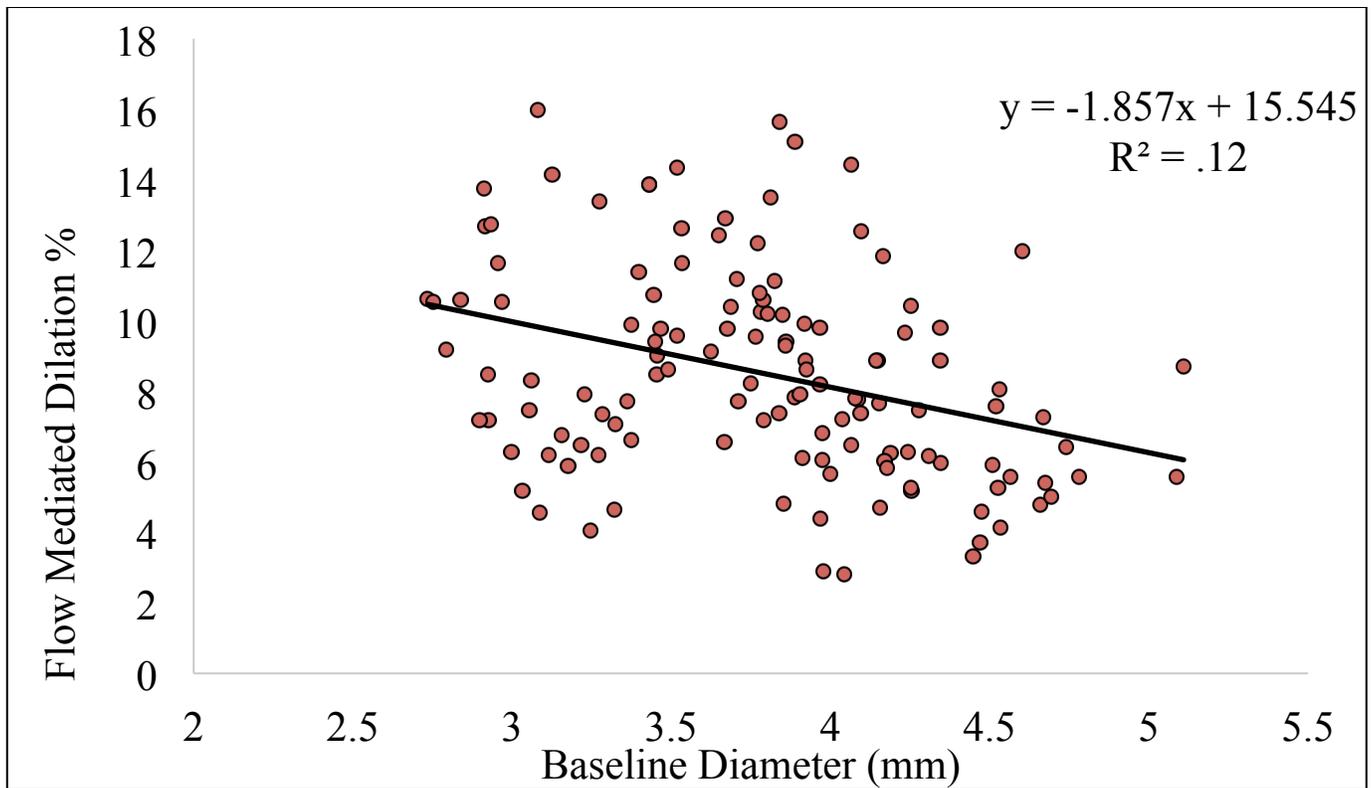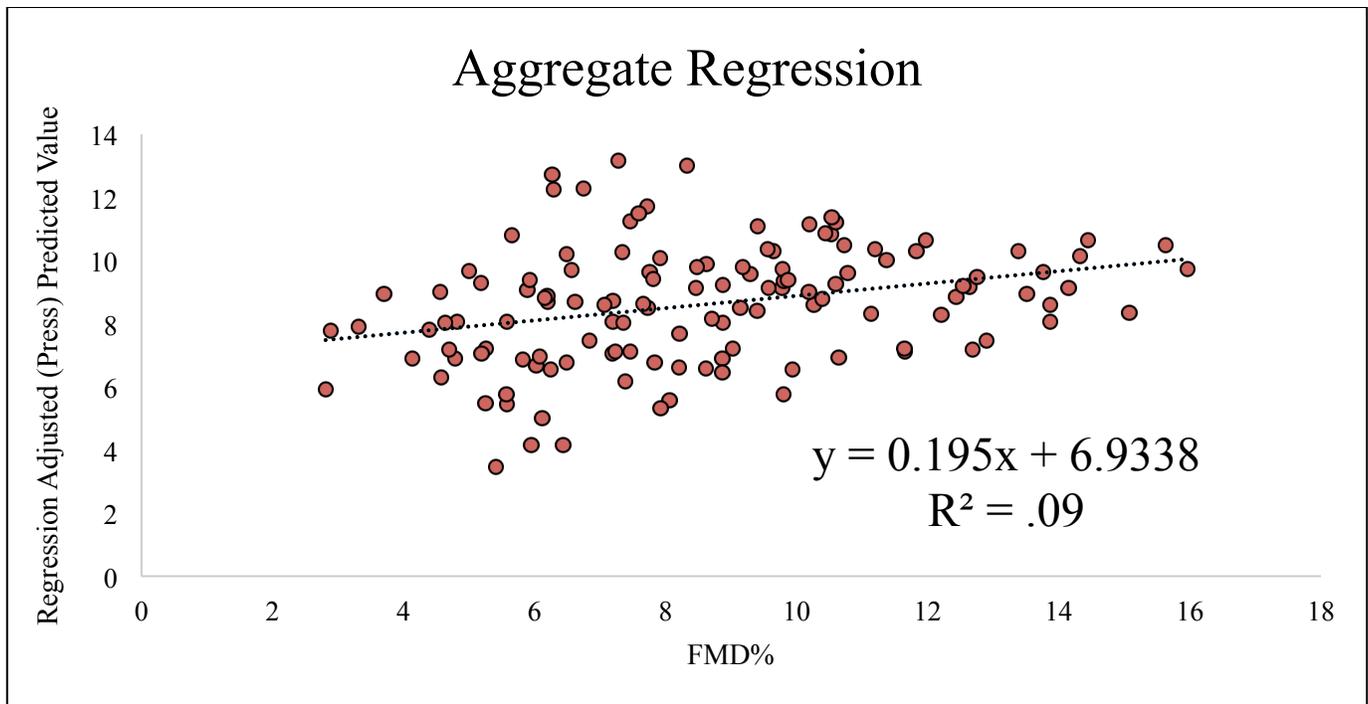
Figure 6.

Linear relationship between baseline diameter and peak diameter that produces the exponent
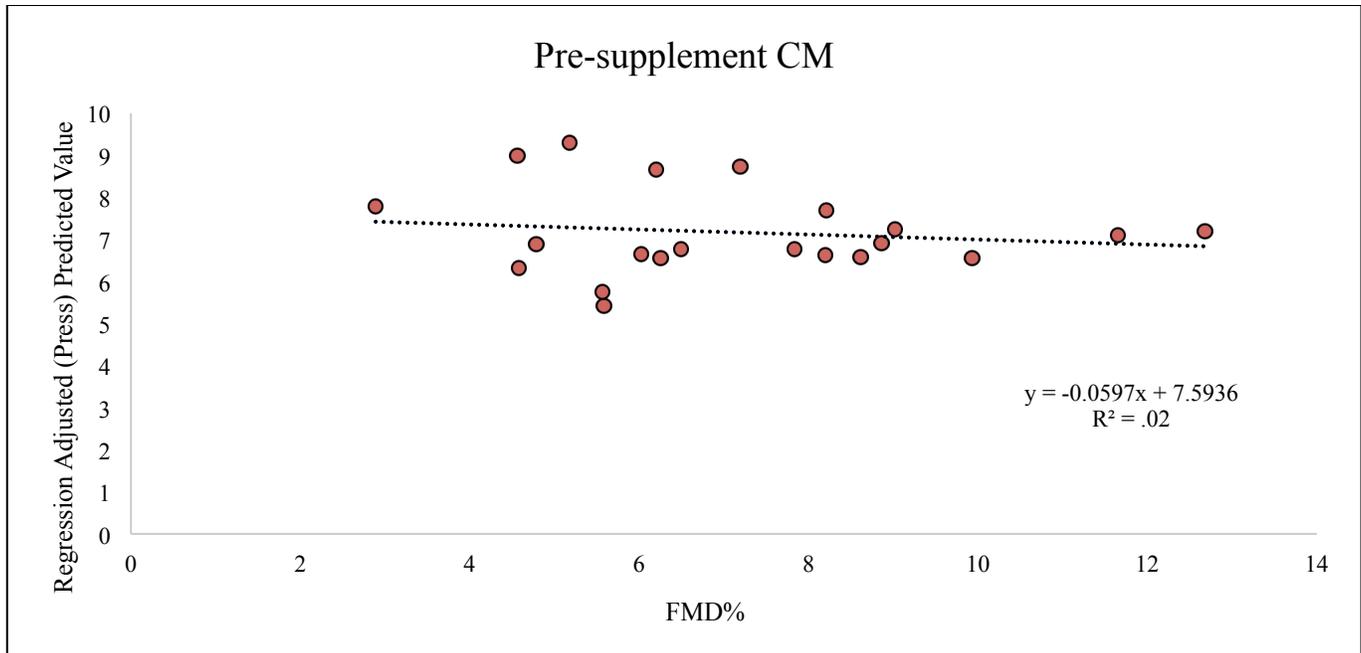
used in allometric scaling

Allometric Scaling FMD Values

$y = 0.9387x + 0.1607$
$R^2 = .97$

Ln (Maximum Diameter (mm))

Ln (Baseline Diameter (mm))

Figure 7.

Aggregate plot of linear regression adjusted FMD% values

Aggregate Regression

$y = 0.195x + 6.9338$

$R^2 = .09$

Figure 8.

Plot of linear regression adjusted FMD% values

Pre-supplement CM

$y = -0.0597x + 7.5936$
$R^2 = .02$

Figure 9.

Plot of linear regression adjusted FMD% values

Post-supplement CM

$$y = -0.1348x + 10.997$$
$$R^2 = .11$$
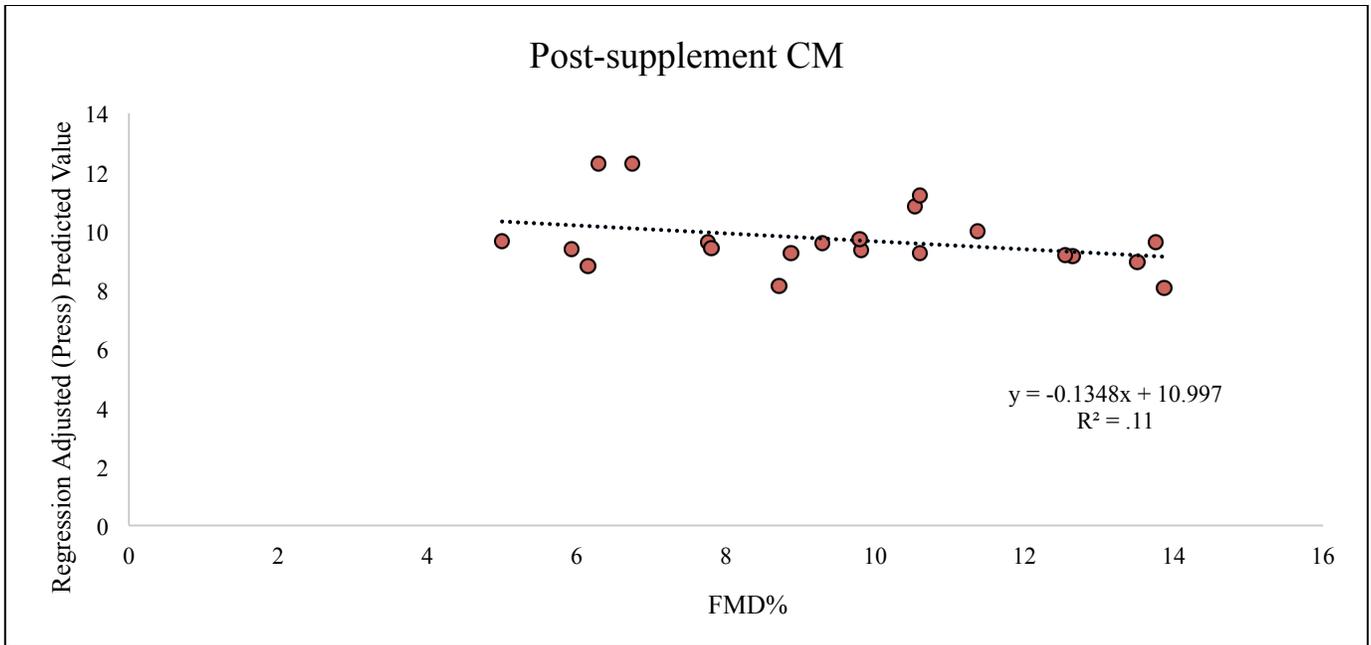
Figure 10.

Plot of linear regression adjusted FMD% values

Pre-supplement Dextrose

$y = 0.1648x + 6.7628$
$R^2 = .07$
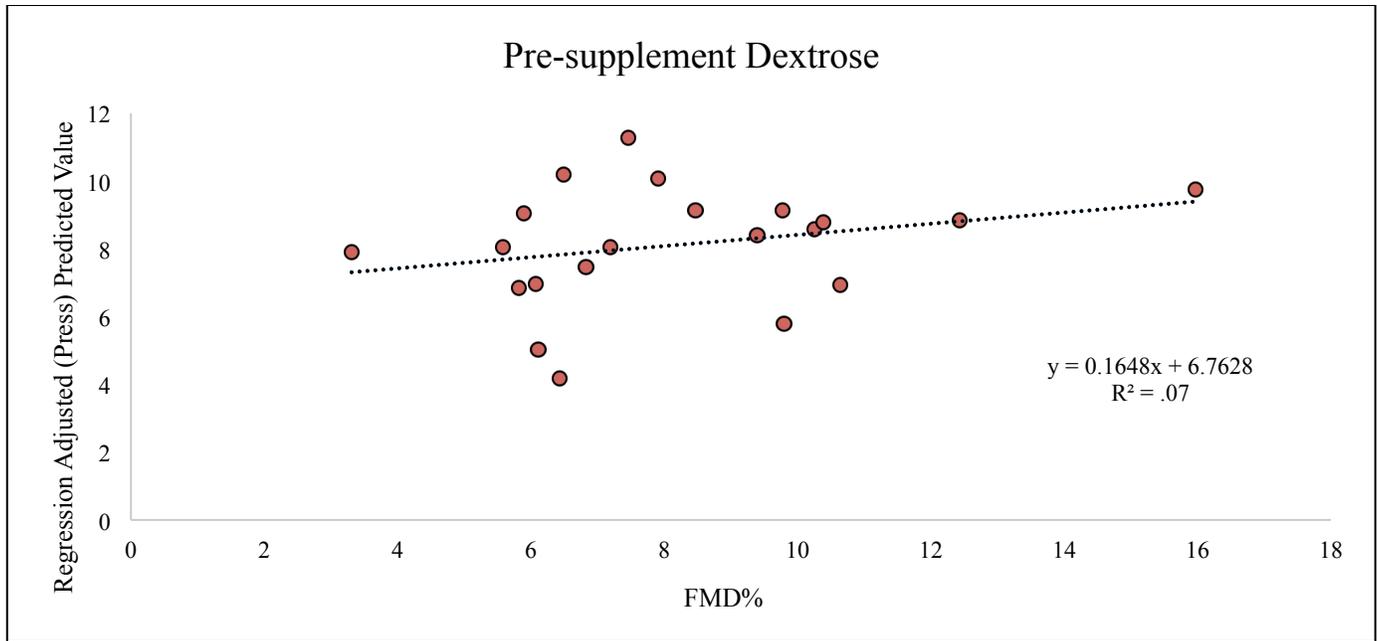
Figure 11.

Plot of linear regression adjusted FMD% values

Post-supplement Dextrose

$y = 0.3273x + 5.4771$
$R^2 = .18$
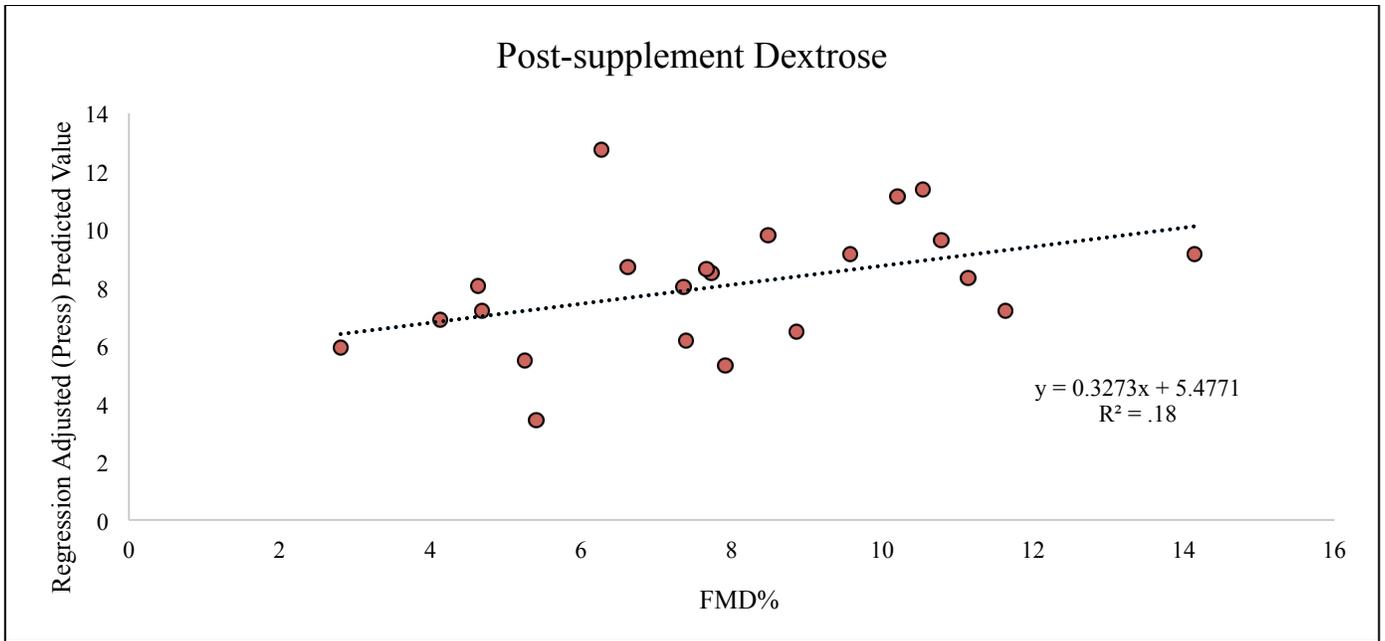
Figure 12.

Plot of linear regression adjusted FMD% values

Pre-supplement Nitrosigine

$y = 0.0733x + 7.4787$
$R^2 = .02$

Y-axis: Regression Adjusted (Press) Predicted Value

X-axis: FMD%
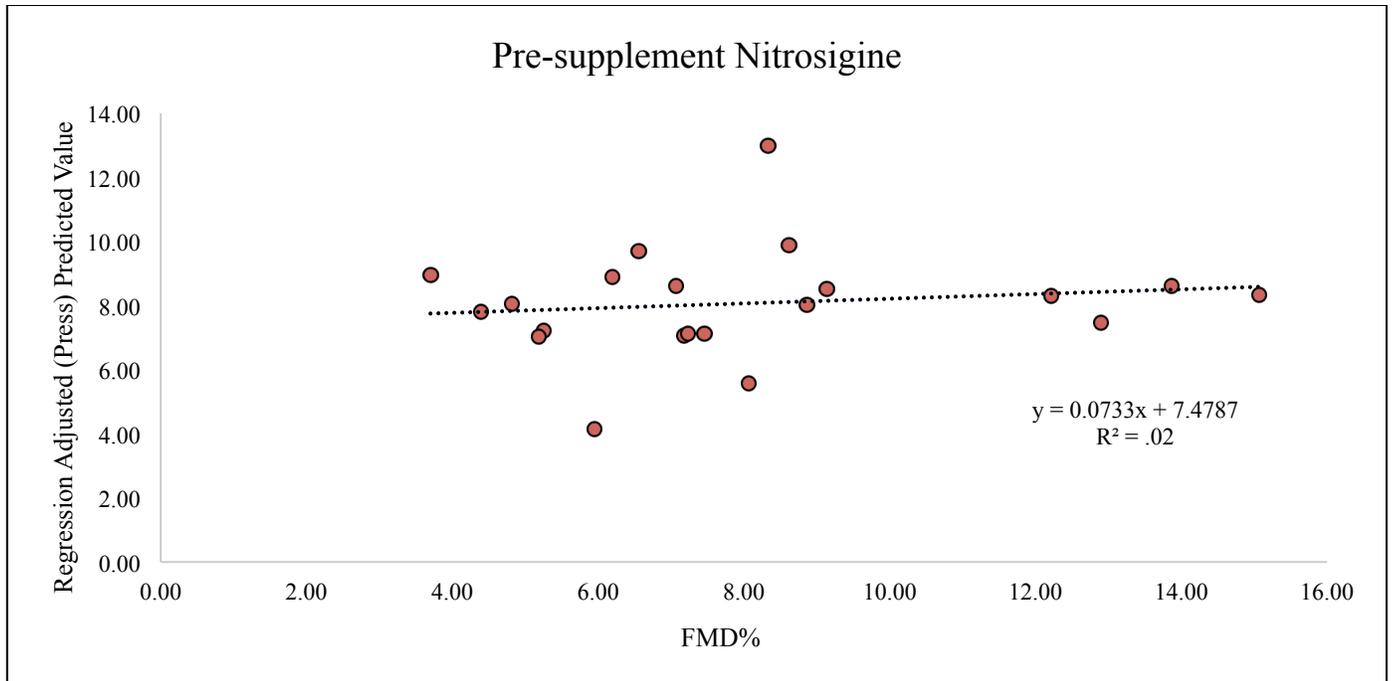
Figure 13.

Plot of linear regression adjusted FMD% values