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# The Effect of creatine supplementation on body composition and total body water measured by multi-frequency bioelectrical impedance

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# The Effect of Creatine Supplementation on Body Composition and Total Body Water Measured by Multi-frequency Bioelectrical Impedance

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## Abstract

**Background:** Acute fluid ingestion causes an increase in estimated body fat percentage (BF%) measurements by single frequency (SF-BIA) and multi-frequency bioelectrical impedance (MF-BIA). However, it is unknown if MF-BIA accurately measures total body water (TBW) and BF% after chronic fluid retention. Creatine supplementation causes fluid retention, and resultant increases in TBW and body mass. Research is needed to determine if MF-BIA is capable of detecting fluid retention secondary to creatine supplementation. **Methods:** 13 male and 14 female subjects (18-22 y) completed one week of creatine monohydrate or maltodextrin supplementation at a dose of 0.3 g/kg body weight. Subjects completed pre-supplementation and post-supplementation measurements of body composition including dual-energy x-ray absorptiometry (DEXA), SF-BIA, and MF-BIA to measure BF%, fat free mass (FFM), and fat mass (FM). Additionally, intracellular water (ICW), extracellular water (ECW), and TBW were estimated by MF- BIA. **Results:** The creatine group had a 2% increase ($p < 0.05$) in TBW between pre- and post-supplementation measured by MF-BIA ($40.4 \pm 9.5$ to $41.2 \pm 9.6$ kg). FFM increased significantly more in the creatine group compared to the placebo group measured by all body composition modes (1.2 kg, 1.9 kg, and 1.1 kg increase in the creatine group measured by SF-BIA, MF-BIA, and DEXA respectively). **Conclusions:** One week of creatine supplementation caused an increase in TBW that was detected by MF-BIA. Changes in body composition that occurred due to the increase in TBW were detected as an increase in FFM measured by SF-BIA, MF-BIA, and DEXA.

# Chapter I

## Introduction

Body composition describes the absolute and relative amounts of FM and FFM (bone, muscle, connective tissue, water). Assessment of body composition is useful for general health, sport and fitness evaluation, and to track progress secondary to a dietary and/or exercise intervention. Excess FM is a major risk factor for many chronic diseases such as cardiovascular disease and type 2 diabetes.[1] Furthermore, obesity is a risk factor for both cardiovascular disease and type 2 diabetes development due to impaired glycemic control from extra adiposity.[1-4] Thus, body composition assessment can be used as a tool for tracking FM loss and BF% which can aid in assessment of risk for chronic disease. Body composition can also be used to track changes in FM and FFM after training or dietary interventions to assess whether weight reductions are due to decreased FM and weight increases are due to increased FFM.[5]

There are many ways to measure body composition, including skin folds, hydrostatic weighing, air displacement plethysmography, DEXA, and bioelectrical impedance analysis (BIA). These methods have varying reliability and validity, with DEXA being the current gold standard.[6] However, DEXA devices are very expensive (>$100k) and are generally only found in medical and research facilities, which limits access to this method. By contrast, BIA, either SF-BIA or MF-BIA, is a more readily available assessment that sends electrical currents of varying frequencies through the body via contact points with electrodes to measure impedance. Resistance to the electrical current changes depending on body composition, as current resistance is much lower for water and muscle as compared to fat mass.[7] SF-BIA devices send one

frequency (~50 kHz) of electrical current and generally utilize fewer contact points with the body (i.e., hand to hand, foot to foot, or hand to foot). Therefore, this current travels only through limited areas, leaving the rest of the body's composition to be estimated. In contrast, MF-BIA utilizes multiple frequencies to differentiate between FM and FFM more accurately, as well as generally using a greater number of contact points with the body (i.e., both hands and feet). SF-BIA currents are only able to pass through ECW, while the multiple frequencies in a MF-BIA device are able to assess both ICW and ECW.[6]

It has been found that in young, active individuals, SF-BIA underestimates BF% compared to DEXA, but may be used in tightly controlled trials to track individual changes in body composition over time.[8-12] However, SF-BIA is affected by hydration status, and consistently overestimates FM and BF% after acute fluid consumption.[7,13-16] The InBody Composition Analyzer is a MF-BIA device that claims to give a comprehensive assessment of body composition while overcoming previous limitations from SF-BIA devices. Few studies have specifically investigated the validity of InBody BIA devices, although Miller et al observed a very high validity coefficient (r=0.94) for an InBody device against DEXA.[17] Despite this strong correlation, average BF% determined by InBody (20.99 ± 9.34%) was significantly lower than DEXA (25.61 ± 10.56%).[17] InBody devices have been found to be reliable, as they produce small individual errors when measurements are taken 24-72 hours apart.[18] However, InBody was found to overestimate FFM, and underestimate BF% and FM compared to DEXA in both males and females.[18] It has also been noted that these inaccuracies tend to be more profound in individuals with FM/FFM values that differ to a larger extent from average

values.[18] These data suggest that InBody devices could be used under specific circumstances to measure body composition due to the small individual error, but the possibility for over/underestimations should be noted.

For measurements of TBW, MF-BIA is thought to be more accurate than SF-BIA especially within different compartments due to the varying frequencies used.[6] A frequency of 50 kHz typically used by SF-BIA can travel through ECW, but may penetrate cell membranes only to a certain degree.[6,19-20] The uncertainty that comes with this measure of ICW is mitigated through the use of MF-BIA devices which not only include additional contact points, but also send multiple different frequencies through the body. MF-BIA typically uses frequencies <50 kHz to measure ECW only, with additional higher frequencies that measure TBW to find ICW as the difference between the two.[6] Therefore, MF-BIA may be able to measure segmental body composition and TBW more accurately compared to SF-BIA.[6] In a meta-analysis by Martinoli et al, it was concluded that MF-BIA accurately estimated TBW while SF-BIA overestimated TBW, as compared to reference values from deuterium oxide dilution ($D_2O$).[21] Conversely, Anderson et al observed that MF-BIA significantly overestimated TBW in both men and women by 5 kg and 4 kg respectively compared to $D_2O$, though high reliability coefficients were observed.[22]

Numerous studies have investigated the effects of varied hydration states on the accuracy of SF-BIA measurements of FM and BF%, but research is lacking regarding MF-BIA. An overestimation of FM was observed following super-hydration using a Tanita single-frequency analyzer compared to baseline measurements in both males and females, suggesting that acute water consumption may cause an overestimation of body

fat percentage when using SF-BIA devices.[15] Similar results have been found when comparing SF-BIA to underwater weighing in studies where subjects underwent exercise-induced fluid loss with subsequent re-hydration.[14,23] All SF-BIA assessments overestimated BF% after fluid consumption and underestimated BF% post-exercise indicating that in these devices, BF% increased or decreased in concert with body water changes.[14] Additionally, during the sequence of euhydration, hypohydration, and re-hydration, SF devices reflect the resulting fluid changes as alterations of FM with resulting changes in BF% (14.4 ± 5.3%, 12.3 ± 5.3%, and 15.5 ± 5.8% respectively).[23] However, because MF-BIA provides additional points of contact with the body that emit multiple frequencies, it is likely more accurate in estimating the content of fluid compartments compared to SF-BIA.[18] Unpublished research from our laboratory assessed body composition with MF-BIA before and immediately after super-hydration. Subjects were given one hour to intake 2 L of fluid before being re-tested and it was found that MF-BIA incorrectly categorized the extra fluid as FM. It is unknown whether long-term, gradual changes in water retention and TBW would be accurately reflected in a MF-BIA measurement. Theoretically, this water retention and subsequent increase in body mass should decrease resistance of the current, and therefore result in changes in FFM.

One way to induce these longer-term adjustments in water retention and TBW is through creatine supplementation. Creatine is a common supplement used to improve short duration, high intensity exercise performance. Increasing muscle creatine stores allows for quicker phosphocreatine resynthesis and therefore ATP production.[24] It is well known that even short-term creatine supplementation causes water retention, which increases TBW and body mass.[24-31] This is likely due to increases in ICW rather than

ECW as increasing muscle creatine changes the osmotic pressure of the intracellular fluid, causing water to follow the osmotically active particles and move into the cell.[26,28,32-33] Therefore, creatine causes water retention in and around the muscle. MF-BIA, and InBody specifically, measures these different fluid compartments and should be able to differentiate this increase in TBW from FM. However, SF-BIA tends to overestimate FM and BF%, especially in super-hydrated states and no research on the effect of creatine supplementation on measures of TBW and BF% has been done using MF-BIA.[23] Therefore, research is needed to determine if InBody devices are capable of accurately categorizing increases in TBW from creatine supplementation.

The InBody analyzer may be able to measure chronic fluid retention secondary to creatine supplementation since the added water will not be in the GI tract, as it would from acute fluid consumption. The purpose of this study is to analyze the effect of creatine supplementation on body composition and TBW measured through MF-BIA. It is hypothesized that creatine supplementation will result in increased FFM measurements through increasing water retention in the intracellular compartment that will be detected by MF-BIA. Learning how accurately InBody can measure TBW after water retention occurs can increase the efficacy of using these measurements, especially for those who regularly supplement with creatine and track body composition measurements.

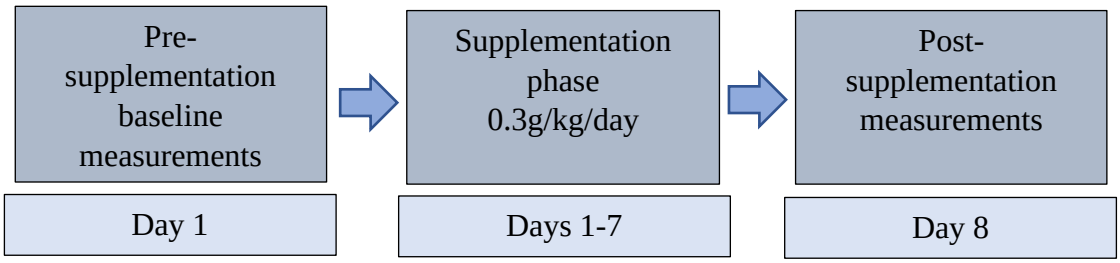
# Chapter II

## Methodology

*Subjects*

32 males and 32 females will be recruited for the study with the following inclusion criteria: 1) aged 18-35 y, 2) participate in exercise designed to increase muscular fitness at least twice per week, and 3) no use of a creatine supplement for at least one month. Subject recruitment will be conducted via word of mouth amongst the Kinesiology Department. Informed consent will be obtained from all participants prior to study participation. All protocols/procedures will be approved by James Madison University's Institutional Review Board prior to the initiation of the study.

*Procedures*




Body composition measurements of all subjects will be taken before the supplementation period begins, and after the supplementation phase. For all measurements, subjects will be asked to come to the lab in the morning after an overnight fast with no fluid consumption. Subjects will also have abstained from alcohol, exercise, and caffeine during the 12 hours prior to testing and will be asked to drink a glass of water 45 minutes before testing.[7] Each testing session will include a DEXA scan using

GE Encore software (GE Lunar; Chicago, Illinois), SF-BIA (OMRON hand-held BIA device; Kyoto, Japan), and MF-BIA (InBodyUSA 770, Seoul, South Korea) to measure BF%, FFM, and FM. Additionally, ICW, ECW, and TBW will be estimated by MF- BIA. Procedures for DEXA and MF-BIA will be completed as described by Schoenfeld et al.[6] Procedures for SF-BIA will begin with calculating subjects' fit index and entering their personal information. They will then be instructed to correctly hold the device with their palms in contact with the electrodes for the duration of the measurement.

Subjects will be split into a creatine supplementation group and a placebo group, each consisting of 16 males and 16 females. The supplementation phase will consist of creatine (Creapure; Trostberg, Germany) or maltodextrin at a dose of 0.3 g of creatine monohydrate per kg of bodyweight administered in a double-blind manner. To allow for an optimal loading phase, creatine dosage will be based on subject bodyweight to allow necessary increases in tissue creatine concentration.[32,34] After baseline measurements are taken, all subjects will be instructed to report to the Human Performance Lab in Godwin Hall every day for all 7 days of the supplementation period to retrieve their dose and return the empty bag from the previous day to track adherence. All subjects will be instructed to mix their supplement with water or other liquids and consume quickly. After the supplementation phase is complete, subjects will be re-tested following the same procedures described previously.

Through the duration of the experiment, subjects will be instructed to maintain their normal dietary habits, fluid consumption, and training. Special emphasis will be placed on subjects' maintaining their normal carbohydrate intake as that may increase fluid retention.[26]

*Statistical Analyses*

A repeated measures analysis of variance will be used to determine changes in dependent measures (BF%, FFM, and FM). Within-subject factors in the model will include body composition methods (DEXA, MF-BIA, and SF-BIA) and creatine supplementation phase (pre vs. post), with supplementation groups and biological sex as between-subject factors. Another repeated measures analysis of variance will be used to compare ICW, ECW, and TBW as measured by MF-BIA with supplementation phase as the within-subjects factor and supplementation groups and biological sex as between-subject factors. Post-hoc testing will be performed using paired t-tests with a Bonferroni correction. The alpha level for all tests will be set at 0.05.

# Chapter III

## Manuscript

## The Effect of Creatine Supplementation on Body Composition and Total Body Water Measured by Multi-frequency Bioelectrical Impedance

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## Abstract

**Background:** Acute fluid ingestion causes an increase in estimated body fat percentage (BF%) measurements by single frequency (SF-BIA) and multi-frequency bioelectrical impedance (MF-BIA). However, it is unknown if MF-BIA accurately measures total body water (TBW) and BF% after chronic fluid retention. Creatine supplementation causes fluid retention, and resultant increases in TBW and body mass. Research is needed to determine if MF-BIA is capable of detecting fluid retention secondary to creatine supplementation. **Methods:** 13 male and 14 female subjects (18-22 y) completed one week of creatine monohydrate or maltodextrin supplementation at a dose of 0.3 g/kg body weight. Subjects completed pre-supplementation and post-supplementation measurements of body composition including dual-energy x-ray absorptiometry (DEXA), SF-BIA, and MF-BIA to measure BF%, fat free mass (FFM), and fat mass (FM). Additionally, intracellular water (ICW), extracellular water (ECW), and TBW were estimated by MF- BIA. **Results:** The creatine group had a 2% increase ($p < 0.05$) in TBW between pre- and post-supplementation measured by MF-BIA ($40.4 \pm 9.5$ to $41.2 \pm 9.6$ kg). FFM increased significantly more in the creatine group compared to the placebo group measured by all body composition modes (1.2 kg, 1.9 kg, and 1.1 kg increase in the creatine group measured by SF-BIA, MF-BIA, and DEXA respectively). **Conclusions:** One week of creatine supplementation caused an increase in TBW that was detected by MF-BIA. Changes in body composition that occurred due to the increase in TBW were detected as an increase in FFM measured by SF-BIA, MF-BIA, and DEXA.

## Introduction

Body composition describes the absolute and relative amounts of FM and FFM (bone, muscle, connective tissue, water). Assessment of body composition is useful for general health, for sport and fitness evaluation, and to track progress secondary to a dietary and/or exercise intervention.[1] Though DEXA is the current gold standard for measuring body composition, these devices are very expensive (>$100k) with limited access. By contrast, bioelectrical impedance (BIA), either SF-BIA or MF-BIA, is a more readily available assessment that may be able to accurately predict FFM and BF%.[2-3]

The InBody Composition Analyzer is a MF-BIA device that claims to give a comprehensive assessment of body composition while overcoming previous limitations from SF-BIA devices. InBody devices have been found to be reliable, as they produce small individual errors when measurements are taken 24-72 hours apart.[4] However, InBody has been found to overestimate FFM, and underestimate BF% and FM compared to DEXA in both males and females. For measurements of TBW, MF-BIA is thought to be more accurate than SF-BIA especially within different compartments due to the varying frequencies used.[5] In a meta-analysis by Martinoli et al, it was concluded that MF-BIA accurately estimated TBW while SF-BIA overestimated TBW, as compared to reference values from deuterium oxide dilution ($D_2O$).[6] Conversely, Anderson et al observed that MF-BIA significantly overestimated TBW in both men and women compared to $D_2O$, though high reliability coefficients were observed.[7]

SF-BIA is affected by hydration status, and consistently overestimates FM and BF% after acute fluid consumption.[8-12] Similarly, FM and BF% are underestimated in studies where subjects underwent exercise-induced fluid loss with subsequent re-

hydration.[9,13] However, because MF-BIA utilizes multiple frequencies, allowing for the differentiation of ICW and ECW, it may be accurate in estimating the content of fluid compartments compared to SF-BIA.[4] Recent unpublished observations from our laboratory suggest that acute fluid ingestion incorrectly increases FM as measured by MF-BIA. However, it is unknown whether long-term, gradual changes in water retention and TBW would be accurately reflected in a MF-BIA measurement. Theoretically, this water retention and subsequent increase in body mass should decrease resistance of the current, and therefore reflect increases in FFM.

Creatine supplementation causes water retention by changing the osmotic pressure of the ICW, increasing TBW and body mass.[14-23] MF-BIA measures these different fluid compartments and theoretically should be able to differentiate this increase in TBW from FM. However, little research on the effect of creatine supplementation on measures of TBW and BF% has been done using MF-BIA. The purpose of this study is to analyze the effect of creatine supplementation on body composition and TBW measured through MF-BIA.

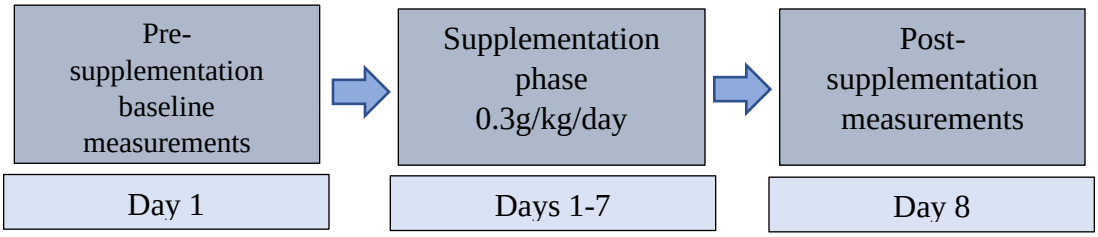
## Methodology

### *Subjects*

27 subjects (N = 14 females, N = 13 males) were recruited for the study with the following inclusion criteria: 1) aged 18-35 y, 2) participate in exercise designed to increase muscular fitness at least twice per week, and 3) no use of a creatine supplement for at least one month (see Table 1 for demographics). Subjects were excluded if they had any known pregnancy or cardiovascular, metabolic, renal, or liver disease. Subjects were

recruited via word of mouth amongst the Kinesiology Department at James Madison University. Informed consent was obtained from all participants prior to study participation. All protocols/procedures were approved by James Madison University's Institutional Review Board prior to the initiation of the study.

P*rocedures*




Body composition measurements of all subjects were taken before the supplementation period began, and after the supplementation phase. For all measurements, subjects were asked to come to the lab in the morning after an overnight fast with controlled fluid consumption as described below. Subjects were also instructed to abstain from alcohol, exercise, and caffeine during the 12 hours prior to testing and were asked to drink a glass of water 45 minutes before testing.[11] Each testing session included a DEXA scan using GE Encore software (GE Lunar; Chicago, Illinois), SF-BIA (OMRON hand-held BIA device; Kyoto, Japan), and MF-BIA (InBodyUSA 770, Seoul, South Korea) to measure BF%, FFM, and FM. Additionally, ICW, ECW, and TBW were estimated by MF- BIA. Procedures for DEXA and MF-BIA were completed as described by Schoenfeld et al.[5] Procedures for SF-BIA began with calculating subjects' fit index and entering their personal information. They were then instructed to correctly hold the device with their palms in contact with the electrodes for the duration of the measurement.

Subjects were randomly assigned to either the creatine or placebo group in a double-blind manner. The supplementation phase consisted of creatine (Creapure; Trostberg, Germany) or maltodextrin at a dose of 0.3 g of creatine monohydrate per kg of bodyweight. To allow for an optimal loading phase, creatine dosage was based on subject bodyweight to allow necessary increases in tissue creatine concentration.[20,24] After baseline measurements were taken, all subjects were instructed to report to the Human Performance Lab in Godwin Hall every day for all seven days of the supplementation period to retrieve their dose and return their empty bag from the previous day to track adherence. All subjects were instructed to mix their supplement with water or other liquids and consume quickly. After the supplementation phase was complete, subjects were re-tested following the same procedures described previously.

Through the duration of the experiment, subjects were instructed to maintain their normal dietary habits, fluid consumption, and training. Special emphasis was placed on subjects' maintaining their normal carbohydrate intake as that may increase fluid retention.[16]

*Statistical Analyses*

A repeated measures analysis of variance was used to determine changes in dependent measures (BF%, FFM, and FM). Within-subject factors included body composition methods (DEXA, MF-BIA, and SF-BIA) and creatine supplementation phase (pre vs. post), with supplementation group as the between-subject factor. Another repeated measures analysis of variance was used to compare ICW, ECW, and TBW as measured by MF-BIA with supplementation phase as the within-subject factor and

supplementation group as the between-subject factor. Post-hoc testing was performed using contrasts via SPSS statistical software. The alpha level for all tests was set at 0.05.

**Results**

29 subjects were recruited and consented to participate in the study. One male subject was excluded due to non-compliance, and one female subject was excluded due to excessive water ingestion prior to post-supplementation measurements. Another male subject was excluded only from the body composition testing because of the inability to obtain a measurement using SF-BIA. The data from 27 subjects (13 males, 14 females) was analyzed (Table 1) and BF%, FM, and FFM were assessed using DEXA, MF-BIA, and SF-BIA.

Analyses of FFM revealed a time x supplement interaction ($p < 0.05$), as well as main effects for time (Post > Pre) and mode (DEXA < MF-BIA < SF-BIA) of assessment ($p < 0.05$; Table 2). Post-hoc analyses revealed greater increases in FFM in the creatine group compared to the placebo group for all modes of assessment ($p < 0.05$; Table 2). Analyses of FM revealed a main effect for mode of assessment (DEXA > MF-BIA > SF-BIA; $p < 0.05$), but no supplement x time interaction ($p > 0.05$; Table 3). Analyses of BF% revealed main effects for both time (Post < Pre; $p < 0.05$) and mode of assessment (DEXA > MF-BIA > SF-BIA; $p < 0.05$), but no supplement x time interaction ($p > 0.05$; Table 4).

TBW, ICW, and ECW were measured by MF-BIA pre- and post-supplementation (Table 5). For ICW, there was a main effect for time (Post > Pre, $p < 0.05$) with a trend for a supplement x time interaction with larger increases in the creatine group ($p = 0.07$).

For TBW, there was a main effect for time (Post > Pre, $p < 0.05$) with a significant supplement x time interaction with larger increases in the creatine group. No statistically significant differences were found for ECW measurements, though there was a trend ($p = 0.06$) for a supplement x time interaction with larger increases in the creatine group compared to the placebo group (Table 5).

**Discussion**

This study examined creatine supplementation on body composition and TBW measured by MF-BIA. It was found that one week of creatine monohydrate supplementation caused a 2% increase in TBW as detected by MF-BIA. Furthermore, this increase in TBW in the creatine group corresponded to an increase in FFM measured by DEXA, SF-BIA, and MF-BIA. Though $D_2O$ was not used as a criterion measure of TBW, it is well established that creatine supplementation increases water retention in the intracellular compartment.[20]

In the present study, each body composition mode detected a significant increase in FFM after 7 days of creatine monohydrate supplementation (DEXA = +1.1%, SF-BIA = +1.2%, MF-BIA = +1.9%). Similarly, it has been found that a nine-week creatine supplementation period caused a 3.8% increase in lean body mass measured through hydrodensitometry.[15] Though this supplementation period was longer and included a training protocol, the results are consistent with the present study where creatine supplementation causes an increase in FFM. Other studies that also included training protocols coupled with creatine supplementation have found similar results with a significant increase in FFM compared to placebo.[14,17,19,21] It is difficult to distinguish if these previously reported changes in FFM were due to the training intervention or the

creatine supplementation. However, as the present study did not include a training protocol, it is likely that some increases in FFM can be attributed to the water retention secondary to the supplementation.

It has been previously found that acute fluid ingestion causes an increase in BF% measured by both SF-BIA and MF-BIA.[9,10,13] The present study, however, aimed to see if chronic fluid retention through creatine supplementation could be accurately detected via MF-BIA. No significant differences in FM were found between pre- and post-supplementation, whereas BF% decreased post-supplementation regardless of supplementation group. As DEXA also did not detect an increase in FM or BF% in the creatine group, it appears that MF-BIA accurately detected increases in TBW from chronic fluid retention, unlike previous results during acute fluid ingestion protocols. Additionally, the decrease in BF% post-supplementation could be explained by the increase in FFM that was found with no change in overall body mass, such that on day seven, a lower percentage of overall body composition was FM.

Consistent with previous findings in the literature, there was a significant increase in TBW after seven days of creatine supplementation measured by MF-BIA.[15,18,20,23] These previously reported increases in TBW were likely due to increases in ICW rather than ECW. This was also seen in the present study as there was a trend towards significant increases in ICW in the creatine group compared to placebo, with no change in ECW in either group. In a similar protocol that used $D_2O$ and measured muscle creatine stores from the vastus lateralis, it was found that the creatine group experienced an increase in muscle creatine stores and TBW.[20] Thus, it is likely that creatine supplementation in this investigation caused an increase in muscle creatine stores,

causing water to follow the osmotically active particles and enter muscle cells to increase ICW. In contrast, some studies have found no change in TBW measured by MF-BIA and SF-BIA with similar supplementation protocols.[17,24] Eliot et al. reported no changes in TBW from creatine supplementation, but this may have been due to their older subject population, as younger individuals tend to experience more changes in body composition and body water from creatine supplementation.[14,17] Additionally, Kreider et al. used only SF-BIA in their analysis of TBW which may have compromised the accuracy of the measurement. They also attributed increases in body mass to increases in muscle mass due to the training protocol rather than from creatine supplementation.[24]

Additionally, MF-BIA measured a 0.8 kg increase in TBW in the creatine group while increases in FFM measured by DEXA, MF-BIA, and SF-BIA were 0.6 kg, 1.1 kg, and 0.7 kg, respectively. From these values, it appears the increases in FFM can be attributed to increases in TBW caused by creatine loading, especially considering that subjects likely did not experience skeletal muscle hypertrophy in just seven days.

Though the previous literature varies in creatine supplementation duration, from the present study it could be concluded that seven days of creatine supplementation at a loading dose of 0.3 g/kg of bodyweight is enough to increase ICW and TBW, and therefore FFM. MF-BIA detected changes in body water, and the body composition changes that occurred were confined to increases in FFM rather than FM. MF-BIA devices such as the InBody are becoming more frequent for general use in health and fitness centers, and therefore the findings of this study are applicable to training efficacy. Athletes and everyday exercisers experience frequent fluctuations in both FM and FFM secondary to dietary and training interventions. If an individual uses creatine supplements

regularly, it should be known that ICW increases, causing a measurable increase in FFM that may not come solely from hypertrophy. The findings of the present study support the idea that the use of MF-BIA devices could allow individuals supplementing with creatine to better understand if increases in FFM are due to changes in TBW or muscle mass. It is clear that MF-BIA detected these changes correctly to a greater degree than in previous literature examining acute fluid ingestion.

**Table 1.** Subject demographics (average ± SD).

| Group (n) | Age | Height (cm) | Weight (kg) |
|---|---|---|---|
| Males (13) | 20.0 ± 1.2 | 180.8 ± 6.6 | 82.9 ± 11.3 |
| Females (14) | 20.1 ± 1.6 | 168.6 ± 6.9 | 63.1 ± 9.7 |
| Total (27) | 20.0 ± 1.4 | 174.5 ± 9.1 | 72.6 ± 14.4 |

**Table 2.** Average (± SD) fat free mass (kg) for creatine and placebo groups pre- and post-supplementation.

| | Creatine # | Placebo |
|---|---|---|
| DEXA pre | 52.4 ± 11.8 | 57.1 ± 10.9 |
| DEXA post* | 53.0 ± 12.1 # | 57.4 ± 10.7 |
| MF-BIA pre | 55.4 ± 13.1 | 60.0 ± 12.3 |
| MF-BIA post* | 56.5 ± 13.3 # | 60.2 ± 11.8 |
| SF-BIA pre | 57.4 ± 12.9 | 62.4 ± 11.1 |
| SF-BIA post* | 58.1 ± 12.9 # | 62.2 ± 11.1 |

* Indicates significant main effect for time (Post > Pre), # indicates significant higher increase in creatine than placebo, $P < 0.05$.

**Table 3.** Average (± SD) fat mass (kg) for creatine and placebo groups pre- and post-supplementation.

| | Creatine | Placebo |
|---|---|---|
| DEXA pre | 17.0 ± 7.9 | 18.7 ± 4.5 |
| DEXA post | 17.0 ± 7.8 | 18.6 ± 4.3 |
| MF-BIA pre | 14.0 ± 7.4 | 15.9 ± 4.5 |
| MF-BIA post | 13.6 ± 7.4 | 15.8 ± 4.4 |
| SF-BIA pre | 12.2 ± 6.0 | 13.5 ± 3.3 |
| SF-BIA post | 12.1 ± 6.1 | 13.8 ± 3.2 |

**Table 4.** Average (± SD) body fat percent for creatine and placebo groups pre- and post-supplementation.

| | Creatine | Placebo |
|---|---|---|
| DEXA pre | 23.9 ± 7.8 | 24.9 ± 5.9 |
| DEXA post* | 23.6 ± 7.6 | 24.7 ± 5.9 |
| MF-BIA pre | 19.7 ± 8.0 | 21.4 ± 6.6 |
| MF-BIA post* | 18.8 ± 8.0 | 21.1 ± 6.2 |
| SF-BIA pre | 17.1 ± 5.7 | 18.1 ± 4.9 |
| SF-BIA post* | 16.7 ± 5.5 | 18.4 ± 4.6 |

* Indicates significant main effect for time (Post < Pre), $P < 0.05$

**Table 5.** Average (± SD) intra-cellular water (ICW), extra-cellular water (ECW), and total body water (TBW) for creatine and placebo groups pre- and post-supplementation measured by MF-BIA. All measurements in kg.

| | Creatine | Placebo |
|---|---|---|
| ICW pre | 25.4 ± 6.1 | 27.4 ± 5.7 |
| ICW post* | 25.9 ± 6.2 | 27.6 ± 5.5 |
| ECW pre | 15.0 ± 3.5 | 16.4 ± 3.3 |
| ECW post | 15.2 ± 3.4 | 16.3 ± 3.0 |
| TBW pre | 40.4 ± 9.5 | 43.8 ± 8.9 |
| TBW post* | 41.2 ± 9.6 # | 43.9 ± 8.5 |

* Indicates significant main effect for time (Post > Pre), # indicates significant higher increase in creatine than placebo, $P < 0.05$.

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# Appendices

## Appendix A

Informed Consent

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Emily Buck and Chris Womack from James Madison University. The purpose of this study is to analyze the effect of creatine supplementation on total body water measured through InBody Composition Analyzer. This study will contribute to completion of Emily Buck's Master's Thesis.

Research Procedures

Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction. This study consists of seven visits to the Human Performance Laboratory in Godwin Hall, Room 217 over the period of 8 days. Two of these visits will involve assessment of your body fat percentage using three separate techniques. You will be asked to refrain from eating or drinking anything except water for 8 hours prior to reporting to the lab. For these visits, you will also be asked to refrain from consuming alcoholic beverages or exercising for 12 hours prior to reporting. The different methods of body composition are described below

> DEXA scan: Your body fat percentage will be determined by a dual-energy x-ray absorptiometry (DEXA) scan. This will involve lying on a cushioned table while the machine scans your entire body with a low-level x-ray.
>
> Hand-held bioelectrical impedance device: You will simply have to hold the device in your hand. It will pass a very mild electrical current through your body that you will not be able to sense.
>
> Multi-frequency bioelectrical impedance device: This device works in the same manner as the hand-held device. You will stand on a device that functions much like a scale and grasp handrails to your side while the measurements are made.

You will either be assigned to the placebo group or the creatine group. Neither you nor the investigators will know which group you are in. If you are in the creatine group, you will take 0.3g of creatine monohydrate per 1 kg of bodyweight for 7 days. You will be asked to come into the lab every day to retrieve your supplement, and will be asked to bring the empty bag back from the previous day. If you are unable to come into the lab, your daily doses will be given to you ahead of time. Testing will occur on day 1 and day 8 when you come into the lab. According to the International Society of Sports Nutrition,

there have been no reported adverse effects or serious harm related to taking creatine at the provided dose of this study.

If you are in the placebo group, you will consume 0.3g per 1 kg of bodyweight of maltodextrin for 7 days. You will be asked to come into the lab every day to retrieve your supplement, and will be asked to bring the empty bag back from the previous day If you are unable to come into the lab, your daily doses will be given to you ahead of time. Testing will occur on day 1 and day 8 when you come into the lab.

Time Required

Your participation will require eight total visits to the Human Performance Lab. Two of these sessions are testing sessions and will last a total of 90 minutes.

Risks

The DEXA test involves exposure to low-level radiation. This exposure is approximately the same as the exposure from a flight across the United States. To minimize risk of COVID-19 exposure, the DEXA machine and both BIA analyzers will be cleaned with disinfectant prior to and after each test. Both the subject and the investigators will wear a mask and keep at least 6 feet apart whenever possible. Investigators will also wear a face-shield and gloves during the data collection. Creatine supplementation includes the risk of weight gain and water retention in your body.

Benefits

Potential benefits from participation in this study include feedback on your current body composition. In addition to your actual scores, you will be given established norms. For individuals randomized to the creatine group, creatine has been known to improve short-duration athletic performance as well as increase muscle mass.

Confidentiality

The results of this research will be presented at relevant regional and national/international conferences. Our findings will also be published in relevant research journals and/or books in the field of exercise science. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the researcher. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind. If you are a student in one of Dr. Womack's or Emily's classes, your participation or lack of participation will not affect your grade in any way.

Questions about the Study

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Emily Buck
Department of Kinesiology
James Madison University
buck3ea@jmu.edu

Christopher Womack
Department of Kinesiology
James Madison University
Telephone: (540) 568-6515
womackcx@jmu.edu

Questions about Your Rights as a Research Subject

Dr. Lindsey Harvell-Bowman
Chair, Institutional Review Board
James Madison University
(540) 568-2611
harve2la@jmu.edu

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am between 18-40 years of age.

This study has been approved by the IRB, protocol # 22-2740.

_____ I verify that I am not pregnant and do not think I am pregnant

_____

Name of Participant (Printed)

_____ _____

Name of Participant (Signed) Date

_____ _____

Name of Researcher (Signed) Date

## Appendix B

Health History Questionnaire

Instructions: Complete each question accurately. All information provided is confidential.

Part I: General Information

1. Subject #

2. Local Phone
Email:_____

3. Gender (circle one) Male Female

4. Date of Birth (Month/ Day/ Year)

Part II: Medical History

5. Circle any that died of heart attack before age 50: Father Mother Brother Sister Grandparent

6. Date of last medical exam: _____ Last physical fitness test: _____

7. Circle operations you have had: Back Heart Kidney Eyes Joint Neck Ears Hernia

Lung Other _____

8. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

| | | |
|---|---|---|
| Alcoholism | Diabetes | Kidney Problems |
| Anemia (sickle cell) | Emphysema | Mental Illness |
| Anemia (other) | Epilepsy | Muscular Injury |
| Asthma | Eye Problems | Neck Strain |
| Back Strain | Gout | Obesity |
| Bleeding trait | Hearing Loss | Orthopedic Injuries |
| Bronchitis, chronic | Heart Problem | Phlebitis |
| Cancer | High Blood Pressure | Rheumatoid arthritis |
| Cirrhosis, liver | Hypoglycemia | Stroke |
| Concussion | Hyperglycemia | Thyroid problem |
| Congenital defect | Infectious Mononucleosis | Ulcer |

Other _____

9. Circle all medications taken in the last six months:

| | | |
|---|---|---|
| Blood thinner | Epilepsy or anti-seizure medication | Nitroglycerin |
| Diabetic pill | Heart-rhythm medication | Other _____ |
| Digitalis | High-blood pressure medication | |
| Diuretic | Insulin | |
| Anabolic steroids/testosterone | Acetaminophen (Tylenol) | |
| Anti-ulcer/heartburn | Oral contraceptives | |
| Acne medication (Accutane) | Arthritis treatment | |
| Anti-anxiety/insomnia | Anti-depressants | |

10. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the

number indicating how often you have each of the following:

5 = Very often 4 = Fairly often 3 = Sometimes 2 = Infrequently 1= Practically never

| | |
|---|---|
| a. cough up blood | f. chest pain |
| 1 2 3 4 5 | 1 2 3 4 5 |
| b. abdominal pain | g. swollen joints |
| 1 2 3 4 5 | 1 2 3 4 5 |
| c. low back pain | h. feel faint |
| 1 2 3 4 5 | 1 2 3 4 5 |
| d. leg pain | i. dizziness |
| 1 2 3 4 5 | 1 2 3 4 5 |

e. arm or shoulder pain j. breathless on slight exertion

1 2 3 4 5 1 2 3 4 5

Part III: Health Related Behavior

11. Do you smoke? Yes No

12. If you are a smoker, indicate the number of smoked per day:

Cigarettes:

40 or more 20-39 10-19 1-9

Cigars or pipes only:

5 or more or any inhaled less than 5, none inhaled

13. Do you exercise regularly? Yes No

14. How many times in a week do you spend at least 30 minutes in moderate to strenuous/vigorous

exercise?

1 2 3 4 5 6 7 days per week

15. Can you walk 4 miles briskly without fatigue? Yes No

16. Can you jog 3 miles continuously at a moderate pace without discomfort? Yes No

17. Weight now: _____ lb. One year ago: _____ lb Age 21: _____ lb

18. To your knowledge, are you currently pregnant or showing signs that you may be pregnant?

_____yes _____no

**Appendix C**

Data Collection Sheet

Preliminary requirements

8 hours fast __

Glass of water 45 minutes before __

No alcohol for 24 hours __

No exercise the day of test __

Initial measurements

Subject # _____

Age_____

Height (cm): _____

Timepoint: ____Pre _____Post

Inbody (must stand for 5 min before each trial)

Weight: _____

SMM (lbs): _____

PBF %: _____

ECW/TBW: _____

Leg lean mass: _____

Intercellular water: _____

Extracellular water: _____

Total Body water:_____

Handheld BIA (Fitness index: normal/athlete)

Fat %: _____

DEXA

Region % fat (Total): _____

Lean mass (lean + BMC): _____

Fat free (lbs): _____

Fat (lbs): ____

Any reported adverse effects post-supplementation:

# Appendix D

FIT Index Determination for SF-BIA

FIT Index = Frequency x Intensity x Time

| Number | Frequency of Exercise |
|---|---|
| 5 | Daily or almost daily |
| 4 | 3 to 4 times per week |
| 3 | 1 to 2 times per week |
| 2 | A few times per month |
| 1 | Less than once per month |

| Intensity | Conditioning Exercise | Sports |
|---|---|---|
| 5 | Cycling - > 12 mph pace | Basketball - competitive |
| | Weightlifting - vigorous, powerlifting or bodybuilding | Boxing |
| | Rowing - moderate to vigorous | Football - competitive |
| | Rowing machines - moderate to vigorous effort | Handball, racquetball, or squash |
| | Aerobic dancing - high impact | Ice hockey |
| | Step aerobics | Karate or kickboxing |
| | Running -> 5.0 mph | Rockclimbing |
| | Rope jumping | Rugby |
| | Rollerblading (roller skating) | Soccer - competitive |
| | Ski machine | Tennis |
| | Stairstepping | Swimming - competitive or lap |
| | Stationary cycling - moderate to vigorous effort | Speed skating - competitive |
| | | Skiing - cross-country<br>Skiing - downhill racing |

| Intensity | Conditioning Exercise | Sports & Recreational Activities |
|---|---|---|
| 4 | Cycling - < 12 mph pace | Archery |
| | Weightlifting - moderate effort | Basketball - shooting baskets |
| | Stationary cycling - light effort | Bowling |
| | Rowing - light effort | Fencing |
| | Calisthenics | Golf |
| | Stretching / Yoga | Gymnastics |
| | Rowing machines - light effort | Horseback riding |
| | Water aerobics or water exercise | Baseball |
| | Aerobic dancing - low impact | Softball |
| | Jogging - < 5.0 mph | Tai Chi |
| | Walking - > 2.5 mph | Volleyball - competitive |
| | Swimming - leisurely | Wrestling - competitive |
| | Rollerblading - leisurely | Ice Skating - < 9 mph |

| Time | Duration |
|---|---|
| 4 | 45 minutes or more |
| 3 | 30 to 44 minutes |
| 2 | 15 - 29 minutes |
| 1 | less than 15 minutes |

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