
Effects of a Nighttime Multi-Ingredient Supplement on Recovery from a Damaging Exercise Protocol

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ABSTRACT

International Journal of Exercise Science 9(4): 471-481, 2016. The purpose of this study was to determine the efficacy of a nighttime multi-ingredient supplement on noninvasive markers of recovery in resistance trained and untrained individuals. Forty-nine participants, both trained (n=25) and untrained (n=24) completed the randomized, double blind, placebo-controlled study. Trained participants were randomly divided into supplement (n=12) and placebo (n=13) groups. Untrained participants were randomly divided into supplement (n=14) and placebo (n=10) groups. Two, 2 (supplement group) × 2 (training status) × 5 (time points) repeated measures analysis of variance (ANOVA) were utilized to determine if an interaction for supplement group and training status existed for peak force (PForce) and delayed onset muscle soreness (DOMS). Four, 2 (supplement group) × 2 (training status) × 4 (time points) repeated measures ANOVAs was employed for SWVL-Long, SWVL-Tera, SWVL-Trans and ROM to determine interactions for supplement group and training status. For significant main effects, pairwise comparisons were utilized to determine at what time-points significant differences occurred. There were no significant interactions for either DOMS or PForce. However, significant main effects of time were observed for both variables (p<0.001). No significant interactions were determined for either training group, or supplement group for SWVL-Tera, SWVL-Trans, SWVL-Long, or ROM. Although the SWVL-Long had a significant main effect of time (p=0.033), post-hoc pairwise comparisons revealed no significant differences between time points. There was no effect of the nighttime multi-ingredient supplement for attenuation of symptoms associated with acute exercise induced muscle damage.

KEY WORDS: Supplementation, eccentric exercise, exercise recovery

INTRODUCTION

Exercise-induced muscle damage (EIMD) is produced in response to multiple

repetitions of a novel movement (i.e. Resistance training), resulting in reduced muscle force production, an increase in muscle swelling and soreness, and an

increased concentration of intramuscular proteins in the blood (12,21). Methods of inducing muscular damage that have been employed previously include eccentric contractions against an external load, downhill running and plyometrics (i.e. depth jumps). The severity of EIMD increases over 24-72 hours, before subsiding (12). EIMD results in an a decrease in muscle strength, range of motion, and both the rate and magnitude of force production, as well as an increase in muscle swelling and soreness (5,12,26). These symptoms can impair performance in subsequent events or training sessions, resulting in an increase in the investigation of recovery modalities to attenuate the effects of EIMD.

Previous research has evaluated EIMD using both invasive and non-invasive measures. Invasive measures often include measurement of blood markers, such as the presence of creatine kinase and myoglobin, while non-invasive measures include measurement of variables such as delayed onset of muscle soreness (DOMS), range of motion (ROM), and return to force (3,11,29).

Not only is the severity of EIMD dependent on the mode, intensity and duration of exercise, but also the training status of the individual (19). Newton and colleagues (19) showed that after a bout of eccentric exercise, resistance trained individuals displayed reduced markers of EIMD than untrained individuals, and returned to baseline values more quickly. Previous research completed by Kanda and colleagues (13) indicated that muscle soreness and serum myoglobin concentrations peak around 72 hours after exercise for untrained individuals.

Decrements to muscular function are more likely to occur as a result of eccentric contraction than from other types of muscular activity (24). However, markers of EIMD are often reduced on subsequent bouts of eccentric exercise in both trained and untrained subjects, suggesting a training effect on response to exercise (18,21).

Strategies aimed at enhancing recovery (e.g. cryotherapy, foam rolling, active recovery, compression) have become increasingly popular and subsequently more researched. Recent research has increasingly examined the use of nutritional supplementation to mitigate muscle damage sustained during exercise (14,28). While the majority of research focuses on the effect of single ingredients such as whey protein, creatine, or branched chain amino acids, the use of multi-ingredient supplements (MIS) has also been employed (4,7,27).

The majority of research concerning MIS has focused on the combinatory effects of supplementation and chronic training to improve measures of performance (17,24). Ormsbee and colleagues (25) found that 4 weeks of loading MIS did not result in reduced levels of soreness or markers of muscle damage following an acute bout of downhill running. However, to our knowledge, no other studies currently exist examining the effect of nighttime multi-ingredient supplement (N-MIS) on markers of muscle damage following an acute bout of damaging exercise.

Previous studies examining the effects of MIS on the repair and recovery of skeletal have primarily used supplements containing ergogenic ingredients (e.g. creatine, beta-alanine, caffeine) (25).

However, proper recovery after exercise is critical for subsequent exercise performance and adaptation, so MIS that contain ingredients purported to increase sleep quality and maximize repair processes may also be beneficial for those engaging in strenuous exercise. Melatonin has been shown previously to improve sleep quality and decrease sleep onset latency (15). Stratos and colleagues (31) reported that following blunt skeletal muscle injury in rat models, melatonin administration enhanced rate and magnitude of muscle force production recovery and quicker proliferation of satellite cells into muscle. However, there is currently a paucity of research evaluating its efficacy in a recreationally active population. The supplement used in the current study also contains L-glutamine, which has been reported to reduce DOMS following damaging eccentric exercise (16). Additionally, supplements that contain branched chain amino acids (BCAAs) may ameliorate performance decrements following damaging exercise. Previous research using BCAAs surrounding damaging exercise reported lower scores for perceived muscle soreness and smaller reductions in maximal force production compared to isocaloric carbohydrate supplementation and placebo (10,30). Therefore, the purpose of this study was to examine the effect of a N-MIS on DOMS, PForce, SWVL-Tera, SWVL-Long, SWVL-Trans and ROM following an acute eccentric muscle damaging protocol in trained and untrained males.

METHODS

Participants

The current study employed a randomized, double-blind, placebo-controlled design. Participants consisted of trained as well as untrained males between the ages of 18-30 years old and were recruited from undergraduate classes on the Georgia Southern University campus. Participants were randomly assigned to either trained-NMIS (n=12; Age: 20.6± 2.3 yrs), trained placebo (trained-PLA) (n=13; Age= 22.3± 2.3 yrs) untrained-NMIS (n=14; Age= 22.4± 2.2 yrs) and untrained-PLA (n=10; Age= 22.4± 2.4 yrs). Prior to participation in this study, all participants signed an informed consent in accordance with the Declaration of Helsinki. This study was approved by the Institutional Review Board of Georgia Southern University. Participant information is included in Table 1.

Table 1. Descriptive Data for Participants (n=49)

Training Status	Supplement Group	Age (yrs)	Height (cm)	Mass (kg)
Untrained	N-MIS (n=14)	22.4 ± 2.2	182.1 ± 7.2	80.4 ± 7.5
	PLA (n=10)	22.4 ± 2.4	181.2 ± 6.7	82.7 ± 11.2
Trained	N-MIS (n=12)	20.6 ± 2.3	179.3 ± 7.2	80.6 ± 12.9
	PLA (n=13)	22.3 ± 2.3	176.4 ± 6.2	78.4 ± 8.6

Protocol

For the purpose of the current study, resistance training was operationally defined as participating in regular lower body resistance exercise, including frequent use ($\geq 1x/week$) of common lower body exercises (i.e. free weight squats, leg press, leg extension, deadlift) for a period of two years or greater. No later than a week prior to the start of the study all trained participants completed a verification testing protocol on a plate-loaded hip sled. In order to achieve "trained" status the

individual successfully completed one rep of 2.04x bodyweight. This placed the individual in the 70th percentile according to the American College of Sports Medicine (1). Non-trained individuals placed in the experimental group were not tested. Untrained individuals were classified as those who participated in less than two days of resistance training per week. All participants were asked to report for all four days of testing within an hour of the first appointment time, and were instructed to minimize all lower body activity, including: weight lifting, running, walking, swimming, cycling, and skating. An overview of the testing flow can be found in Figure 1.

Lower body muscle soreness of the individual was assessed at Pre-, Post-, 24-hours, 48-hours, and 72-hours. Participants were asked to complete the Lower Extremity Functional Scale (LEFS) upon arrival to the testing laboratory. LEFS is a twenty-question Likert scale inventory that assesses the participants' ability to complete everyday tasks (4). The test-retest reliability of the LEFS is 0.94 (4).

Participants lay prone on a firm, examining table with feet hanging off one end. Using the participant-determined dominant leg, researchers placed the axis of an extendable goniometer about the lateral epicondyle of the tibia and extended the ends to the greater trochanter of the femur and the lateral malleolus of the ankle. Participants were then asked to flex at the knee (without assistance), bringing the heel as close to the gluteus as possible. For reference, when a participant's leg was parallel to the ground the associated angle would be 0°, and when the lower leg was perpendicular to the

ground the angle would be 90°. This motion was repeated three times, with the greatest ROM being recorded.

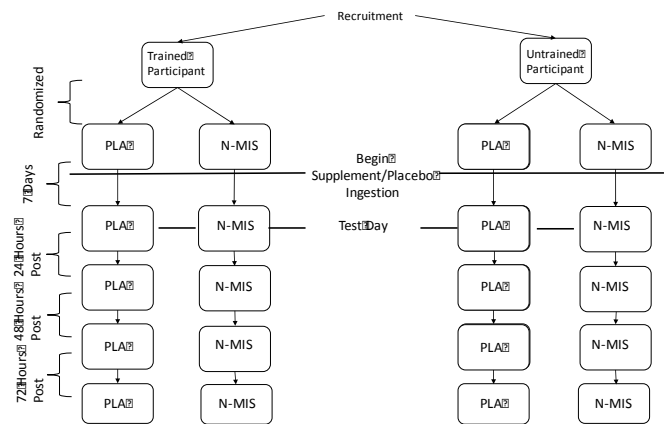


Figure 1. Experimental Flow

Upon arrival to the laboratory on the first testing day, researchers made three marks with a permanent marker on the quadriceps of the participant's dominant leg. Marks were made at 30%, 60% and 70% of the distance (cm) from the anterior superior iliac spine to the lateral epicondyle of the tibia, along the vastus lateralis. If confusion of the muscular border was present, researchers palpated the muscle border for verification of vastus lateralis. Three images were taken of the vastus lateralis using a Terason T3200 (Terason, MA, USA) brightness mode ultrasound. Panoramic images were made using Terascape software (Terason, MA, USA). Panoramic images were initiated at the 30% mark, and terminated at the 70% mark, with the transducer head parallel to the thigh and the transducer was manipulated to completely visualize the fascicle. The use of panoramic ultrasound imaging of the vastus lateralis has been previously validated compared to magnetic resonance imaging (2). Longitudinal and transverse images were taken at the 60% mark, with

the transducer head oriented parallel and perpendicular, respectively. This is a similar location on the vastus lateralis to that used by Fukunaga (9) which used 50% of the distance from greater trochanter to lateral epicondyle of the tibia.

Swelling of the vastus lateralis (SWVL) was determined by the distance (cm) between the fascial borders of the vastus lateralis. All distance measurements were made using the software's measurement calipers. Two measurements were taken from the widest segment of the muscle, with the mean value representing the SWVL for all three images. Due to the nature of the panoramic swelling of the vastus lateralis (SWVL-Tera), horizontal measurements on the image were made each testing day to ensure accurate placement of the SWVL distance measurement. A trained researcher took all images and measurements.

Peak force (PForce) of the knee extensors was assessed using an isokinetic dynamometer (Biodex, NY, USA). Participants were set up on the isokinetic dynamometer to assess the peak isometric force of their self-selected dominant leg. Participants were asked to maximally extend and maximally flex at the knee. These points were then designated in the software as ROM reference points for away and toward, respectively. Biodex protocols for isometric testing were performed with the knee oriented at 45°. Participants were asked to complete two sets of five repetitions; the first and second reps were at 50% & 75% of their perceived maximal force, respectively. Reps three, four, and five were all completed at 100% perceived maximal force, with the highest peak force

value between the two sets were recorded. Rest period between sets was 3-5 minutes.

The fatiguing protocol was completed on the same isokinetic dynamometer and consisted of 50 eccentric repetitions of the knee extensors against a load of 120% of the participant's maximum voluntary contraction (MVC), as determined by the previous measurement of peak force. The angular excursion was 170°-40° at an angular velocity of 60°/sec. Participants were instructed to maximally resist every repetition. Strong verbal encouragement was given to the participant via the research team.

In a double-blind fashion, participants were randomly assigned to either the N-MIS or placebo (PLA) group. Participants met with the research team no later than one week prior to the first testing day to receive seven nights' worth of N-MIS (5.7g/day) or PLA (5.7g/day) in individually packaged doses. This dose is the general serving size per the manufacture recommendations. The seven day "loading" phase was utilized as a result of previous research demonstrating the efficacy of BCAA containing supplements after only a week of ingestion (11). The placebo was a non-caloric powder matched for color, and taste to ensure blinding. The ingredient label as reported by the manufacturer for the N-MIS is provided in Figure 2. When participants returned for completion of the exercise protocol as well as baseline muscular assessment, empty bags were returned to ensure compliance.

After completion of exercise testing and muscular assessment, as well as muscular testing on subsequent days, one day of

supplementation was provided, with empty bags returned the following day. Supplementation was consumed at night immediately before bed. Participants were asked to refrain from alcohol, maintain a regular diet and not initiate any new dietary changes or supplements throughout the seven-day loading of the supplement and during the four testing days. Additionally, participants were required to complete a daily dosing log to ensure compliance for the supplementation.

Supplement Facts		
Serving Size: 5.7 g		
Servings Per Container: 30		
	Amount Per Serving	% DV*
Calories	5	
Total Carbohydrate	1 g	<1%
Vitamin D (as Cholecalciferol)	500 IU	125%
Vitamin B6 (as Pyridoxine Hydrochloride)	10.5 mg	525%
Magnesium (as Magnesium Aspartate)	125 mg	31%
Zinc (as Zinc Aspartate)	30 mg	200%
Copper (as Copper Glycinate)	10 mcg	1%
Boron (as Boron Citrate)	2 mg	**
Arnold's Dream Proprietary Blend	3,555 mg	**
Night Growth Matrix		
L-Glycine, Gamma Amino Butyric Acid (GABA), 5-HTP (5-Hydroxytryptophan), Mucuna Pruriens 20% L-Dopa, Horny Goat Weed (Epimedium Sagittatum) Stem and Leaf Extract, N-Acetyl-5-Methoxytryptamine (Melatonin).		
Muscle Recovery Matrix		
L-Glutamine, BCAA Nitrate 3:1:2 Blend (L-Leucine, L-Valine, L-Isoleucine), Fenugreek (Trigonella Foeniculum Greacum) Proprietary Seed Extract Standardized to 50% Saponins.		
*Percent Daily Value Based on a 2,000 Calorie Diet		
**Daily Value Not Established		

Figure 2. N-MIS supplement facts and ingredient list

Statistical Analysis

A two (supplement group) x two (training status) x five (time points) repeated measures analysis of variance (ANOVA) was utilized to determine if an interaction for supplement group and training status existed for PForce and DOMS. Due to differing time-frames for SWVL-LONG, SWVL-Tera, SWVL-Trans and ROM a separate repeated measures ANOVA was conducted. The additional ANOVA and different time points was employed due to the potential for post-exercise blood pooling as a result of

repeated muscle actions. This blood pooling, while important for muscular hypertrophy and training adaptations, may have increased the likelihood of committing a Type I error by falsely stating that the natural state of engorgement was a part of the tissue repair process. Thus, the second ANOVA: a two (supplement group) x two (training status) x four (time points: Baseline, 24hrs, 48hrs, 72hrs) repeated measures ANOVA was employed for SWVL-Long, SWVL-Tera, SWVL-Trans and ROM to determine if interactions for supplement group and training status. If a significant main effect was determined, pairwise comparisons were utilized to determine at what time-points significant differences occurred. Baseline group differences were determined using one-way ANOVA. All analyses were performed with SPSS version 20.0 (SPSS, Inc., Chicago, IL). Data is presented as mean \pm standard deviation (SD). The alpha level was set at $p \leq 0.05$.

RESULTS

There were no between group differences at baseline for either DOMS or PForce. Additionally, there were no significant interactions (training status or supplement) for either DOMS or PForce. Due to the lack of group effect all data was pooled to assess for significant effects of time for the entire cohort. Pooled data resulted in significant effects of time for both DOMS (Table 2) and PForce (Table 3). Both DOMS scores and PForce values were significantly lower at all time points compared to baseline, suggesting the protocol used in the current study effectively induced muscle damage regardless of training or supplementation status.

There were no between group differences at baseline for any of the variables. No significant interactions were determined for either training group, or supplement group for SWVL-Tera, SWVL-Trans, SWVL-Long, or ROM. Although the SWVL-Long had a significant main effect of time ($p=0.033$), post-hoc pairwise comparisons revealed no significant differences between time points.

Table 2. DOMS Scores Pairwise Comparison Results

Time Point	Mean LEFS Score
Baseline	77.4 ± 5.6
Immediately Post	57.9 ± 19.5**
Baseline	77.4 ± 5.6
24-hours post	64.9 ± 15.2**
Baseline	77.4 ± 5.6
48-hours post	58.9 ± 20.2**
Baseline	77.4 ± 5.6
72-hours post	63.7 ± 20.3**
Immediately Post	57.9 ± 19.5
24-hours post	64.9 ± 15.2*
Immediately Post	57.9 ± 19.5
48-hours post	58.9 ± 20.2
Immediately Post	57.9 ± 19.5
72-hours post	63.7 ± 20.3*
24-hours post	64.9 ± 15.2
48-hours post	58.9 ± 20.2**
24-hours post	64.9 ± 15.2
72-hours post	63.7 ± 20.3
48-hours post	58.9 ± 20.2
72-hours post	63.7 ± 20.3*

*denotes a significant difference at $p < 0.05$;

**denotes a significant difference at $p < 0.001$.

Table 3. Peak Force Scores Pairwise Comparison Results

Time Point	Mean Peak Force (Nm)
Baseline	211.20 ± 44.89
Immediately Post	164.37 ± 55.054**
Baseline	211.20 ± 44.89
24-hours post	187.92 ± 50.102**
Baseline	211.20 ± 44.89
48-hours post	178.31 ± 70.90*
Baseline	211.20 ± 44.89
72-hours post	176.8 ± 73.91*
Immediately Post	164.37 ± 55.054
24-hours post	187.92 ± 50.102**
Immediately Post	164.37 ± 55.054
48-hours post	178.31 ± 70.90
Immediately Post	164.37 ± 55.054
72-hours post	176.8 ± 73.91
24-hours post	187.92 ± 50.102
48-hours post	178.31 ± 70.90
24-hours post	187.92 ± 50.102
72-hours post	176.8 ± 73.91
48-hours post	178.31 ± 70.90
72-hours post	176.8 ± 73.91

*denotes a significant difference at $p < 0.05$;

**denotes a significant difference at $p < 0.001$.

DISCUSSION

Findings of the present study demonstrated that supplementation with N-MIS did not attenuate decrements in muscle function and markers of recovery for PForce, DOMS, swelling of the vastus lateralis, or ROM following an acute bout of eccentric exercise. Furthermore, no differences in recovery were observed between training status groups.

Classically, two common symptoms from eccentric muscle damage have been an increase in DOMS and a decrease in force production capability (11,20,22,32,33). The current study demonstrated a significant

effect of time for both DOMS and PForce, indicating that the protocol did induce muscle damage. Disruption of Z-discs and sarcomeres following repeated eccentric exercise has been shown to cause damage, and death to muscle fiber. In agreement with the previous literature, the current study determined that significant decreases in muscular force and significant decreases in muscle function occur following an unaccustomed eccentric exercise bout (11,20,22,25,32,33). Due to this finding we can infer, non-invasively, that there was disruption of muscular contractile properties of the knee extensors as a result of the 50-repetition exercise protocol completed.

A significant main effect of time on the longitudinal ultrasound measurement was found across all time points, indicating a swelling response. Acute myofibril injury leads to increases in inflammation and edema (27). This finding of swelling in close temporal proximity (less than 2-days) to the eccentric damaging protocol corroborates the findings of Crenshaw et al. (7) and Friden et al. (8) who found significant levels of swelling via intramuscular pressure using a catheter and biopsy, respectively. The current study also found that the measurements made using the transverse (SWVL-Trans) and panoramic (SWVL-Tera) transducer placement resulted in no significant changes in cross sectional area. There is a possibility of an inconclusive result as a consequence of the inherent challenges with the application of ultrasound as a means to analyze intramuscular swelling. This may be, in part, due to the kinematics of ultrasound and the resultant image output only displaying changes in muscular cross

sectional area as a result of increasing border distances (33).

Previous research investigating the effectiveness of nutritional supplementation as a recovery modality has drawn mixed conclusions. In agreement with the present study, Ormsbee and colleagues (25) found that a multi-ingredient supplement did not attenuate any markers of muscle damage following a downhill running protocol in trained runners. Contrastingly, a single ingredient branched chain amino acid supplementation intervention, compared to placebo, after a 7-day loading period resulted in decreased CK concentration, decreased severity of DOMS, smaller loss of force and improved time to recovery for peak force following an eccentrically damaging drop-jump protocol in highly resistance-trained males (10). Previous research that has established a beneficial effect of supplementation with BCAAs has traditionally used a larger overall dose (~3-10g) compared to the dosing of the proprietary blend in the current study (11,28). It is possible that this reduced dosage did not provide adequate amounts of leucine for muscle protein synthesis to be maximized. It is important to note that, despite the sub-optimal dosing of BCAAs, a trend towards a significant time x supplement group interaction was found ($p=0.063$). It may be that the short duration and small sample sizes of the current study failed to demonstrate the significant effect.

The present study found no significant effect of training status on any of the non-invasive markers of muscle damage employed. Contrastingly, Newton et al. (20) demonstrated a protective effect of habitual

resistance training to eccentrically induced muscle damage of the elbow flexors and its effects on muscle function. Previous studies have postulated that despite the submaximal loading in the eccentric phase during traditional resistance training, it may be that the repeated submaximal efforts result in adaptive processes thus reducing the risk for future injury (23). It is possible that due to the unaccustomed nature of the mode of exercise, both habitual exercisers and non-exercisers in the present study were subjected to a novel training stimulus, of which the body has not yet adapted to (6,20).

The current study was limited due to the inability to control for exercise and dietary intake during the four-day testing period. Although all participants were instructed to refrain from any type of lower body exercise, and to maintain a consistent diet throughout the testing period, compliance was based on self-report rather than direct supervision. Furthermore, the current study only used non-invasive measures to assess muscle damage and recovery, making it hard to compare the results of this study to previous ones. The small sample size and short duration also pose as limitations to the current study. Future studies should aim to analyze both invasive and non-invasive measures of muscle damage using a larger sample size.

The protocol utilized in the current study was sufficiently difficult to induce skeletal muscle damage to the active musculature. Present data suggest that acute supplementation with NMIS offered no effect on non-invasive markers of recovery in untrained and recreationally trained males. An investigation into chronic

supplementation of NMIS is warranted to determine what, if any, effect on performance or recovery exists. While speculative, it is possible that an ergogenic effect of the supplement may occur following multiple stressors and myofibrillar damaging events. Future research may seek to utilize a sleep quality survey or analysis software to determine any acute effects on sleep quality from NMIS supplementation. Additionally, no participants in the current study reported any adverse effects from NMIS ingestion.

ACKNOWLEDGEMENTS

This project was supported through an unrestricted educational grant awarded by the International Society of Sports Nutrition and funded through Musclepharm Corporation.

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