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Research article

Heavy Resistance Training and Peri-Exercise Ingestion of a Multi-Ingredient Ergogenic Nutritional Supplement in Males: Effects on Body Composition, Muscle Performance and Markers of Muscle Protein Synthesis

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Abstract
This study determined the effects of heavy resistance training and peri-exercise ergogenic multi-ingredient nutritional supplement ingestion on blood and skeletal markers of muscle protein synthesis (MPS), body composition, and muscle performance. Twenty-four college-age males were randomly assigned to either a multi-ingredient SizeOn Maximum Performance (SIZE) or protein/carbohydrate/creatine (PCC) comparator supplement group in a double-blind fashion. Body composition and muscle performance were assessed, and venous blood samples and muscle biopsies were obtained before and after 6 weeks of resistance training and supplementation. Data were analyzed by 2-way ANOVA ($p \leq 0.05$). Total body mass, body water, and fat mass were not differentially affected ($p > 0.05$). However, fat-free mass was significantly increased in both groups in response to resistance training ($p = 0.037$). Lower-body muscle strength ($p = 0.029$) and endurance ($p = 0.027$) were significantly increased with resistance training, but not supplementation ($p > 0.05$). Serum insulin, IGF-1, GH, and cortisol were not differentially affected ($p > 0.05$). Muscle creatine content was significantly increased in both groups from supplementation ($p = 0.044$). Total muscle protein ($p = 0.038$), MHC 1 ($p = 0.041$), MHC 2A, ($p = 0.029$), total IRS- ($p = 0.041$), and total Akt ($p = 0.011$) were increased from resistance training, but not supplementation. In response to heavy resistance training when compared to PCC, the peri-exercise ingestion of SIZE did not preferentially improve body composition, muscle performance, and markers indicative of MPS.

Key words: Whey protein, carbohydrate, creatine, amino acids, muscle strength, muscle hypertrophy, men.

Introduction

Of late, a common approach in the exercise/sport nutrition supplement industry is the development of multi-ingredient products with the intent of simultaneously up-regulating multiple ergogenic-related physiological mechanisms in order to increase muscle mass, strength, and performance. For example, a multi-ingredient product that could potentially increase muscle strength and mass while concomitantly improving metabolic function, oxidative stress, and body composition may prove to be superior to a single-ingredient product. Many multi-ingredient products on the market contain whey protein, amino acids, carbohydrate, and creatine, and a number of these products have shown to augment physiological responses to resistance training (Candow et al., 2006; Shelmadine et al; Spillane et al., Willoughby et al., 2007). Positive outcomes emanating from clinical studies involving these type of multi-ingredient products are challenging because it is difficult to determine which ingredient(s) contained within the product may be inducing the ergogenic outcome. Nevertheless, since more of these types of products are appearing on the market, more research is necessary to substantiate their alleged effectiveness and efficacy.

A multi-ingredient product available on the market with a comprehensive list of ingredients is SizeOn Maximum Performance (Gaspari Nutrition, Inc., Lakewood, NJ), which contains a variety of ingredients alleged to augment the effects of resistance training. This product has recently been shown to be preferentially effective when compared to a protein/carbohydrate/creatine comparator at improving body composition and muscle performance (Schmitz et al., 2010). This multi-ingredient product contains a blend of vitamins and minerals containing vitamin C, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, calcium, phosphorus, magnesium, sodium, and potassium. These vitamins and minerals help maintain normal cellular function during stress-inducing situations such as exercise (Fenech and Ferguson, 2001). In addition, the product contains a blend of carbohydrates with different rates of absorption. This blend consists of the disaccharides iso-maltulose, trehalose, as well as monomeric glucose. Apparently, the alleged rationale for adding three different types of carbohydrates is to ensure proper glycogen replenishment in response to exercise due to the different rates in which each type of carbohydrate is absorbed (Lina et al., 2002; Sola-Penna and Fernandes, 1998; Jentjens and Jeukendrup, 2003). Apparently, to enhance glycogen replenishment, pterostilbene is included in the ingredient profile. Pterostilbene, an extract of blueberries, appears to enhance insulin sensitivity by enhancing hepatic enzymes associated with glucose uptake (Pari and Satheesh, 2006). In addition, the product contains a blend of hydrolyzed whey protein with free-form and branched-chain amino acids (BCAAs). Hydrolyzed whey protein and BCAAs have been shown to enhance the skeletal muscle protein synthesis in response to exercise (Borsheim et al., 2002; Manninen, 2009; Tang et al., 2007; Paddon-Jones et al., 2006). The product also contains creatine monohydrate and its salts (magnesium creatine chelate, disodium creatine phosphate). Creatine and its salts have been shown to enhance muscle mass and strength via increased protein synthesis in skeletal muscle tissue (Rawon and Volek, 2003; Sakkas et al., 2009; Selsby et al., 2004). Finally, SizeOn Maximum Performance contains a blend of elec-

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trolytes: sodium glycerophosphate, calcium glycerophosphate, potassium glycerophosphate, and the amino acids L-taurine, L-alanyl-L-glutamine, and magnesium glycyl glutamine. This blend of electrolytes and amino acids apparently act as an osmoregulator to ensure proper cellular hydration for optimal cellular function (Fumarola et al., 2005; Goodman et al., 2009).

The purpose of this study was to determine the effects of the peri-exercise nutritional supplement, SizeOn Maximum Performance, versus a conventional protein/carbohydrate/ creatine comparator supplement on indices of muscular adaptations to resistance training in young men. We hypothesized that SizeOn Maximum Performance would preferentially improve muscle strength and mass and body composition, along with increasing blood and skeletal muscle markers associated with muscle anabolism.

Methods

Experimental approach

In a randomized, double-blind, parallel design, non-resistance-trained males participated in a 4-day/week heavy resistance training program for 6 weeks in conjunction with the peri-exercise ingestion of either a multi-ingredient SizeOn Maximum Performance or protein/carbohydrate/creatine comparator supplement. Body composition and muscle performance were assessed, along with venous blood samples and muscle biopsies being obtained, before and after the 6-week intervention.

Participants

Twenty-four apparently healthy, resistance trained [regular, consistent resistance training (i.e. thrice weekly) for at least 1 year prior to the onset of the study], males between the ages of 18-35 and a body mass index between 18.5-30 $kg \cdot m^{-2}$ volunteered to participate in the double-blind study. Enrollment was open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and who had not consumed any nutritional supplements (excluding multi-vitamins) 3 months prior to the study were allowed to participate. All participants provided written informed consent and were cleared for participation by passing a mandatory medical screening. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for the Protection of Human Subjects in Research of Baylor University. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Helsinki Code.

Assessment of body composition and muscle performance

A testing session was performed prior to the first dose of supplement and beginning of the resistance training program (day 0) and on day 43, after 42 days of supplementation and resistance training. Body and composition and muscle performance were determined during these two testing sessions.

The determination of the one-repetition maximum (1-RM) for the angled leg press and knee extension exercises was based upon our previous procedures (Shelmadine et al; Spillane et al., Willoughby et al., 2007). As a warm-up, an estimated 50% 1-RM was utilized to complete 10 repetitions. After a 2.5 min rest period, a load of 70% of estimated 1-RM was utilized to perform five repetitions. At this point, the weight was gradually increased until a 1-RM was reached, with a 2.5 min rest period in between each successful lift. Test-retest reliability of performing these strength assessments on subjects within our laboratory has demonstrated low mean coefficients of variation and high reliability for the angled leg press (2.3%, intra-class r = 0.94) and knee extension (0.82%, intra-class r = 0.93), respectively. In order to assess muscle endurance, using the bench press and angled leg press exercises, participants performed as many repetitions as possible with 75% of their 1-RM (Spillane and Willoughby, 2012).

Total body mass (kg) was determined on a standard dual beam balance scale (Detecto, Webb City, MO). Percent body fat, fat mass, and fat-free mass, were determined using dual-energy x-ray absorptiometry (DEXA; Hologic Discovery Series W, Waltham, MA) based on our previous guidelines (Shelmadine et al., 2009; Spillane et al., 2009; Willoughby et al., 2007). Baseline hemodynamic measurements at rest were completed. Heart rate was determined by palpation of the radial artery using standard procedures. Blood pressure was assessed in the supine position after resting for 5-min using a mercurial sphygmomanometer using standard procedures.

Dietary analyses

For the 72 hours immediately prior to reporting to the lab for testing at Day 0 and 43, participants were instructed to record their dietary intake. During each of these 72-hour time periods, participants were instructed to not change their usual dietary habits. The dietary data were analyzed with the Food Processor dietary assessment software (ESHA Research, Salem, OR, USA) for determination of the average intake of total food energy and intake of the macronutrients.

Venous blood sampling and muscle biopsies

Venous blood samples and muscle biopsies were obtained during the testing sessions at Day 0 and 43. Blood was collected from the antecubital vein into a 10 ml Vacutainer tube. Blood samples were allowed to stand at room temperature for 10 minutes and then centrifuged. The serum was removed and frozen at -80°C for later analysis.

Percutaneous muscle biopsies (50-70 mg) were obtained from the middle portion of the vastus lateralis muscle of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For the two biopsies, attempts were made to extract tissue from approximately the same location as the initial biopsy by using the pre-biopsy scar, depth markings on the needle, and a successive incision that was made approximately 0.5 cm to the former from

medial to lateral. After removal, adipose tissue was trimmed from the muscle specimens and immediately frozen in liquid nitrogen and then stored at -80°C for later analysis.

Supplementation protocol

In double-blind fashion, participants were assigned a 6-week (42-day) supplementation protocol consisting of the oral ingestion of either a 50 grams/day of a comparator (PCC) supplement or SizeOn Maximum Performance (SIZE) (Gaspari Nutrition, Inc., Lakewood, NJ). Both supplements contained 39 grams of maltodextrose, 7 grams of whey protein, and 4 grams of creatine monohydrate. Additionally, SizeOn contains vitamin C, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, calcium, phosphorus, magnesium, sodium, and potassium, pterostilbene, BCAAs, sodium glycerophosphate, calcium glycerophosphate, potassium glycerophosphate, and the amino acids L-taurine, L-alanyl-L-glutamine, and magnesium glycyl glutamine.

Both supplements, which were identical in color, texture, smell, and taste, were mixed in 30 ounces of water and half of the total daily dosage (15 ounces) was ingested 15 minutes prior to each exercise session and the remaining half (15 ounces) were ingested upon beginning exercise and completely consumed within 20 minutes into the exercise session. The supplements were ingested 4 times per week on exercise days only. Supplementation compliance was monitored by participants returning empty containers of their supplement on day 43, and also by completing weekly a supplement compliance questionnaire.

Resistance training protocol

Identical to weeks 1-6 for the protocol previously described (Schmitz et al., 2010), participants engaged in a supervised, linearly-periodized 4-day/week resistance-training program split into two upper and two lower extremity workouts each wk. However, unlike the protocol of Schmitz et al. (2010) which involved 9 weeks or resistance training, we chose to assess the effectiveness of resistance training and supplementation for only six weeks. Prior to each workout, participants performed a standardized series of stretching exercises. The participants then performed an upper-body resistance-training program consisting of nine exercises (bench press, lat pull, shoulder press, seated rows, shoulder shrugs, chest flies, biceps curl, triceps press down, and abdominal curls) twice per week and a seven exercise lower extremity program (leg press, back extension, step ups, leg curls, leg extension, heel raises, and abdominal crunches) performed twice per week (Table 1). Participants performed 3 sets of 8-10 repetitions with as much weight as they could lift per set (typically 60-75% of 1RM) for weeks 1-3 and 3 sets of 4-8 repetitions with approximately 75-90% 1-RM for weeks 4-6. Rest periods between exercises and sets lasted no longer than 2 minutes. As participants underwent increases in strength, the training load was increased on the respective exercise so that they continued to maintain a training load of 70%-80% 1RM throughout the course of the study. Resistance training was conducted at the Student Life Center at Baylor University and supervised by study personnel.

Assessment of serum cortisol, insulin, IGF-1, and GH

From the two blood samples obtained at day 0 and day 43, serum samples were analyzed for cortisol, insulin, GH (Alpha Diagnostics, San Antonio, TX, USA), and IGF-1 (Enzo Life Sciences, Plymouth Meeting, PA, USA) using commercially-available enzyme-linked immunoabsorbent assay (ELISA) kits. For cortisol, this kit has a sensitivity of 0.4 $\mu g \cdot dl^{-1}$ and no cross-reactivity with epiandrosterone, 17-a-hydroxyprogesterone, progesterone, testosterone, or estradiol. The insulin kit has a sensitivity of 0.05 $\mu IU \cdot ml^{-1}$ and has no cross-reactivity with C-peptide, bilirubin, or hemoglobin. For GH, this kit has a sensitivity of 0.2 $ng \cdot ml^{-1}$ and no cross-reactivity with human chorionic gonadotropin (HCG) or prolactin. The sensitivity of the IGF-1 kit is 48.5 $pg \cdot ml^{-1}$, and does not cross-react with IGF-2, and IGFBPs 2-4, insulin, or GH. Absorbance's, which were directly proportional to the concentration of hormone in the sample, were measured in duplicate at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA). A set of standards of known concentrations of each hormone was utilized to construct a standard curve by plotting the net absorbance values of the standards against the respective protein concentrations. By applying a linear curve using data

Table 1. Resistance training program utilized in the study.

		WEEK 1-2-3	WEEKS 4-5-6	Rest
Day 1 and 3	1. Bench Press	**12,10,8**	**8,6,4**	1.5-2 minutes
	2. Lat pulldown	**12,10,8**	**8,6,4**	1.5-2 minutes
	3. Shoulder Press	**12,10,8**	**8,6,4**	1.5-2 minutes
	4. Seated Rows	**12,10,8**	**8,6,4**	1.5-2 minutes
	5. Shoulder Shrugs	**3x12**	**3x8**	30-60 sec.
	6. Chest Flys	**3x12**	**3x8**	30-60 sec.
	7. Bicep Curls	**3x12**	**3x8**	30-60 sec.
	8. Triceps Press down	**3x12**	**3x8**	30-60 sec.
	9. Abdominal Crunches	**125 Weighted Reps**	**125 Weighted Reps**	none
Day 2 and 4	1. Leg Press	**12,10,8**	**8,6,4**	1.5 -2/ 5min.
	2. Step-ups	**12,10,8**	**8,6,4**	1.5 -2/ 5min.
	3. Leg Extension	**3x12**	**3x8**	30-60 sec.
	4. Leg Curl	**3x12**	**3x8**	30-60 sec.
	5. Back Extension	**3x12**	**3x8**	30-60 sec.
	6. Heel/Calf Raises	**3x12**	**3x8**	30-60 sec.
	7. Abdominal Crunches	**200 Unweighted Reps**	**200 Unweighted Reps**	none

reduction software (Microplate Manager, Bio-Rad, Hercules, CA, USA), the serum concentrations of each hormone were calculated. The overall intra-assay percent coefficients of variation were 6.2%, 6.8%, 7.4%, and 5.8% for cortisol insulin, GH, and IGF-1, respectively.

Assessment of muscle total creatine

Total muscle creatine levels were analyzed spectrophotometrically by the diacetyl/α-napthtol reaction as previously described (Spillane et al., 2009). Briefly, approximately 10-15 mg of muscle tissue was cut and placed in a microfuge tube, and then placed in a vacuum centrifuge (Savant ISS110 SpeedVacTM Concentrator, Thermo Scientific, Milford, MA, USA) to be spun for 18 hours. After sufficient muscle drying, the samples were then placed in an ultra-low freezer at -80°C. Dried muscle was powdered by grinding on a porcelain plate with a pestle. Connective tissue was removed and discarded, whereas powdered muscle was placed into pre-weighed microfuge tubes. Powdered muscle immersed in a 0.5 M perchloric acid/1 mM EDTA solution on ice for 15-minutes, while periodically vortexing. Samples were then spun at 15,000 rpm at 4˚C for 5-minutes. The supernatant was transferred into a microfuge tube and neutralized with 2.1 M KHCO3/0.3 M MOPS solution and then centrifuged again at 15,000 rpm for 5-minutes. In order to determine muscle total creatine concentration, supernatant from the above reaction was combined with ddH2O and 0.4 N HCl and heated at 65˚C for 10-minutes to hydrolyze phosphate groups. The solution was then neutralized with of 2.0 N NaOH and the samples were allowed to incubate at room temperature allowing for color formation, which was detected by a spectrophotometer at 520 nm. Then the samples were run in duplicate against a standard curve of known creatine concentrations by applying a linear curve using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA, USA). The coefficient of variation was 5.67%.

Skeletal muscle cellular extraction

Based on our previous approach (Shelmadine et al., 2009; Spillane et al., 2009), approximately 20 mg of each muscle sample was weighed and subsequently homogenized using a commercial cell extraction buffer (Biosource, Camarillo, CA) and a tissue homogenizer. The cell extraction buffer was supplemented with 1mM phenylmethanesulphonylfluoride (PMSF) and a protease inhibitor cocktail (Sigma Chemical Company, St. Louis, MO) with broad specificity for the inhibition of serine, cysteine, and metallo-proteases.

Assessment of skeletal muscle proteins

From the two muscle tissue samples obtained at day 0 and day 43, total muscle protein was further isolated from the skeletal muscle cellular extracts with repeated incubations in 0.1% SDS at 50˚C and separated by centrifugation, and protein content was determined spectrophotometrically based on the Bradford method at a wavelength of 595 nm (Bradford, 1976). A standard curve was generated using bovine serum albumin (Bio-Rad, Hercules, CA, USA), and based on our previous approach, myofibrillar protein content was expressed relative to muscle wet-weight (Shelmadine et al., 2009; Spillane et al., 2009).

The assessment of total IRS-1, Akt, p70S6K, and 4EBP-1 was determined using commercially-available ELISA kits (Life Technologies, Grand Island, NY, USA) (Ferreira et al., 2014). The sensitivities of these kits are < 260 $pg \cdot ml^{-1}$, < 0.1 $pg \cdot ml^{-1}$, < 0.10 $ng \cdot ml^{-1}$, and < 0.34 $ng \cdot ml^{-1}$, respectively, for IRS-1, Akt, 4EBP-1, and p70S6K. Absorbance's, which were directly proportional to the concentration of protein in the sample, were measured in duplicate at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA). A set of standards of known concentrations of each protein was utilized to construct a standard curve by plotting the net absorbance values of the standards against the respective protein concentrations. By applying a linear curve using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA), the concentrations of each protein were calculated. The overall intra-assay percent coefficients of variation were 7.62%, 6.30%, 6.54%, and 4.59% for IRS-1, Akt, 4EBP-1, and p70S6K, respectively.

The MHC protein isoform composition was determined under denaturing conditions using an Experion Pro260 automated electrophoresis system (Bio-Rad, Hercules, CA, USA) using the principles of SDS-PAGE and LabChip (Caliper Life Sciences, Hopkinton, MA, USA) technology (Spillane et al., 2009; Willoughby et al., 2007). The Experion Pro260 analysis kit has a resolution and quantitation of 10-260 kDa proteins while also separating and detecting 2.5–2,000 $ng \cdot \mu l^{-1}$ protein. The Experion Pro260 system combines electrophoresis, staining, de-staining, imaging, band detection, and basic data analysis into a single, automated step. Gel images were then processed and displayed on a computer monitor and MHC bands identified by migration relative to the molecular weight marker (data not shown). The density of the MHC bands was determined using Experion Imaging software (Bio-Rad, Hercules, CA, USA), expressed in arbitrary density units.

Reported side effects from supplements

At the testing session on day 43, participants reported by questionnaire whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the protocol of the study.

Statistical analysis

Data were analyzed with separate 2 (group) x 2 (time) analysis of variance (ANOVA) using SPSS for Windows Version 20.0 software (SPSS, Chicago, IL). Significant differences among groups were identified by a Tukey HSD post-hoc test. However, to protect against Type I error, the conservative Hunyh-Feldt Epsilon correction factor was used to evaluate observed within-group F-ratios. An *a-priori* power calculation showed that 10 participants per group was adequate to detect a significant difference between groups in the dependent variable of muscle strength and the independent variable of resistance training, given a type I error rate of 0.05 and a power of 0.80. The index of effect size utilized was partial Eta squared ($\eta 2$), which estimates the proportion of variance

Table 2. Dietary intake variables before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Total Calories (kcals·day^{-1})	SIZE	2485.8 (804.5)	2566.3 (936.7)	.618	.509	.323
	PCC	2848.4 (786.2)	2446.5 (845.8)			
Protein (g·day^{-1})	SIZE	128.5 (54.1)	120.8 (46.4)	.544	.632	.968
	PCC	136.9 (34.2)	130.4 (67.8)			
Carbohydrate (g·day^{-1})	SIZE	265.7 (103.9)	304.4 (99.4)	.950	.620	.064
	PCC	319.9 (90.1)	253.6 (91.5)			
Fat (g·day^{-1})	SIZE	101.3 (40.3)	94.4 (47.9)	.205	.499	.916
	PCC	118.1 (44.9)	108.6 (33.2)			

Dietary caloric and macronutrient intake for the PCC (n = 12) and SIZE (n = 12) groups. No significant differences were detected for any of the dietary variables (p > 0.05).

in the dependent variable that can be explained by the independent variable. Partial Eta squared effect sizes were determined to be: weak = 0.17, medium = 0.24, strong = 0.51, very strong = 0.70 (O'Connor et al., 2007). For statistical procedures, a probability level of ≤ 0.05 was adopted throughout the study.

Results

Subject demographics

Twenty-six participants began the study; however, two dropped out due to reasons unrelated to the study. As a result, 24 participants completed the study. The PCC group (n = 12) had a mean (±SD) age of 20.61 ±2.46 years, height of 1.78 ± 0.04 m, and total body mass of 83.75 ±13.22 kg. The SIZE group (n = 12) had an age of 21.38 ±4.07 years, height of 180.58 ±5.20 cm, and total body mass of 8.63 ±13.52 kg.

Dietary analyses, supplement compliance, and reported side effects

The completed dietary intake forms were used to analyze the average daily caloric and macronutrient consumption (Table 2). Neither group significantly increased their caloric intake during the course of the study (p > 0.05). Furthermore, there were no significant group x test interactions indicating there to be no differences between groups for total calories (p = 0.323, effect size = 0.022) or for the intake of protein (p = 0.968, effect size = 0.001), carbohydrate (p = 0.064, effect size = 0.074), and fat (p = 0.916, effect size = 0.001).

In regard to compliance, PCC and SIZE were 88.32 ±9.12 % and 91.72 ±7.18 % compliant to the resistance training program, respectively. For supplementation compliance, PCC and SIZE were 93.94 ±3.55 % and 95.05 ±2.65 % compliant to the supplementation protocol, respectively. Over the course of the 6 weeks, three participants in PCC and two in SIZE reported side effects. For PCC, all participants reported feelings of nausea, 2 reported a rapid heart rate, and 1 reported shortness of breath. For SIZE, both participants reported dizziness, headache, and rapid heart rate, 1 participant reported feelings of nausea, shortness of breath, and nervousness.

Body composition

There were no significant differences in total body mass between groups (p = 0.866, effect size = 0.001) or as a result of resistance training (p = 0.788, effect size = 0.002). In addition, there were no significant changes occurring in total body water as a result of training (p = 0.863, effect size = 0.018) and supplementation (p = 0.655, effect size = 0.004). Fat mass was unchanged with resistance training (p = 0.325, effect size = 0.012) and supplementation (p = 0.448, effect size = 0.020). However, fat-free mass was significantly increased in both groups in response to training (p = 0.037; effect size = 0.130), but there were no significant differences between groups (p = 0.082, effect size = 0.100) (Table 3).

Muscle Performance

For muscle strength, there were no significant differences in upper-body strength with resistance training (p = 0.510, effect size = 0.009) and supplementation (p = 0.115, effect size = 0.002). However, lower-body strength demonstrated a significant increase with resistance training (p = 0.029, effect size = 0.095), but there was no significant

Table 3. Body composition before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Body Mass (kg)	SIZE	83.75 (13.22)	85.01 (13.22)	.866	.788	.948
	PCC	84.63 (13.52)	86.39 (13.77)			
Body Water (kg)	SIZE	44.17 (5.12)	45.26 (5.02)	.655	.358	.653
	PCC	45.52 (5.94)	46.24 (5.34)			
Fat Mass (kg)	SIZE	13.67 (5.12)	14.06 (17.05)	.448	.325	.384
	PCC	14.01 (7.33)	14.33 (7.73)			
Fat-Free Mass (kg)	SIZE	60.64 (8.02)	61.61 (7.89)	.082	.037	.849
	PCC	61.27 (6.89)	62.46 (6.57)			

Body composition for the PCC (n = 12) and SIZE (n = 12) groups. * denotes a significant increase at Day 43. Resistance training increased fat-free mass (p = 0.037) for both groups; however, there were no differences associated with supplementation (p > 0.05).

Table 4. Muscular performance before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Upper-Body Strength ($kg \cdot kg^{-1}$)	SIZE	1.24 ±0.144	1.28 ±0.143	.736	.510	.901
	PCC	1.26 ±0.282	1.30 ±0.272			
Upper-Body Endurance (reps)	SIZE	20.23 ±2.45	21.23 ±2.86	.840	.422	.946
	PCC	20.53 ±5.75	21.38 ±4.48			
Lower-Body Strength ($kg \cdot kg^{-1}$)	SIZE	5.06 ±0.508	5.23 ±1.12 *	.102	.029	.404
	PCC	4.81 ±0.585	5.14 ±0.649 *			
Lower-Body Endurance (reps)	SIZE	28.84 ±8.93	32.31 ±8.55	.658	.027	.869
	PCC	29.69 ±12.92	34.15 ±12.42			

Upper-body and lower-body strength and endurance for the PCC (n = 12) and SIZE (n = 12) groups. * denotes a significant increase at Day 43. Resistance training increased lower-body strength (p = 0.029) and endurance (p = 0.027) for both groups; however, there was no difference associated with supplementation (p > 0.05).

difference with supplementation (p = 0.102, effect size = 0.055) (Table 4).

Regarding muscle endurance, there were also no significant differences in upper-body endurance with resistance training (p = 0.422, effect size = 0.014) and supplementation (p = 0.840, effect size = 0.001). Although, lower-body endurance demonstrated a significant increase with resistance training (p = 0.027, effect size = 0.091), but there was no significant difference with supplementation (p = 0.658, effect size = 0.004) (Table 4).

Serum insulin, IGF-1, GH, and cortisol

Serum insulin demonstrated no significant differences with resistance training (p = 0.760, effect size = 0.002) and supplementation (p = 0.905, effect size = 0.001). In regard to IGF-1, there were no significant differences with resistance training (p = 0.950, effect size = 0.001) and supplementation (p = 0.361, effect size = 0.017). Similarly, no significant differences were noted for GH with resistance training (p = 0.819, effect size = 0.001) and supplementation (p = 0.169, effect size = 0.032). Cortisol also demonstrated no significant differences with resistance training (p = 0.182, effect size = 0.037) and supplementation (p = 0.608, effect size = 0.006) (Table 5).

Total muscle creatine, total muscle protein, and MHC protein isoform content

Total muscle creatine levels significantly increased in both groups (p = 0.044, effects size = 0.082) during the course of resistance training p = 0.020, effect size = 0.108); however, there was no significant Group x Test interaction (p = 0.279, effect size = 0.024). Total muscle protein content was significantly increased in both groups with training (p = 0.038, effect size = 0.092); however, there was no significant difference between groups (p = 0.230, effect size = 0.030).

For MHC1 (p = 0.041, effect size = 0.085) and MHC2A (p = 0.029, effect size = 0.095), both isoforms were significantly increased in both groups with training; however, there was no significant change for MHC2X (p = 0.158, effect size = 0.041). There was no significant difference between groups for MHC 1 (p = 0.878, effect size = 0.001), MHC 2A (p = 0.657, effect size = 0.004), MHC2X (p = 0.884, effect size = 0.001) (Table 6).

Total IRS-1, Akt, p70S6K, and 4EBP-1

For total IRS-1 (p = 0.041, effect size = 0.084) and Akt (p = 0.011, effect size = 0.087), both proteins were significantly increased in both groups with training; however, there was no significant change for total P70S6K (p = 0.403, effect size = 0.015) and 4EBP-1 (p = 0.276, effect size = 0.025). There was no significant difference between groups for total IRS-1 (p = 0.073, effect size = 0.065), Akt (p = 0.442, effect size = 0.034), P70S6K (p = 0.136, effect size = 0.046), and 4EBP-1 (p = 0.431, effect size = 0.013) (Table 7).

Discussion

The purpose of this study was to determine the effects of SizeOn Maximum Performance versus a conventional protein/carbohydrate/creatine comparator supplement on indices of muscular adaptations to resistance training in young men. We demonstrated that a daily 50 gram dose of the multi-ingredient ergogenic dietary supplement

Table 5. Serum variables before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Insulin ($\mu IU \cdot ml^{-1}$)	SIZE	18.84 (5.57)	21.1 (7.4)	.905	.760	.433
	PCC	20.22 (10.72)	19.2 (4.2)			
IGF-1 ($pg \cdot ml^{-1}$)	SIZE	3317.9 (740.4)	3207.6 (822.7)	.361	.950	.750
	PCC	3491.4 (1267.8)	3565.5 (1215.5)			
HGH ($pg \cdot ml^{-1}$)	SIZE	206.8 (277.7)	275.9 (281.3)	.169	.819	.891
	PCC	229.8 (280.3)	277.1 (245.1)			
Cortisol ($pg \cdot ml^{-1}$)	SIZE	3120.8 (204.7)	3051.9 (160.6)	.608	.182	.727
	PCC	3109.5 (214.0)	2992.4 (362.4)			

Serum variables for the PCC (n = 12) and SIZE (n = 12) groups. There were no significant differences associated with resistance training (p > 0.05) or supplementation (p > 0.05).

Table 6. Muscle protein levels before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Total Creatine (µmol·mg^{-1})	SIZE	3.48 (.75)	4.80 (2.42) *†	.044	.020	.279
	PCC	3.12 (.72)	3.61 (.562) *†			
Total Protein (µg·mg^{-1})	SIZE	.070 (.024)	.083 (.039) *	.230	.038	.623
	PCC	.089 (.026)	.096 (.029) *			
MHC 1 (arbitrary density units)	SIZE	1101.7 (391.0)	1394.1 (419.1) *	.878	.041	.873
	PCC	1137.6 (536.6)	1398.4 (531.6) *			
MHC 2A (arbitrary density units)	SIZE	1084.7 (611.9)	1336.5 (538.7) *	.657	.029	.501
	PCC	1278.5 (484.9)	1504.7 (342.6) *			
MHC 2X (arbitrary density units)	SIZE	892.9 (521.4)	836.2 (385.9)	.884	.158	.120
	PCC	990.7 (188.3)	767.0 (199.0)			

Muscle protein levels for the PCC (n = 12) and SIZE (n = 12) groups. * denotes a significant increase at Day 43. † Denotes a significant increase for Group. Both supplement groups underwent significant increases in total creatine levels (p = 0.044). Resistance training increased total protein content (p = 0.038) as well as the protein content of Type I (p = 0.041) and Type IIA (p = 0.029) MHC.

SizeOn Maximum Performance for 42 days combined with resistance training did not increases muscle mass and strength, nor did it elevate serum hormones and growth factors, augment skeletal muscle signaling pathway markers indicative of muscle protein synthesis, or increase total muscle and MHC protein content when compared to an equivalent daily dose of a protein/carbohydrate/creatine comparator.

The outcomes we observed in body composition and muscle strength in both groups are similar to those we observed in our previous studies involving both four (Shelmadine et al., 2009) and 10 weeks of heavy resistance training (Willoughby et al., 2007). These data help to substantiate the effectiveness of the resistance training protocol in our current study. However, the changes we observed in the current study do not agree with a similar study also using SizeOn Maximum Performance which used the identical training program as the current study, but with nine weeks of resistance training (Schmitz et al., 2010). In the current study, both groups were highly compliant to the resistance training program and the supplementation protocol, and there were no significant differences between groups in either of these compliance variables. In addition, neither group underwent significant changes in their dietary intake during the course of the study and nor none of the dietary variables assessed were significantly different between groups. In the Schmitz et al. (2010) study both groups significantly increased muscle strength and endurance and fat-free mass, while decreasing fat mass; however, these effects were preferentially improved in the group supplemented with SizeOn Maximum Performance. However, in our current study, we observed no preferential effects from SizeOn Maximum Performance supplementation. Unlike the Schmitz et al. (2010) study, in the current study resistance training sessions were directly supervised by study personnel. Therefore, in lieu of the lack of agreement between the two studies, the most conceivable explanation for this incongruence in results certainly could be the lack of supervision of the resistance training program in the Schmitz et al. (2010) study and the shorter resistance training duration involved in our current study.

Supplement dosage may also explain the lack of difference between the two supplements. Both supplement groups were receiving four grams of creatine daily. This dose for creatine is lower than the typical five to seven grams/day which has been demonstrated by a number of studies to be effective at inducing ergogenic benefits in conjunction with resistance training (Volek et al., 1999; Willoughby and Rosene, 2001). In the Willoughby and Rosene (2001) study, five grams of creatine each day with resistance training resulted in preferential increases in muscle strength and mass that mirrored increases in total muscle protein content and MHC protein composition. In the present study, we observed resistance training-induced increases in these variables, but none were significantly different between groups. However, since we did not include a placebo group, it makes it difficult to specifically determine if our outcomes may have been due to the supplementation protocol, and not simply by resistance training.

In addition, both groups were receiving whey

Table 7. Total Phopshoprotein levels of insulin signaling pathway intermediate before and after SizeOn maximum performance supplementation and resistance training. Values are means (±SD).

Variable	Group	Day 0	Day 43	Group (p ≤ .05)	Test (p ≤ .05)	Group x Test (p ≤ .05)
Total IRS-1 (ng·mg^{-1})	SIZE	.657 (.157)	.807 (.285) *	.073	.041	.154
	PCC	.610 (.127)	.767 (.149) *			
Total Akt (ng·mg^{-1})	SIZE	.365 (.160)	.498 (.111) *	.442	.011	.142
	PCC	.371 (.120)	.505 (.141) *			
Total p70S6K (ng·mg^{-1})	SIZE	.014 (.004)	.019 (.011)	.136	.403	.467
	PCC	.015 (.005)	.013 (.003)			
Total 4EBP-1 (ng·mg^{-1})	SIZE	.076 (.039)	.096 (.042)	.431	.276	.779
	PCC	.091 (.057)	.102 (.071)			

Muscle phosphoprotein levels for the PCC (n = 12) and SIZE (n = 12) groups. * denotes a significant increase at Day 43. Resistance training increased total IRS-1 (p = 0.041) and Akt (p = 0.011) protein content.

protein and maltodextrose from their respective supplement at a daily dose of seven and 39 grams, respectively. Incidentally, seven grams of whey protein possesses an amount of leucine far below the so-called "leucine threshold" of three to four grams, necessary for increasing MPS (Breen and Phillips, 2011). As such, we have previously demonstrated that a 10-gram dose of whey protein following resistance exercise has been shown to be ineffective at increasing the activity of signaling intermediates, such as IRS-1, Akt, p70S6K, and 4EBP-1, indicative of MPS (Willoughby et al., 2011). However, Tang et al. (2007) did find that the combination of 10 grams of whey protein with 21 grams of fructose carbohydrate was more effective than 31 grams of carbohydrate (21 grams as fructose and 10 grams as maltodextrin) at increasing MPS following resistance exercise. We also previously demonstrated that five grams of leucine combined with 120 grams of carbohydrate (as maltodextrin) following resistance exercise was no more effective than 120 grams of carbohydrate at increasing the activity of the MPS signaling intermediates, IRS-1, Akt, mTOR, and P70S6K (Ferreira et al., 2014).

In regard to the 39 grams of carbohydrate from each supplement in the current study, several studies have demonstrated that the addition of anywhere between 30 grams to 90 grams of carbohydrate to a protein dose that is known to effectively stimulate MPS (20-25 grams) has no additive or synergistic effect on MPS and muscle protein breakdown (Glynn et al., 2013; Koopman et al., 2007; Staples et al., 2011).

We have previously shown (Willoughby et al., 2007) that 10 weeks of resistance training combined 40 grams of carbohydrate (maltodextrose) or a protein supplement (16 grams of whey, 12 grams of casein, 12 grams of essential amino acids) equally provided one hour before and immediately following resistance exercise were both effective at increasing muscle strength and mass and total muscle and MHC protein content, whereas the protein supplement was preferentially more effective. From this study, our data are indicative of the ergogenic nature of both protein and carbohydrate supplementation when combined with resistance training. Indeed, similarly in the current study we demonstrated that both groups effectively increased muscle strength and mass over the course of the study, presumably as a result of their respective supplement. However, without the inclusion of a true placebo group, we can only assume that these results would have been different to resistance training alone. The same issue arises for the Schmitz et al. (2010) study. Even though they did report a preferential, ergogenic effect for SizeOn Maximum Performance, without comparison to a true placebo group their results should be interpreted with caution, as the likelihood that their results may not be any different than only resistance training is conceivable.

In the current study, the primary difference between the two supplements was the inclusion of the various vitamins and minerals, pterostilbene, BCAAs, L-taurine, and L-alanyl-L-glutamine in SizeOn Maximum Performance. Therefore, if any difference occurred it would most likely be due to this difference between supplements. While much is known about the beneficial effects of BCAAs on muscle protein synthesis and breakdown, less is known about pterostilbene and exercise performance, and there appear to be no published studies on this topic.

It has been shown that seven days of L-taurine supplementation, while producing a 13-fold increase in plasma taurine levels, did not increase muscle taurine content or alter substrate metabolism during prolonged exercise in humans (Galloway et al., 2008). Therefore, in this scenario it is unlikely that L-taurine supplementation would have beneficially impacted muscle performance. However, a study involving 28 days of supplementation with a multi-nutrient product containing BCAAs, L-taurine, anti-inflammatory plant extracts, and B vitamins in middle-aged men (Dunn-Lewis et al., 2011) demonstrated decreases in markers indicative of systemic inflammation along with increases in vertical jump power and isometric grip strength. In regard to the dipeptide L-alanyl-L-glutamine, a study sought to examine the efficacy of acute supplementation during hydration stress in endurance exercise (Hoffman et al., 2010). Results demonstrated that L-alanyl-L-glutamine provided a significant ergogenic benefit by increasing time to exhaustion during mild hydrate stress, likely due to an enhanced fluid and electrolyte uptake.

Limitations

In view of the results presented herein, our study does possess three possible limitations. One limitation may be the sample size. While a sample size of 24 is somewhat small, indeed it is notably larger than many other studies in the literature employing a very similar experimental design. We did perform an *a-priori* power analysis; therefore, our study should be adequately powered. The second limitation is the issue of bioavailability, as we did not assess the serum levels of any of the product's ingredients; although whey protein, creatine, BCAAs, carbohydrate, minerals, and B vitamins are known to be quite bioavailable. The third limitation is supplementation compliance. Even though participants returned the empty containers and reported their compliance, we cannot be entirely sure as to their complete level of compliance. The fourth limitation is the failure to include a placebo group. Even though we observed significant effects in both groups, we cannot be sure that they may not be any different that resistance training alone. Despite our confidence in the reliability and validity of our data, in lieu of these limitations, our results with respect to the apparent lack of effectiveness of SizeOn Maximum Performance should be interpreted with some amount of caution.

Conclusion

Based on the outcomes and limitations of the present study, it is clear that more research needs to be conducted on SizeOn Maximum Performance supplementation in humans regarding its ability to increase the activity and content of protein signaling intermediates involved in muscle protein synthesis, along with its potential ability to increase muscle strength, mass, and performance. However, based on the results of the current study we con-

clude that 42 days resistance training combined with SizeOn supplementation, at a daily dose of 50 g, does not increase the content of signaling intermediated indicative of MPS or total muscle and MHC protein content, nor does it preferentially increase skeletal muscle mass and strength in resistance-trained males when compared to an equivalent daily dose of a supplement containing protein, carbohydrate, and creatine.

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Key points

- In response to 42 days of heavy resistance training and either SizeOn Maximum Performance or protein/carbohydrate/creatine supplementation, similar increases in muscle mass and strength in both groups occurred; however, the increases were not different between supplement groups.
- The supplementation of SizeOn Maximum Performance had no preferential effect on augmenting serum insulin, IGF-1, and GH, or in decreasing cortisol.
- While resistance training was effective in increasing total creatine content in skeletal muscle, myofibrillar protein, and the content of total IRS-1 and Akt, it was not preferentially due to SizeOn Maximum Performance supplementation.
- At the daily dose of 50 g, SizeOn Maximum Performance supplementation for 42 days combined with resistance training does not increases muscle mass and strength due to its ability to elevate serum hormones and growth factors or in its ability to augment skeletal muscle signaling pathway markers indicative of muscle protein synthesis when compared to an equivalent daily dose of protein/carbohydrate/creatine.

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