Effects of Probiotic (*Bacillus subtilis*) Supplementation During Offseason Resistance Training in Female Division I Athletes

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Abstract

Toohey, JC, Townsend, JR, Johnson, SB, Toy, AM, Vantrease, WC, Bender, D, Crimi, CC, Stowers, KL, Ruiz, MD, VanDusseldorp, TA, Feito, Y, and Mangine, GT. Effects of probiotic (*Bacillus subtilis*) supplementation during offseason resistance training in female Division I athletes. *J Strength Cond Res* XX (X): 000–000, 2018—We examined the effects of probiotic (*Bacillus subtilis*) supplementation during offseason training in collegiate athletes. Twenty-three Division I female athletes (19.6 ± 1.0 years, 67.5 ± 7.4 kg, and 170.6 ± 6.8 cm) participated in this study and were randomized into either a probiotic ($n = 11$; DE111) or placebo ($n = 12$; PL) group while counterbalancing groups for sport. Athletes completed a 10-week resistance training program during the offseason, which consisted of 3–4 workouts per week of upper- and lower-body exercises and sport-specific training. Athletes consumed DE111 (DE111; 5 billion CFU/day) or PL supplement daily for the entire 10-week program. Before and after training, all athletes underwent 1 repetition maximum (1RM) strength testing (squat, deadlift, and bench press), performance testing (vertical jump and pro-agility), and isometric midthigh pull testing. Body composition (body fat [BF]%) was completed using BODPOD and bioelectrical impedance analysis, as well as muscle thickness (MT) measurement of the rectus femoris (RF) and vastus lateralis using ultrasonography. Separate repeated-measures analyses of variance were used to analyze all data. Significant ($p \leq 0.05$) main effects for time were observed for improved squat 1RM, deadlift 1RM, bench press 1RM, vertical jump, RF MT, and BF%. Of these, a significant group × time interaction was noted for BF% ($p = 0.015$), where greater reductions were observed in DE111 (−2.05 ± 1.38%) compared with PL (−0.2 ± 1.6%). No other group differences were observed. These data suggest that probiotic consumption in conjunction with post-workout nutrition had no effect on physical performance but may improve body composition in female Division I soccer and volleyball players after offseason training.

Key Words gut microbiota, body fat, athletic performance, sport

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Introduction

Interactions between the gut microbiota and host play an important role in the regulation of a multitude of physiological processes. Current evidence suggests that gut-host communication affects cognition (25), epithelial protection, mitochondrial function (3), and may shape metabolic and immune network activity (2,6). Strenuous physical exertion elicits both localized muscular disruptions and systemic physiological stress. Research evidence suggests that high-intensity exercise may be linked to an impaired gut barrier, resulting in endotoxin translocation, proinflammatory cytokine production, and impaired nutrient absorption (37,38). For athletes, maintaining a healthy gut barrier is important because gastrointestinal dysfunction and impaired nutrient absorption have been suggested to adversely affect acute exercise performance and blunt subsequent training adaptations (4).

Probiotics consist of live microorganisms that use benefits to their host primarily by supporting the proliferation of beneficial gut microflora (2). Furthermore, probiotics modulate the frequency of the tight junction proteins that regulate the permeability of the intestinal paracellular pathway (17). By enhancing intestinal barrier function, probiotics serve as preventative agents to defend against adverse effects of pathogens (17), promoting positive effects on digestion and

immune health (6,22,34,41). In addition, it seems that the beneficial effects of probiotics may be strain specific, with most of probiotic studies investigating *Bifidobacterium* and *Lactobacillus* strains in various special groups (e.g., diabetic and obese) of the general population (2,16,17,29). It is noteworthy that probiotics of the *Bacillus* strain have been shown to be well tolerated in healthy populations (8) and have garnered attention recently for their potential beneficial effects in a recreationally active population (12). However, additional human trials evaluating the efficacy of these probiotic strains are needed to provide evidence-based recommendations to patient and clients.

Currently, data on the efficacy of probiotic administration in the athletic population are limited. A bulk of the current literature shows promising effects of probiotics for prevention of acute and chronic illness in endurance athletes during times of intense training (6,41,42). However, much less is known about the potential benefits of probiotics that may confer to athletes who regularly engage in resistance exercise. Recently, it has been reported that coingestion of a probiotic supplement and protein after muscle-damaging exercise resulted in improved perceived recovery, decreased muscle soreness, and tended to decrease markers of muscle damage during 72 hours of recovery (12). In addition, it was shown that 21 days of probiotic supplementation attenuated circulating interleukin-6 concentrations and range of motion decrements in the initial 48 hours after a damaging bout of eccentric resistance exercise (11). Taken together, it seems that probiotics may have immunomodulatory properties that could aid in the acute regenerative capacity of skeletal muscle repair and functional recovery. Improved acute recovery could potentially allow for increased training capacity in subsequent exercise bouts, leading to enhanced training adaptations.

Although studies have evaluated the potential benefit of probiotics on acute recovery from resistance exercise, to date, we have a limited understanding regarding effects of probiotics on chronic adaptations to resistance training. Thus, the objective of the current study was to determine the effects of *Bacillus subtilis* (DE111) probiotic supplementation on muscle thickness (MT) and strength, body composition, and athletic performance in Division I female volleyball and soccer athletes.

Methods

Experimental Approach to the Problem

A study overview is presented in Figure 1. Participants reported to the Human Performance Lab (HPL) on 2 separate occasions at the beginning and end of the 10-week training intervention after a 10-hour overnight fast. In addition, athletes were instructed to report to all performance and laboratory testing in a hydrated state while abstaining from caffeine, alcohol, and vigorous exercise for at least 24 hours. Activity more than light running, stretching, or calisthenics was defined as vigorous (e.g., anaerobic conditioning, sprinting, or resistance training). When athletes reported for testing, these conditions were confirmed verbally by an investigative team member. During laboratory visits (session 1), the participants were tested for body composition, muscle architecture, and isometric power. Assessments were conducted in the morning, and each participant reported to the laboratory at the same time of day before and after intervention. After session 1 testing, athletes reported to their strength and conditioning coordinator on 2 separate occasions before and after training, to measure 1 repetition maximum (1RM) for bench press, squat, and deadlift along with testing vertical jump and pro-agility (sessions 2 and 3). Performance testing with the strength and conditioning coach took place over the course of 2 days before and after training and was conducted after all laboratory procedures. After 1RM testing, participants were randomized into a probiotic (volleyball = 6 and soccer = 5) and a placebo group (volleyball = 4 and soccer = 8) matched for squat 1RM.

Subjects

Twenty-three Division I female athletes (mean ± *SD*, 19.6 ± 1.0 years, 67.5 ± 7.4 kg, and 170.6 ± 6.8 cm) from the university's volleyball ($n = 10$) and soccer ($n = 13$) teams participated in this double-blind, placebo-controlled, randomized study. After an explanation of all procedures, risks, and benefits, each participant provided their written informed consent before participation in this study. The research protocol was approved by the institutional review board of Lipscomb University before participant enrollment. Exclusion criteria included the use of medication or other probiotic supplementation, ergogenic aids, or suffering from any medical, muscular, or metabolic contraindications. No restrictions were put on athlete exercise outside of their offseason training protocol. However, because of the time commitment of regular offseason training, athletes verbally reported negligible exercise outside of team activities.

Procedures

Body Composition: Air Displacement Plethysmography. Body density and fat-free mass (FFM) were estimated using air displacement plethysmography using the BODPOD (COSMED, Rome, Italy). Before each test, the BODPOD was calibrated according to the manufacturer's instructions using a 2-point calibration. Before testing, athletes were instructed to wear a sports bra, tight-fitting compression shorts, and a swimming cap, as well as to remove all metal, including jewelry and watches. Body mass (BM) was measured to the nearest 0.01 kg using the system's calibrated scale. All athletes were instructed to sit in the chamber, breathe normally, and to minimize any movement. A minimum of 2 trials were performed. If measurements were not within 150 ml of each other, a third trial was conducted. Thoracic gas volume was estimated using BODPOD software, which uses standard prediction equations and has demonstrated no difference compared with measured lung volumes (21).

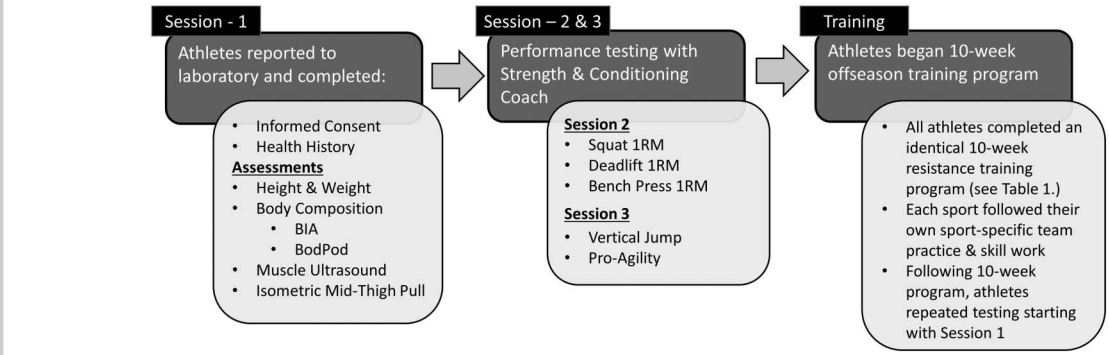

Figure 1. Study overview. All testing during sessions 1–3 occurred over the course of 5 days. All session 1 testings were completed before 2 and 3. Session 2 and session 3 occurred at least 24 hours apart. Ten-week training program began the following Monday after all testing sessions. 1RM = 1 repetition maximum; BIA = bioelectrical impedance analysis.

Body Composition: Bioelectrical Impedance Analysis. Total body water (TBW) was determined using multifrequency bioelectrical impedance analysis (BIA) using the InBody 570 Body Composition Analyzer device (Biospace, Inc., Seoul, Korea). Body composition from BIA is obtained from the measures of resistance and reactance when an electrical current travels throughout the body. Before each assessment, the participants' hands and feet were thoroughly cleaned with InBody provided tissues. Age, height, and sex were manually entered, whereas a scale positioned within the device assessed BM. The participant was then instructed from the software to stand fully erect on the measurement electrodes situated on the platform and to hold hand electrodes, with arms extended, without touching the sides of their body. Participants were asked to refrain from moving or talking until the assessment was completed. It has previously been shown that BIA is a valid measurement tool for determining TBW when compared with a deuterium oxide technique (1).

Body Composition: Three-Compartment Model (3C-W). The criterion percent body fat (%BF) was estimated using the 3-compartment water (3C-W) model described by Siri (32). The equation includes measurements of body density (from the BODPOD), TBW (from the BIA), and BM. The equation for %BF is listed below:

$$\%BF = \{(2.118/\text{Body density}) - (0.78 \times \text{TBW[L]}/\text{BM[kg]}) - 1.354\} \times 100.$$

Muscle Ultrasonography. Noninvasive measurements of MT were collected using B-mode ultrasound imaging with a 12-MHz linear probe (LOGIQ P5; General Electric Co., Wauwatosa, WI, USA). Measurements for the rectus femoris (RF) were taken at 50% of the distance from the anterior, inferior suprailiac spine to the most proximal point of the patella. Vastus lateralis (VL) measurements were taken in the same fashion as previously stated; however, the sampling location is determined by 50% of the straight-line distance between the greater trochanter and the lateral epicondyle of the femur (14). Before image collection, participants lay supine for 5 minutes, and the probe was coated with a water-based conduction gel. For measurements of MT, the probe was oriented longitudinally in the sagittal plane parallel to the muscle tissue without depressing the skin. Once images were collected, analysis was completed using Image J software (version 1.45 s; National Institutes of Health, Bethesda, MD, USA). Muscle thickness was determined from the still image as the distance between the inferior border of the superficial aponeurosis and the superior border of the deep aponeurosis. Intraclass correlation coefficients ($ICC_{3,k}$) standard error of the mean (*SEM*), and minimal difference (MD) for the ultrasound technician were calculated for the RF MT ($ICC_{3,k} = 0.99$, $SEM_{3,k} = 0.02$, MD = 0.07 cm) and VL MT ($ICC_{3,k} = 0.99$, $SEM_{3,k} = 0.05$, MD = 0.14 cm) from analysis of 10 individuals separated by 24 hours.

Dynamic Strength Testing. One-repetition maximum strength was assessed in the bench press, squat, and deadlift exercises. All 1RM testing was performed using methods previously described (36). Before testing, each athlete completed a general warm-up led by the strength and conditioning coach, which included jogging and a dynamic warm-up. Each athlete performed 2 warm-up sets using a resistance of approximately 40–60% and 60–80% of her perceived maximum, respectively. For each exercise, 3–4 subsequent trials were performed to determine the 1RM. A 3–5-minute rest period was provided between each trial. Trials not meeting the range of motion criteria for each exercise or where proper technique was compromised were discarded.

Isometric Strength Testing. The isometric midthigh pull (IMTP) test was used to assess peak force (PF) and rate of force development from 0–250 ms (RFD 250 ms).The midthigh position was determined for each participant before

Table 1. Ten-week offseason resistance training program.*

Day 1	Set × reps	Day 2	Set × reps	Day 3	Set × reps
Phase I (weeks 1–2)					
Bench press	4 × 3–5	Hang clean	4 × 3–5	Power clean high pull	4 × 3–5
Band pull apart	60 reps	Squat	4 × 3–5	Terminal knee extension	4 × 12
Eccentric pull-ups	2 × 8	Box jump	5 × 3	Deadlifts	4 × 3–8
1-arm DB shoulder press	4 × 8	Glute-ham raise	3 ×:45	Incline DB bench	4 × 6
Inverted row	3 ×:30	Isometric goblet squat	3 ×:45	Hanging knee to chest	3 × 8
DB shrugs	3 ×:30	Barbell glute bridge	3 ×:45	Keiser 1-arm rot. press	3 × 8
Front raise	3 ×:30	Good mornings	3 ×:45	TGU	3 × 8
Incline DB row	3 ×:30			DB row	3 × 8
Phase II (weeks 3–6)					
Bench press	4 × 3–5	Hang clean	4 × 3–5	Power clean	4 × 3–5
Band pull apart	3 × 20	Squat	4 × 3–5	Ankle touches	3 × 3
Band assist pull-ups	3 × 8	Hip flexor stretch	:15	Deadlifts	4 × 3–5
DB shoulder press	3 × 10	Box jump	3 × 3	Incline DB bench	3 × 8
TRX row	3 × 10	Swiss ball leg curl	3 × 10	Swiss ball pike	3 × 10
Ext. rotation	3 × 10	Single-leg squat	3 × 5	1-arm rot. press	3 × 10
Lateral raise	3 × 10	Vertimax jumps	3 × 3	Kettle bell windmill	3 × 10
Keiser pull-down	3 × 10	Cross-over step-ups	3 × 8	Landmine row	3 × 10
Push-up	3 × 10				
Phase III (weeks 7–10)					
Bench press	4 × 3–5	Hang clean	4 × 3–5	Jump power shrugs	4 × 3–5
Band pull apart	4 × 20	Squat	4 × 3–5	Terminal knee extension	3 × 12
Band assisted pull-ups	4 × 6	Hip flexor stretch	:15	Deadlifts	4 × 3–5
DB push press	3 × 6	Box jump	4 × 3	Lateral band walk	3 × 5
Keiser 1-arm row	3 × 10	Swiss ball leg curl	3 × 10	Band press	3 × 5
DB shrugs		Single-leg squat	3 × 5	Kettlebell Halo	3 × 5
Face pulls	3 × 10	Vertimax jumps	3 × 3	Unsupported row	3 × 5
TRX push-up	3 × 10	Step-up	3 × 8		

*DB = dumbbell; TGU = Turkish get-up; TRX = total resistance exercise.

testing by marking the midpoint distance between the knee and hip joints. Each participant was instructed to assume their preferred deadlift position by self-selecting their hip and knee angles. The height of the barbell was then adjusted up or down to make sure it is in contact with the midthigh. An overhand grip with lifting straps was used to ensure that grip strength did not limit their capacity to pull maximally. The participants were instructed to pull upward on the barbell as hard and as fast as possible and to continue their maximal effort for 6 seconds. The force-time curve for each trial is recorded by a force plate (PASCO, Roseville, CA, USA) with a sample rate of 1,000 Hz similar to previous work (36). Peak force was defined as the highest force achieved during the 6-second isometric test minus the participant's body mass in Newtons. The RFD was then calculated with the following equation: RFD = ΔForce/ΔTime. The RFD equation was applied to the predetermined time band of 0–250 ms, which is in accordance with previous studies demonstrating high reliability (20).

Performance Testing. A vertical jump testing station (Uesaka Sport, Colorado Springs, CO, USA) was used to assess vertical jump height (±1.27 cm). Before the test, each athlete's standing vertical reach height was determined by colored squares located along the vertical neck of the device. These squares correspond with similarly colored markings on each horizontal tab, which indicate the vertical distance from the associated square. Vertical jump height was determined by the indicated distance on the highest tab reached after 3 maximal, countermovement jump attempts performed from a standing position with feet shoulder width apart.

For the pro-agility test, 3 cones were placed parallel, 5 meters apart. The athletes set up for the test in a straddle position facing the middle cone. On their ready, the athletes were instructed to pivot to their right and accelerate as quickly as possible to a cone 5 m away and then on touching the first cone, pivot again to their left, and sprint the 10-m distance to the furthest cone. On touching this cone, the athletes once again pivoted to the right to return to the middle cone as quickly as possible. During each change in direction, the athletes were asked to touch the ground next to the cone. Trials where the athlete failed to touch the ground were discarded. Athletes were allowed for 3 attempts, and the fastest time measured in seconds was recorded.

Table 2. Strength and performance changes after 10 weeks of offseason training.*†

Variable	Group	Before	After	Time	Time × group
Strength measures					
Squat 1RM (kg)	DE111	73.3 ± 11.2	87.1 ± 12.6	$p < 0.000$	$p = 0.394$; $n^2 = 0.043$
	PL	74.1 ± 15.3	93.4 ± 19.0		
Deadlift 1RM (kg)	DE111	85.0 ± 14.5	96.0 ± 11.2	$p < 0.000$	$p = 0.343$; $n^2 = 0.056$
	PL	81.8 ± 13.1	90.6 ± 16.4		
Bench press 1RM (kg)	DE111	45.3 ± 8.0	48.0 ± 8.5	$p < 0.000$	$p = 0.633$; $n^2 = 0.012$
	PL	42.8 ± 5.3	46.9 ± 6.3		
Performance measures					
Vertical jump (cm)	DE111	50.8 ± 5.9	53.3 ± 6.1	$p < 0.000$	$p = 0.405$; $n^2 = 0.041$
	PL	54.2 ± 7.8	56.0 ± 8.4		
Pro-agility (sec)	DE111	5.07 ± 0.23	5.11 ± 0.21	$p = 0.077$	$p = 0.794$; $n^2 = 0.004$
	PL	4.97 ± 0.17	5.04 ± 0.19		
IMTP PF (N)	DE111	1,570.3 ± 303.7	1,598.1 ± 282.6	$p = 0.150$	$p = 0.351$; $n^2 = 0.049$
	PL	1,482.5 ± 208.7	1,607.6 ± 221.5		
IMTP RFD 250 ms (N)	DE111	3,450.5 ± 1833.0	3,336.0 ± 1,676.5	$p = 0.923$	$p = 0.761$; $n^2 = 0.005$
	PL	3,045.4 ± 1,340.5	3,104.8 ± 1,311.9		

*1RM = 1 repetition maximum; IMTP = isometric midthigh pull; PF = peak force; RFD = rate of force development.
†All data presented as mean ± *SD*.

Supplementation Protocol. Participants were required to consume a probiotic (DE111) or placebo (PL) once a day for 10 weeks. The probiotic supplement consisted of 5 billion colony-forming units *B. subtilis* (DE111; Deerland Enzymes, Kennesaw, GA, USA). On training days, supplementation occurred immediately after workout as a study investigator provided each athlete with their individual supplement (DE111 or PL), and each athlete consumed a protein and carbohydrate recovery drink (Gatorade Recover; Gatorade Co., Chicago, IL, USA) consisting of 45 g of carbohydrates, 20 g of protein, and 2 g of fat. This recovery drink was chosen to maximize postprandial muscle protein synthesis (26) and to remain within the National Collegiate Athletic Association (NCAA) macronutrient guidelines for nutritional support. On weekend or nontraining days, athletes were provided their supplement in individually label bags, were required to consume their supplement with a normal meal, and were asked to return their empty bags.

Dietary Logs. During the training and supplemental intervention, participants were asked to complete a 3-day food log (2 weekdays and 1 weekend day) on 2 separate weeks. No dietary restrictions were placed on the athletes besides abstaining from other supplemental use, and athletes refrained after any special dietary program (e.g., Atkins, ketogenic, and vegan). Dietary recalls were used to provide an estimate of total kilocalorie intake (kcal) and

Table 3. Body composition changes after 10 weeks of offseason training.*

Variable	Group	Before	After	Time	Time × group
Body mass (kg)	DE111	70.0 ± 8.4	69.7 ± 7.6	$p = 0.171$	$p = 0.055$; $n^2 = 0.181$
	PL	66.6 ± 5.1	68.2 ± 5.4		
Fat-free mass (kg)	DE111	114.2 ± 10.4	116.2 ± 11.0	$p = 0.098$	$p = 0.631$; $n^2 = 0.013$
	PL	114.3 ± 8.2	117.8 ± 8.4		
Body fat (%)	DE111	25.06 ± 3.98	23.01 ± 2.94	$p < 0.000$	$p = 0.015$; $n^2 = 0.289$
	PL	21.0 ± 5.36	20.0 ± 5.25		
Rectus femoris thickness (cm)	DE111	2.22 ± 0.29	2.29 ± 0.27	$p = 0.015$	$p = 0.500$; $n^2 = 0.024$
	PL	1.98 ± 0.32	2.09 ± 0.28		
Vastus lateralis thickness (cm)	DE111	1.75 ± 0.31	1.71 ± 0.22	$p = 0.623$	$p = 0.308$; $n^2 = 0.151$
	PL	1.42 ± 0.28	1.49 ± 0.23		

*All data presented as mean ± *SD*.

macronutrient distributions (carbohydrate, protein, and fat) of the athlete's typical weekly diet. All dietary analysis was completed using the MyFitnessPal application (Under Armour Inc., Baltimore, MA), which contains a large, detailed U.S.-branded food database.

Offseason Training Protocol. All athletes completed the same periodized (traditional linear) resistance training program for 10 weeks (3 $d \cdot wk^{-1}$) (Table 1). The program incorporated upper- and lower-body workouts centered on 3 core lifts (bench press, squats, and deadlifts) and commonly referred to as the "Wendler 5/3/1" (40). This program organizes progressions on each core exercise over 4-week segments (i.e., 1 week of 3 sets of 5 repetitions, followed by 1 week of 3 sets × 3 repetitions, followed by 1 week of 1 × 5/3/1 repetitions, and then finally a lighter "unloading" week of 3 sets × 5 repetitions). Accessory lifts followed a higher volume pattern (i.e., 3–4 sets and 8–12 repetitions). In addition to strength training, the athletes participated in team conditioning, agility, jumping, and sprint work (3 sessions $\cdot wk^{-1}$). These workouts consisted of approximately 30–40 minutes of sport-specific skill development and conditioning-related work. All training sessions were performed under the supervision of a certified strength and conditioning specialist.

Statistical Analyses

Statistical evaluation of performance, anthropometric, and subjective data was accomplished using separate 2-way (group × time) repeated-measures analysis of variance (RMANOVA). Before the RMANOVA, all data were assessed for normal distribution, homogeneity of variance, and sample independence. When a significant group × time interaction was observed, independent-samples *t*-tests were performed for each dependent variable at each testing session between groups; dependent-samples *t*-tests within each group were performed; and delta scores were calculated and independent-samples *t*-tests between groups were performed. Group differences were further assessed through effect sizes (η^2_p; partial eta squared). Effect sizes were interpreted as small (0.01–0.059), medium (0.06–0.139), or large (>0.14) as previously recommended (7). An alpha level was set at $p \leq 0.05$, and all analyses were performed using SPSS version 24.0 (SPSS, Inc., Chicago, IL, USA).

Results

After 10 weeks of resistance training, significant main effects for time ($p < 0.001$) were observed for squat 1RM, deadlift 1RM, bench press 1RM, and vertical jump. However, there was no main effect for time for pro-agility, IMTP PF, or IMTP RFD 250 ms. In addition, no significant group × time interactions were observed for any measure of strength or athletic performance. All measures of strength and performance before and after 10 weeks of offseason resistance training are presented in Table 2.

Body composition changes after 10 weeks of resistance training are presented in Table 3. No significant ($p > 0.05$) main effect for time or interaction was observed for BM. However, a trend toward an increase in FFM across groups was observed ($p = 0.098$) with no differences seen between groups. A significant main effect for time ($p < 0.05$) and a significant group × time interaction ($p = 0.015$) were observed for BF%. Delta BF% scores (POST − PRE values) further indicated that the DE111 group experienced greater decrease in BF% (−2.05 ± 1.38%) compared with PL (−0.2 ± 1.6%; Figure 1). A significant main effect was observed for RF thickness ($p = 0.015$) with both groups experiencing an increase in MT compared with PRE values. However, only 57% of the participants experienced an increase in MT, which exceeded the minimal difference (0.07 cm) calculated in our ICCs. In addition, no main effect for time was observed for VL MT, and no interactions were seen for RF nor VL thickness between treatment groups.

No significant differences in average daily caloric intake were observed between the DE111 (1836.4 ± 233.8 kcals) and PL (1804.1 ± 182.1 kcals) groups. In addition, no significant differences were seen between groups in carbohydrate (DE111: 238.4 ± 42.9 vs. PL: 215.1 ± 48.5 g), protein (DE111: 91.0 ± 14.7 vs. PL: 94.5 ± 16.3 g), and fat (DE111: 60.5 ± 8.2 vs. PL: 63.1 ± 8.7 g) intakes. Furthermore, both DE111 and PL supplements were well tolerated, and no adverse events were reported.

Discussion

The major finding of this study was that probiotic supplementation with a post-workout recovery drink resulted in greater reductions in BF percentage after 10 weeks of resistance training compared with a placebo. Furthermore, our data showed that 10 weeks of offseason training resulted in significant improvements in 1RM strength (bench press, squat, and deadlift) and vertical jump height with DE111 supplementation, providing no additional benefit compared with placebo. In addition, we observed no difference between groups in pro-agility time, IMTP PF, IMTP RFD, and MT. To the best of our knowledge, this is the first study to investigate the effects of probiotic supplementation on resistance training adaptations in college athletes.

After 10 weeks of training, both groups experienced improvements in BF percentage similar to values previously reported in female collegiate basketball players after eight weeks of offseason resistance training and protein supplementation (35,44). Our findings also revealed greater reductions in BF% in the probiotic group (−2.1%) compared with placebo (−0.2%). Currently, there is a significant gap in the literature regarding probiotics and body composition in healthy adults. However, a growing body of evidence suggests that in overweight and obese individuals, modulation of the gut microbiota produces favorable reductions in BF mass (16,28,31,34). In healthy, normal-weight adults, probiotic supplementation has been reported to attenuate

increases in BF mass during a prolonged high-fat diet (29). Furthermore, it has been observed that just 3 days of a hypercaloric diet (3,400 kcal) has the capacity to alter the gut microbiome, resulting in an additional energy harvest of 150 kcals in lean and obese individuals (15). Taken together, although the participants in our study on average did not report high average daily caloric or fat intake, it is possible that the probiotic supplement reduced energy storage after potential episodic overfeedings during the 10 weeks. An increase in weight as little as 2% of BM has been previously shown to impair vertical jump and sprinting performance (10). In addition, improvements in body composition have reported to be modest over multiple training seasons in female athletes (33), and accumulation of fat mass is often experienced in the offseason (24). Therefore, the findings of this study may prove useful to athletes seeking to alter body composition, as well as those in weight-restricted or aesthetic competitions.

It is important to note that although the underlying mechanisms of probiotic-induced improvements in body composition were outside the scope off this investigation, evidence suggests that gut microbiota composition has wide reaching effects on the human body (3,19). These microorganisms beneficially modulate intestinal permeability, which may play a role in the absorption of protein post-workout after acute muscle breakdown. It has been previously reported that high-intensity interval training and resistance exercises increase markers of intestinal damage (30,37) and may impair dietary protein digestion and absorption during postexercise recovery (38). This impairment in absorption may lead to a reduced capacity for amino acid uptake and may blunt training adaptations. In this study, increased protein absorption in the probiotic group may have contributed to the improvements in body composition by increased dietary protein-induced thermogenesis (43) and altered satiety signaling (39). On average, our athletes had a daily consumption of 1.6 $g \cdot kg^{-1}$ of protein including the provided post-workout nutrition (20 g of protein). Although the supplemental protein allowed these athletes to meet recommended range of protein intake for supporting lean muscle accretion (1.4–2.0 $g \cdot kg^{-1} \cdot d^{-1}$), intakes above this reference range have been suggested for additional improvements in body composition (13). Thus, improved amino acid uptake in the probiotic group may have allowed for more efficient protein digestion, simulating the effects of a higher daily protein intake. Nevertheless, future work is needed to investigate potential underlying mechanisms for the observed improvements in body composition.

Based on a previous study using a probiotic of the *Bacillus* strain (18), we speculated that probiotic supplementation would promote improved dietary protein absorption and utilization, resulting in enhanced muscular adaptations after training. Although previous literature examining acute protein absorption highlights the relevancy of probiotic supplementation (18,38), it seems that coadministration of protein and probiotics do not augment the increase in RF or VL muscle size from 10 weeks of resistance training in trained athletes who habitually consumed adequate dietary protein. There was a significant time effect for an increase in RF MT, with no significant increase seen in VL MT. However, because of the amount of participants that did not exceed the RF minimal difference (43%), these data may have little practical importance. These findings are in agreement with a previous investigation reporting no change in VL thickness after 14 weeks of a periodized resistance training program in Division I softball players (27). Conversely, previous work has shown improvements in both RF and VL thickness after strength training programs of various lengths (5,9). Dietary recalls revealed the athletes' overall caloric intake was about 400–600 calories below what would be predicted for an active female population (23). Thus, although the athletes were able to meet protein recommendations, overall caloric intake may not have been sufficient to observe substantial hypertrophy as also indicated by the lack of statistical improvement in FFM. However, both groups experienced improvements in 1RM strength measurements after training, with no differences observed between experimental groups. These data are in agreement with previous investigations reporting similar strength adaptations after offseason resistance training (35,44). Furthermore, no improvements in IMTP PF or IMTP RFD were observed. Although previous work has investigated the relationship between IMTP and athletic performance (36), only 1 study has reported improvements in IMTP performance after chronic resistance training (20). It is possible that 10 weeks is not a sufficient training duration to see improvements in IMTP performance in trained collegiate athletes. Although we verbally confirmed athletes' adherence to pretesting guidelines, we did not quantify hydration status, and it is possible that variations in hydration status may have affected physical performance testing. Nevertheless, much more work is needed in examining various daily caloric and protein intakes, protein types (e.g., soy, pea, and casein), and longer training periods are needed to advance our understanding of the potential benefits of probiotics on muscular adaptations in college athletes.

Practical Applications

In summary, we report that supplementation with the probiotic DE111 may improve body composition in female collegiate athletes in conjunction with offseason resistance training. Although no performance advantages were observed, these data are of interest to a wide array of athletes and coaches attempting to optimize body composition changes during offseason training. In addition, as acute and chronic resistance training–induced stressors have the potential to negatively impact immune, neuroendocrine, and gut health, promoting an optimal microbiota could benefit athletes based on previous literature. Nevertheless, further research is needed to investigate the potential benefits of

probiotics in relation to protein absorption, acute exercise recovery, body composition, and training-induced muscular adaptation in athletes.

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