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### "THE PHYSIOLOGICAL EFFECTS OF TRAINING IN ARTISTIC-ATHLETES"

By

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#### ABSTRACT OF THE DISSERTATION

# THE PHYSIOLOGICAL EFFECTS OF TRAINING IN ARTISTIC-ATHLETES By: DAVID JOHN SANDERS

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Dancers are often overlooked as athletes because of the artistic nature of their event. However, they must perform physiologically demanding choreography with precise technique, while maintaining a pleasing aesthetic. Because of the demands of dance performance, the artistic-athlete presents a unique challenge to practitioners striving to optimize their health and performance.

Managing athlete health and performance requires testing and monitoring to evaluate the demands of training, how the athlete is responding to these demands, and determine areas that can be improved upon. Heart rate monitoring (HRM) is a viable means to monitor training as training load (TL) and exercise energy expenditure (EEE) is assessed, and they are reflective of the internal physiological training stress endured by the athlete. The effects of training can be observed through changes in various performance markers and blood biomarkers, and the success of training can be determined whether the desired outcome was achieved or not. However, HRM is limited to the time the athlete is wearing a monitor. Thus, inclusion of blood biomarkers provides a thorough assessment of one's physiological response to on- and off-stage stressors.

Therefore, the purpose of this dissertation is to observe the physiological stress (i.e. TL and blood biomarkers) of training in the artistic-athlete, and examine interventions to optimize their health and performance (i.e. resistance training). We

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hypothesize the physiological demand of training will be low (relative to other sports), however blood biomarkers will change with cumulative TL, reflective of overall stress, related to inadequate energy intake and suboptimal recovery.

#### AIMS AND OBJECTIVES

*Aim 1:* To investigate the physiological demands of a typical college-level ballet and modern class through training load (TL) and exercise energy expenditure (EEE) using a 5-zone heart rate based monitoring system. Further, the differences in physiological stress were assessed between 2 differenct class types. Two ballet and two modern dance classes were monitored for TL and exercise EEE using the Polar Team<sup>2</sup> Pro System. In addition, a physiological profile of cross-disciplined collegiate female dancers was determined from performance testing done at baseline. Performance testing included body composition, vertical jump, anaerobic power, and aerobic capacity. This study will provide information about the physical demands placed upon female college dance majors, and their current fitness status.

*Aim 2:* To investigate the effects of an 8-week progressive overload resistance-training (RT) program on body composition, muscular strength and power, and aerobic fitness in collegiate female dancers. The RT group met 3x/week in addition to their required dance curriculum, while the control group maintained their regular dance training. RT will be performed at a moderate intensity of ~65% 1RM. This study will provide information in regard to the role of RT in facilitating enhancements in muscular strength and power, and body composition that may yield positive adaptations for performance in this population. *Aim 3:* The purpose of this study is to evaluate the cumulative effects of semster long training in conjunction with changes in performance and blood biomarkers associated with health, performance, and recovery in elite, adolescent ballet dancers. Training load will encompass heart rate monitoring (HRM) from training (exercise energy expenditure) and activities of daily living (non-exercise energy expenditure) from the Polar HRM

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system. This study will encompass the entire semester including training and staged performances. The primary aim of this study is to evaluate the effects of cumulative stress on various biomarkers and performance outcomes in both male and female athletes. The secondary aim is to evaluate the differences in male vs. female dancers to investigate potential differences in both workload factors as well as any physiological changes seen in the biomarkers and performance. This study will provide real world data that can lead to sex specific recommendations on biomarker utilization. The concurrent analysis of both males and females under similar stress, training loads, and environment provides a unique insight on physiological response to season long stress.

### ACKNOWLEDGEMENTS

Aim 1 and Aim 2 of the current manuscript have been published in academic

journals, and represents original work. Below are references in their respective order:

- 1. Sanders DJ, Walker AJ, Prior K, Poyssick AN, Pellegrino JK, Arent SM. Physiological Profile and Training Demands in Collegiate Dancers. *J Strength Cond Res* XX(X): 000-000, 2019. doi: 10.1519/JSC.000000000003107
- Sanders DJ, Walker AJ, McFadden BA, Bozzini B, Cintineo H, Cardaci T, Arent SM. The Effect of Resistance Training on Body Composition and Performance Variable in Collegiate Dancers. *Comparative Exercise Physiology*, 0 (0): 1-8, 2020. https://doi.org/10.3920/CEP190074

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#### **1.1 Introduction**

Sports medicine is a constantly expanding field. Historically, sports medicine practitioners have specialized in the management and treatment of injuries and other maladaptation's to exercise training (12). However as the field has expanded a broader definition of 'medicine of exercise' has been applied by the authors Bruckner and Khan (12). As such, sport science has emerged as a sub-domain of sports medicine to include the practice of injury prevention and performance optimization. Progressive overload during training is necessary to elicit physiological adaptations that will enhance human performance. This model of stress and adaptation was initially described by Hans Selye (61), and further adapted by Matveyev as the foundation of training periodization (45). Selye's general adaptation syndrome (GAS) includes three phases that occur in sequential order: alarm, resistance and exhaustion (61). The initial alarm, or stressor, causes a temporary decrease in performance that is overcome and surpassed during the resistance phase. If the stressor persists for too long, the exhaustion stage is reached yielding performance and physiological decrements that is synonymous with overreaching and/or overtraining. Periodization was developed to optimize performance through systematically programed alterations of training volume and intensity to prevent progression to the exhaustion and elicit a supercompensatory effect. Further, recovery time is programmed for performance enhancement, as well as maintenance of overall health. Nutrient intake, and timing of macronutrient consumption, can augment recovery by enhancing tissue repair, muscle protein synthesis, and mood (36). When athletes are overstressed, or under-recovered, maladaptation's to training may occur manifesting as performance decrements and

unfavorable changes in psychological and physiological responses (46,56). Psychological maladaptation can include disruptions in sleep, appetite, irritability, restlessness, staleness, lack of motivation and depression. Physiological maladaptation may present as perturbations of the hypothalamic-pituitary-adrenal axis (HPA axis), hypothalamic-pituitary-gonadal axis (HPG axis), sympathetic-adrenalmedullary axis, or the immune system (46,56). Recovery from these symptoms can take weeks to years depending on their severity and inter-individual differences (46). During a training cycle an athlete is in constant flux through the Stress-Recovery model as trainers and coaches attempt to maximize an athlete's performance capability prior to competitive events (63).

#### **1.1.1 Optimizing Athlete Health and Performance: An Integrated Model**

"Citius, Altius, Fortius," or faster, higher, stronger has been the motto of the Olympic games since the establishment of the International Olympic Committee in 1894. However, athletes have been seeking ways to improve performance since the legendary Milo of Croton. As knowledge was gained about biological mechanisms and their roles in exercise and training adaptations, coaches and athletes began to recognize the efficacy of a scientific approach to training (51). Periodization was developed to systematically plan alterations in training stress, but recently authors have considered other nutritional, psychological, and social stressors to aid in performance optimization of the free-living athlete (51). Thus, the free-living athlete is a psychosociophysiological system that requires a multifaceted approach to optimize performance (63).

Training load (TL) is altered throughout a program by manipulation of volume, intensity, time, type, and frequency of training (63). Phases of increased TL are used to obtain desired physiological adaptations that ultimately lead to performance improvement, despite an initial acute performance decrement (51). Increases in physiological stress are necessary for performance to improve. Training load cannot be increased indefinitely or too rapidly, as too great of an increase can negatively impact performance from symptoms of overtraining. Also, if TL is decreased or too low, performance can be inhibited due to detraining. Thus, quantification of TL is necessary to assist coaches and sports scientist in appropriately prescribing the intensity of training sessions (63). Heart rate monitoring (HRM) is a common technique to objectively quantify TL as heart rate corresponds to the physiological stress of training. Bannister and associates developed the concept of training impulse (TRIMP), which is the product of training duration, average heart rate (i.e. intensity), and a non-linear metabolic adjustment factor that is based on the blood lactate curve and the duration of a training session (5). A critical limitation of TRIMP is that only aerobic training intensities can be adequately determined (63). However, HR can be used to indirectly assess intensity via caloric expenditure because of the linear relationship between HR, oxygen consumption, and energy expenditure (1). Thus, exercise intensity, defined as the amount of energy expended per minute (1), can be used to determine the physiological stress of training. Further, monitoring exercise energy expenditure enables athletes and practitioners to strategically address energy intake (EI) to augment performance adaptation, and energy balance (EB) to optimize athlete health.

Nutrition can be utilized by athletes in several ways to optimize training adaptations and performance. Mujika et al. describe several themes to manipulate nutrient intake between and within days, two of which include periodization of EI in conjunction with changing needs from competition or training goals (i.e. energy balance), and arrangement of nutrient intake over the day in relation to training to enhance the metabolic interaction between exercise and nutrition (i.e. nutrient timing) (51). In this regard, nutrition will impact performance through physiological variables (e.g. muscle glycogen levels, muscle protein synthesis, etc...), and body composition. When energy expenditure chronically exceeds EI, inadequate energy is leftover to maintain health and performance. Low energy availability (LEA), an underlying concept of Relative Energy Deficiency in Sport, leads to the down regulation of physiological functions that are not necessary for immediate survival (25). Specifically, hormones related to sexual development and reproduction appear most sensitive to LEA. Disruption may occur as upstream as the hypothalamus, where secretion of gonadotropic releasing hormone is diminished leading to decreased levels of the sex hormones testosterone and estradiol (25). LEA influences short- and longterm injury risks as down regulation of anabolic hormones may impair one's ability to recover between training bouts, or lead to stress fractures related to low bone mineral density (25). In contrast, sufficient nutrient intake can augment health and performance by favorably influencing the cellular and hormonal milieu to restore homeostasis (51). Ultimately, nutrition can be used to ameliorate physiological stress from training and improve health and performance, or nutrition can compound the physiological stress increasing risk for illness and performance decrement.

Periods of intensified training have been associated with disturbances in mood and sleep quality (37,48). Morgan et al. were amongst the first to demonstrate the relationship between increased TL and mood disturbances. The investigators repeatedly reported dose-response relationship throughout a competitive season, and further determined that the disturbances were primarily due to increased fatigue and decreased vigor (48). Interestingly, similar outcomes have been reported over shorter periods of intensified training. Killer et al. found significant decreases in sleep quality and mood state due to 9-days of intensified training in highly trained cyclist, independent of carbohydrate consumption (37). When using psychological measures to assess an athlete's response to training, other non-training stressors may inherently influence results. Morgan et al. compared mood changes over a training semester of collegiate swimmers and non-athletes, and reported increased mood disturbances in swimmers and no changes in mood in non-athletes (48). Thus, the authors suggest that the mood disturbances reported in swimmers is associated with TL independent of other stressors common to college students (48). In contrast, Killer et al. reported an increase in lifestyle stress scores due to intensified training (37). These findings indicate a relationship between training and non-training stressors. Though it is unclear the extent non-training stress provokes psychological symptoms, it is prudent to account for non-training stressors (e.g. work, school, finances, friends, family, etc...) when determining the cumulative stress of an athlete (35,63). Athletes will react and adapt differently over individual time frames, even when TL is identical (63). As such, a holistic approach to athlete management enables practitioners to

evaluate training and non-training related variables in order to optimize athlete health and performance.

#### 1.1.2 The Artistic-Athlete

Dancers primarily view themselves as an artist due to the nature of their event (2). This bias has led to dancers athleticism to be overlooked. However, a successful performance can be as physically demanding as it is artistically. Artistically, choreography must be executed with precise technique to be aesthetically pleasing. Physiologically, choreography requires sufficient muscular strength, power, and endurance for satisfactory performance. Athletes demonstrate the high physiological demands throughout a routine performing numerous leaps, kicks, turns, and lifts through extreme ranges of motion that may place extra stress on the body. Additionally, the artistic-athlete needs to perform while maintaining aesthetic appeal. The demands of the artistic-athlete present a unique challenge to health and performance management because practitioners must be aware of the physiological and body composition needs of the artistic-athlete.

High-level dancers are exposed to a high work volume, with training occurring upwards of 6-days per week at the professional level. A typical training day is comprised of 6-8 hours of technique class, variations, and choreography rehearsal (74). Workloads continue to increase during the 4-8 week performance season as well, with dancers completing 8-10 performances per week, while continuing to take daily technique classes and rehearse other shows (74). At the professional level, it is the goal of artistic directors to have their dancers prepared to perform their best on opening night and throughout their performance season. At the vocational level, a top priority of training is to develop highly skilled dancers capable of coping with and adapting to the diverse demands of dancing professionally (74). The following sections examine the physiological demands of dance training and other physiological considerations for the artistic-athlete in order to optimize health and performance.

#### **1.2 Training Load Monitoring**

Athlete monitoring and tracking TL is popular in sport, but has yet to be systematically applied to the artistic-athlete. TL is quantified in numerous ways, typically incorporating training frequency, duration, and intensity (7). Training diaries and questionnaires can be used to assess weekly, monthly, or yearly TL. However, the reliability and validity of exercise intensity is limited, especially when compared with monitoring techniques that incorporate a physiological variable (7)

Other commonly used techniques include monitoring heart rate (HRM), oxygen consumption, or accelerometry. Since the 1980s, HRM has been utilized to quantify the internal workload (i.e. effort), of an individual to perform a given task (1,30). This enables coaches and practitioners to modify training programs to optimize performance and minimize the risk of injury (1). Several methods can be used to assess TL from heart rate including training impulse, summated heart rate zone score, and proprietary algorithms developed by manufacturers (1). Importantly, HRM can estimate exercise energy expenditure (EEE) while training due to the relationship between HR and  $VO_2(1)$ . Energy expenditure provides a marker of absolute (i.e. total expenditure) and relative (i.e. rate of energy expenditure) training intensity. Recent

advancements in accelerometry have expanded assessment of physical load through movement related metrics such as jumps, acceleration, and decelerations. The combination of HRM and accelerometry provides a comprehensive assessment of TL that enables a scientific approach to understanding athletes training responses and performance readiness (30).

Previous investigations have characterized TL of various aspects of a dance class. Cohen et al. investigated the oxygen consumption and caloric expenditure response during barre and center floor exercises using ECG and the Douglas bag technique during a 60-min professional ballet class (20). In females, the researchers reported an average intensity of 37.7±7.7 and 45.9±9.5% of VO<sub>2</sub>max and caloric expenditure of 3.96±1.0 and 4.86±1.1 kcal•min<sup>-1</sup> for barre (28 minutes) and center floor exercises (32 minutes), respectively. In males, a greater intensity was observed 38.3±4.0 and  $54.6\pm7.0\%$  of VO<sub>2</sub>max and  $5.85\pm1.04$  and  $8.38\pm1.54$  kcal•min<sup>-1</sup> for barre and center floor exercises, respectively (20). Also in male and female professional ballet dancers, Schantz and Åstrand reported similar rates of oxygen consumption of 36% of VO<sub>2</sub>max for barre and 46% for center floor (60). Similar TL has been reported in response to modern dance class as well. Wyon et al. reported energy expenditure of  $4.73\pm0.81$  kcal•min<sup>-1</sup> and  $6.67\pm1.95$  kcal•min<sup>-1</sup> in female and male dancers. respectively (75). These early investigations of the intensity of ballet and modern class demonstrate the low intensity of dance training.

Subsequent investigations have sought to determine differences in TL between class, rehearsal and staged performances. Using telemetric gas analysis in male and female modern dancers, Wyon et al. reported significantly greater VO<sub>2</sub> and rate of energy expenditure (REE) during a performance compared to class and rehearsal (75). Surprisingly, the authors reported lower  $VO_2$  and REE during rehearsal than class. This most likely occurred due to increased time at lower workloads as choreography is reviewed. In high-level female ballet dancers, Rodrigues-Krause et al. reported a significantly greater VO<sub>2</sub> in rehearsal than class  $(51.9\pm7.3 \text{ vs. } 38.8\pm5.9\% \text{ VO}_2\text{max})$ respectively)(57). The conflicting results between the two studies are likely due to differing interpretations of the term rehearsal. Rodrigues-Krause et al. described their rehearsal as performance of a specific ballet routine that is more similar to Wyon's use of collecting performance during a dress rehearsal (57,75). As such, both studies report a significantly greater TL from performing than training. Comparison of TL from dance class and performance demonstrates a deficit in physiological demands, which suggests class inadequately prepares dancers for the physiological requirements of performance. Further, the low TL of dance class may lead to the observation of lower than expected physiological characteristics such as muscular strength and VO<sub>2</sub>max.

Few investigations have studied the daily TL of high-level dancers. Using accelerometry, Twitchett et al. investigated the demands of a working day in professional female ballet dancers. The authors reported that ballet dancers spend a majority of their training day (~90%) at a moderate work intensity (3-6 METS) or less (< 3 METS) (67). This translates to a VO<sub>2</sub> of approximately 21 ml·kg<sup>-1</sup>·min<sup>-1</sup>, which is similar to values obtained by Cohen et al. (20,67). Brown et al. used accelerometry to assess energy expenditure (EE) in preprofessional contemporary dancers over a 7-day period, and reported an average EE of 2784±569 kcal•day<sup>-1</sup>. Together, these studies demonstrate the low TL of dance training, especially when compared to other high-level athletes (62).

Regardless of the technique used to assess TL, it is useful in tracking an athlete's physiological response to a training session, and to sufficiently progress TL in order to optimize performance. Further, this information can be utilized by a dancer's support staff to design training and nutritional interventions if necessary.

#### **1.3 Performance**

Assessing and monitoring changes in physiological characteristics is essential to determine an athlete's strengths and weaknesses, their adaptation to training, and evaluate program efficacy. Dance is an intermittent, power-endurance activity requiring sufficient anaerobic power, muscular strength, flexibility, and body composition for successful performance (38). The low intensity of dance training discussed in the previous section may minimize improvement in performance characteristics, leading to lower than expected fitness levels in dancers. Koutedakis et al. have reported that professional dancers are similar in strength and fitness to healthy, sedentary individuals, despite rigorous training (38).

Researchers have studied the physiological characteristics of dancers, and they have focused on those who primarily train in a single discipline (i.e. ballet or contemporary/modern). Initial investigations of aerobic fitness reported a marginally greater capacity in female ballet dancers than sedentary controls (41.5±6.7 vs. 36.8±5.5 ml·kg<sup>-1</sup>·min<sup>-1</sup>) (54). Chatfield et al. reported similar outcomes comparing professional modern dancers to healthy, but sedentary individuals (43.6±2.2 vs.

 $36.4 \pm 4.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (17). Interestingly, the researchers found no differences between groups in knee or ankle strength or anaerobic power and capacity (17). However, disparity in physical capacity has been observed between levels of dancer. Greater aerobic capacity has been found in professional level ballet and modern dancers when compared with their collegiate counterparts, with a greater discrepancy found between professional and university modern dancers (3,68). Further, greater muscular strength and power has been reported in professional ballet and modern dancers when compared to lower level dancers (4,9,68).

Body composition is of high concern to dancers, as low body fat or lean appearance is favored by directors and choreographers (68). Male and female professional dancers have been found to have lower body percentages than healthy, but sedentary individuals (17,68). Professional ballet dancers have been found to have lower body fat than their university level counterparts (41,68,72). Also, lower body fat has been reported in professional female ballet dancers compared to contemporary dancers (41). Interestingly, the authors reported no differences in body composition between male ballet and contemporary dancers (41).

Regular performance testing including body composition, strength, power, and aerobic capacity should be incorporated by dance practitioners to optimize athlete health and performance. Changes in any variable, or lack there of, may be indicative of issues that have negative health consequences and ultimately inhibit one's ability to perform. Further, outcomes from performance testing enable practitioners to individualize any training, psychological, or nutritional plans for their athletes (51).

#### **1.4 Perceptual Measures**

Along with TL and performance monitoring, it is recommended to monitor perceptual measures of well-being and sleep quality (52). Recent evidence suggests the interplay between overall athlete load, athlete health, and performance can reliably be tracked through mood and wellness surveys (46,52). Research has consistently demonstrated an increase in mood disturbances with an increase or overload in TL (37,48). Mood disturbances have been shown to present with symptoms of decreased vigor, increased fatigue, tension, anger, depression, restlessness, and loss of interest (46). Mood disturbances suggests flux through the stress:recovery paradigm towards under-recovery, which may be further compounded by disruptions in sleep quality, latency, and efficiency (16,46,58).

The subjective nature of assessing athlete mood and sleep quality comes with several considerations that should be taken into account. Athlete buy-in is critical to collecting reliable data, as athletes may alter their responses in order to be viewed favorably. Researchers may aid in this process by administering questionnaires that minimize athlete burden, maintain confidentiality of athlete responses, and promptly provide feedback (52). Numerous reliable and valid tools exist for monitoring athletes' psychometric responses to training. Importantly, practitioners must consider the specificity of the questionnaire to their environment (52).

The profile of mood states (POMS) is one of the most studied questionnaires in human performance (52). POMS assessment enables insight into a person's mood state, and was shown to change due to increases in TL in a series of studies by Morgan and colleagues (48). One study in dancers investigated the effect of a 5-week performance season on POMS in professional male and female ballet dancers. Liederbach et al. reported a significant increase in mood disturbance from week 1 to week 4, due to an increase in fatigue and decrease in vigor (40). Similar changes in POMS to other athletes were found in this study due to the high volume of dance performed, as the participants had 6-hours of classes and rehearsals prior to an evening performance throughout the study (40). Use of POMS with athletes has been criticized because it was not developed with the consideration of athletic populations (52). The multi-component training distress scale (MTDS) was developed specifically to monitor the psycho-behavioral responses (mood disturbances, perceived stress, and behavioral symptoms) of athletes to training epochs (44). Although not as thoroughly researched as POMS, the brevity (22 questions) and inclusion of multiple constructs has made the MTDS a promising self-report measure for future research (52).

Successful athletic performance requires integration of the physiological, psychological, and technical components (63). Improving one's mood state prior to competition may have a positive outcome on results, as positive mental health is associated with high performance levels and vice versa (47). Thus, monitoring mood state in conjunction with other physiological and performance will help practitioners optimize their athlete's health and performance.

#### **1.5 Nutritional Considerations**

Nutrition and training dynamically interact to support physiological adaptations that enhance performance capacity. Conversely, malnutrition can impede performance capabilities and be detrimental to athlete health and well-being. At it's most fundamental level, energy balance (caloric intake = caloric expenditure) is desired for athletes in order to maintain health and performance. Energy availability (EA) expands upon energy balance by determining the energy remaining for other physiological process after accounting for the energy cost of exercise (EA= (EIexercise expenditure)/fat free mass) (23). Changes in EEE occur throughout a training cycle, and create differences in energy needs as well as macro- and micronutrient, and fluid requirements (51). Further, EI must support the body composition needed for optimal performance while meeting training load and health requirements (51). Fueling for sport, while safely meeting body composition needs demonstrates the difficult yet delicate balance between ideal health and the short- and long-term health consequences of low energy availability (LEA) (50).

A range of nutritional strategies may be utilized around training and competition to address physiological or biochemical factors that may promote optimal performance (51). Beginning with macronutrient consumption meeting EEE requirements, recommendations for intake range from 3-12 g/kg BW/day, 1.2-2 g/kg BW/day, and 20-35% of EI for carbohydrates (CHO), protein (Pro), and fats, respectively (66). Each macronutrient provides numerous functions needed for performance such as providing energy (CHO and fat), substrates for cell function (fat and Pro), or immune system support (CHO and Pro). In particular, CHO intake can vary day to day based on the intensity of that days training intensity. CHO recommendations for low, moderate, high, and very high training intensities are 3-5 g/kg BW/day, 5-7 g/kg BW/day, 6-10 g/kg BW/day, and 8-12 g/kg BW/day, respectively (36,66). CHO requirements increase with exercise intensity due to the increase in EE from training, thus it is prudent for the athlete and the practitioner to monitor caloric expenditure and adjust EI accordingly. In contrast, Pro and fat intake can remain stable, as long as the aforementioned minimum recommendations are met. One caveat to that being a potential need to decrease fat mass via caloric restriction. In that case, protein intake should be increased to preserve LBM through the upregulation of muscle protein synthesis (34,66).

CHO is vital for the power-endurance athlete because of its role in glycoloysis and the intermediate energy supply. Further, sufficient CHO is necessary in order to maximize intramuscular muscle glycogen (IMG) and liver glycogen stores, which can be achieved by consuming 7-10g/kg BW of CHO (14). Restoration of these stores is a fundamental goal of recovery between training and competition, especially when multiple events occur in a congested fixture (13). A post-exercise window of opportunity exists to maximize rates of glycogenesis due to increased glucose uptake via increased translocation of the Glut-4 transport protein to the cell membrane, increasing glucose uptake (14). Co-ingestion of CHO with Pro, creatine, and/or caffeine may further amplify the rate of glycogenesis (14,36). CHO consumption, or a mixture of CHO and Pro, in the peri-workout period is advantageous beyond restoring energy balance. Evidence suggests consumption of food-stuffs before, during, and after a training session promotes a favorable hormonal milieu for recovery (36). Peri-exercise EI promotes the anabolic environment through increased insulin and decreased cortisol levels (36). In contrast, restricting caloric intake may impair recovery as greater cortisol levels post exercise promotes a catabolic environment and reduces glycogenesis. Chronically low levels of glycogen has been

related to mood disruptions and performance impairment, underpinning the glycogen hypothesis of the overtraining syndrome (46). For the artistic-athlete, manipulating CHO intake to meet fuel needs may enable the athlete to live freely at or close to their desired weight for performance, reducing or eliminating the need for aggressive dieting for weight lose that may impair performance (65).

Protein consumption provides the amino acids (AAs) necessary to repair and rebuild skeletal muscle and other connective tissue damaged during exercise. Evidence suggests that the protein needs of athletes are 50-175% greater than sedentary controls, dependent upon the damage elicited from training (34). With that, a positive nitrogen balance (synthesis > breakdown) is needed to optimize recovery, while even greater protein consumption may facilitate increases in muscle mass. Pro consumption itself up-regulates muscle protein synthesis (MPS). Athletes can utilize this physiological response by consuming a 20-40g bolus of Pro every 3-4 hours throughout the day for increased levels of MPS and other performance benefits (34). Though the efficacy of timing Pro consumption to maximize the post-training 'anabolic window' has been debated, best practices for optimal health and performance advocate Pro consumption in the peri-exercise period, especially when multiple events occur in a congested fixture (34). Van loon and colleagues investigated the rate of AAs digestion and incorporation into muscle tissue and found that  $\sim 11\%$  of the AAs consumed from a 20g protein bolus are synthesized into muscle in 5 hours post-consumption (27). The researchers demonstrate the importance of AA availability, as they are rapidly integrated into existing tissue. Increased Pro intake may be necessary during times of high TL or calorie restriction. Greater Pro intake (≥

3g/kg/bw) will attenuate muscle protein breakdown and preserve LBM while in a hypocaloric state, facilitating body composition needs of the artistic-athlete and other weight-class athletes (34). Several investigations have found similar Pro consumption between dancers of different backgrounds, and all groups reported consumption quantities on the low end of the recommendation range ( $\leq 1.4g/kg/day$ ) (10,11,18,19). Interestingly, Brown et al. reported lower body weight, lower %BF in dancers who consumed more Pro and total calories than others (10). Thus, Pro consumption of  $\geq 2g/kg/day$  may facilitate body composition adaptation necessary for artistic-athletes to enhance their power-to-weight ratio, while consuming enough calories to meet their energy needs.

Lipid consumption is essential for various physiological processes such as the production of steroid hormones, cell membrane maintenance, and absorption of fat soluble vitamins (8). As such, athletes are recommended to consume at least 15-20% of their caloric intake from fat in order to maintain these processes (65,66). Restrictive or low fat dieting (< 20% of total calories) for body composition may impair health due to decreased absorption of fat-soluble vitamins (A, D, E, and K), and reduced consumption of essential fatty acids (n-3 and n-6) (66). Dancers and other weight-class athletes are prone to restrictive eating behaviors that attenuate physiological adaptation (59,65). Systematic monitoring of TL enables athletes and practitioners to assess energy expenditure and meet energy needs through diet. Athletes can be educated on the recommended macronutrient intakes for athletic performance, empowering them to make proper food choices while shopping and dining.

Energy availability acknowledges that dietary energy is expended on several fundamental physiological processes such as cell maintenance, thermoregulation, growth, reproduction, immunity, and locomotion (42). Because EA accounts for EEE, it can be viewed as an input to the body's other metabolic processes (42). Low energy availability, the underlying concept of RED-S, has been linked to numerous health decrements, especially in female athletes (49). Loucks et al. performed the initial investigations of LEA, and found 4-days of very low EA (10 kcal/kg LBM/day) lead to decreased pulsatility of luteinizing hormone independent of exercise stress in young, healthy women (43). Other researchers have found similar disturbances in females due to 5 days of low EA (30 kcal/kg LBM/day), as well as markers of bone turnover and reduced blood glucose (15). Classification of EA zones is presented in *Table 1*.

Though these experiments were short in duration, chronically low EA may manifest as dysfunction in the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes, low bone mineral density, depression, or immune suppression (49). Performance impairments have been postulated through indirect mechanisms of LEA such as impaired glycogen and muscle protein synthesis, and reduced training quality from injury or illness (49). Despite the importance of these associations, only one study has directly investigated the impact of LEA on performance. Vanheest et al. reported a 10% decrement in 400m swimming velocity in young, female swimmers with ovarian suppression (i.e. HPG dysfunction), while their eumenorrheic counterparts improved 8% over a 12 week competitive season (69). Much of the research and recommendations for athletes related to LEA has been performed in female athletes, however there is evidence demonstrating men are susceptible as well. In men, LEA may manifest as testosterone deficiency described as the exercise-hypogonadal male condition (29,33). The role of nutrition in optimizing athlete health and performance is robust, and, in conjunction with TL, many of these outcomes can be assessed through blood-based biomarkers.

**Table 1:** Classification of EA Zones Derived From Burke et al. (15)

Energy Availability Range	Classification and Comments
>45 kcal/kg FFM/day	Support growth and body mass gain
~45 kcal/kg FFM/day	Optimal.
	Maintains weight, provides adequate
	energy for physiological function
30-45 kcal/kg FFM/day	Subclinical or reduced.
	Tolerable for short periods of time,
	planned weight-loss
<30 kcal/kg FFM/day	Low.
	Health implication with impairment of
	many body systems

### **1.6 Biomarkers**

Biomarkers are measurable products or substances of an organism that can be used as an indicator of athlete health, performance, and/or recovery (39). While TL monitoring can detect an athlete's response to training stress, biomarker monitoring is emerging as a viable method to assess an individual's response to overall stress (training and non-training stress), and identify balance between stress and recovery (39). Systematic biomarker assessment throughout training and performance periods is necessary to assess positive or negative adaptations because a single assessment does not provide sufficient insight into an athlete's health status (39). Systematic assessment may also enable practitioners to intervene prior to noticeable health or performance decrements. Currently, no gold standard for biomarker assessment exists for optimizing athlete health and performance. However, monitoring markers, in conjunction with TL, related to sex hormones, muscle breakdown and inflammation, metabolism, and other health markers hold promise. Practitioners can use multiple biomarkers in order to design training and nutrition programs to optimize athlete performance.

#### **1.6.1 Anabolic Adaptation**

Biomarkers related to the functioning status of the HPA- and HPG-axis are commonly used as indicators of physiological adaptation because of their roles in fostering an anabolic or catabolic environment. Downstream effects of cortisol, testosterone, and estradiol have been thoroughly researched (39). Sex hormones have demonstrated sensitivity to training and nutrition, which suggests that proper signaling of these hormones is essential for optimizing athlete health and performance. Other biomarkers of interest include growth hormone (GH) insulin-like growth factor 1 (IGF-1), sex-hormone binding globulin (SHBG), and pituitary hormones luteinizing hormone (LH) and follicular stimulating hormone (FSH) (39). Cortisol is a glucocorticoid hormone secreted by the adrenal gland in response to a physiological or psychological stressor (28). During exercise, cortisol is secreted to mediate lipid oxidation, gluconeogenesis, and proteolysis providing substrates for fuel utilization and other physiological processes (32). Increases in cortisol due to an acute bout of exercise are typically transient in nature, returning to baseline levels or slightly below rapidly after the termination of exercise (28). When TL or stress increases without adequate recovery, resting cortisol is elevated due to the disruption of homeostasis, impairing recovery from training by suppressing glycogenesis, protein synthesis, and the immune system (39). Athletes may be able to tolerate this increase for short periods of time. However if left unchecked, athletes may progress to the overreached or overtrained state, with additional dysfunction of the HPA axis (46).

Testosterone is a steroid hormone secreted from the Leydig cells that has anabolic and anticatabolic physiological effects (70). Post exercise, testosterone facilitates protein synthesis, glycogenesis, as well as red blood cell production (39). High training volume has been associated with suppressed testosterone levels, especially in male athletes (29,39). The ratio of testosterone to cortisol (T:C ratio) is considered a relative indication of the anabolic-catabolic state of an athlete, and is more sensitive to TL than either measure independently (39). Fluctuations in the T:C ratio may be indicative of upstream dysfunction of the HPG-axis. Suppression of the reproductive axis may originate at the hypothalamus from decreased pulsatility of gonadotropinreleasing hormone (73). Further, LEA and TL have been found to alter pulsatility of LH and FSH in athletes, ultimately diminishing concentrations of testosterone, estradiol, and progesterone (22,25,39,43,73).

Estradiol is a steroid hormone primarily produced in the follicles of the ovaries that is indicative of female athlete health. Also, estradiol has varying effects on performance through enhancement of glucose uptake in the liver and muscle, and lipid oxidation and utilization (53). Increases in TL and EEE, with inadequate caloric intake yielding LEA has been found to decrease estradiol concentrations and have a negative impact on female reproductive health (49). Suppression of estradiol may lead to menstrual cycle disruptions such as oligomenorrhea or amenorrhea, as energy deficiency increases the frequency of menstrual disturbances in a dose-response manner (73). Further, amenorrheic athletes have demonstrated lower bone mineral densities (BMD) than their eumenrrheic counterparts (24,71). Low BMD at a young age may lead to early on-set osteoporosis, or cause stress fractures that may inhibit the athlete from training.

Other biomarkers that provide additional insight into the anabolic-catabolic balance include SHBG, GH, IGF-1, and vitamin D. SHBG protects and transports sex-hormones throughout the body, and increases due to training in male and female athletes (39). Increases in SHBG with concomitant decreases in testosterone suggests insufficient recovery (39). GH increases circulating concentrations of IGF-1, and these hormones work in concert to promote anabolism by promoting muscle protein synthesis and lipid oxidation, while inhibiting protein breakdown (25,39). These biomarkers have also demonstrated sensitivity to EA, as low EA has been found to increase GH and suppress IGF-1 (43). As such, GH is elevated to maintain euglycemia via lipid oxidation (25). Also, decreased IGF-1 concentrations has been associated with greater risk of fracture in females (39). Vitamin D is a steroid like substance (29) that is involved in bone maintenance and muscle protein synthesis (39). Thus, deficiencies in vitamin D may lead to decreased bone density and reduced rates of muscle protein synthesis.

#### **1.6.2 Muscle Breakdown and Inflammation**

Creatine kinase (CK) is a biomarker frequently used as an indicator of muscle damage because it leaks out of the muscle membrane after a damaging bout of exercise (39). Persistently high CK values in response to training may indicate inadequate recovery, increasing risk of injury or diminished performance. CK is a relevant marker to monitor in dancers due to the strong eccentric component of dancing (55). Although CK is one of the few biomarkers with athlete specific values, practitioners must take into account recent TL when assessing CK values, as changes in acute workloads will effect CK concentrations.

Other markers used as indicators of recovery are the cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF)- $\alpha$  (39,64). Cytokines have been shown to increase due to muscular contraction, diminished muscle glycogen, prolonged stress, injury, and muscle damage in order to up-regulate an immune response (64). Researchers suggest that TNF-  $\alpha$  acts in a proinflammatory role at the onset of the initiation of the inflammatory response, and acts locally at the site of distress. Then, IL-6 is synthesized to modulate local and systemic inflammation, acting in an antiinflammatory role (64). Chronic increases in circulating cytokines indicate inadequate recovery from stressors which has been shown to lead to depressed moods, sleep disturbances, and fatigue that may ultimately have a negative impact performance (64).

#### **1.6.3 Metabolic Health**

Optimizing athletes' health and performance requires an adequately functioning metabolism, especially in aesthetic or weight-class athletes. Diminished resting metabolic rates (RMR) have been found in male and female athletes with low EI, which suggests alterations to the hormonal milieu to conserve energy for maintenance of body composition and other physiological functions (25). Reductions in RMR would increase the difficulty for athlete's to reach the body composition or weight desired for performance, which may increase the likelihood of an athlete to take drastic (legal or illegal) measures to achieve their weight that may be detrimental to their health and performance (65).

The thyroid hormones triiodothyronine (T3) and thyroxine (T4) are the primary hormones regulating metabolism. Research also suggests the hypothalamic-pituitarythyroid (HPT) axis interacts with leptin, an adipocyte hormone that effects energy status, reproductive function, and body mass (6). Leptin interacts with the HPT axis by inhibiting thyroid releasing hormone production at the hypothalamus, potentially reducing hypothalamic drive (6). Together, these hormones have been found to be sensitive to energy availability. Baylor and Hackney found decreased concentrations of free T3, thyroid stimulating hormone, and leptin in collegiate female athletes due to an intense period of training (6). The authors speculate that the observed changes

occurred as a means of energy conservation, i.e. decreasing RMR to preserve energy for necessary physiological processes. As such, HPT axis dysfunction may coincide with HPG axis dysfunction. Cross-sectional studies of male and female athletes have reported significantly lower free T3 in amenorrheic women and low testosterone males, compared to their eumenorrheic and normal testosterone counterparts (31). Interestingly, these symptoms may also manifest as an increase in circulating cholesterol, especially in women. Friday et al. reported significantly greater LDL and HDL cholesterol in amenorrheic athletes compared to eumenorrheic athletes (26). Potentially, the higher than expected cholesterol level is due to a decrease in steroid hormone production as cholesterol serves as the backbone of steroid hormone production. Hypercholesterolaemia was also observed in ketogenic diet adapted, highly trained, male ultra-endurance runners (21). Together, hypercholesterolaemia in exercisers may be related to carbohydrate availability. However, more research in male and female athletes at risk of LEA is needed to better elucidate the role of cholesterol.

### **1.7 Conclusions**

Athletes train to maximize their performance capabilities through physiological adaptations and skill acquisition. Training load is periodically increased at particular times in order to optimize performance for specific events. Comprehensive monitoring (e.g. physiological, psychological, nutritional) of athletes during these times of increased load, in particular, will enable coaches and practitioners to make informed decisions about the athletes training and nutrition to prevent maladaptation (63). The principle of individualization suggests athletes will respond differently to similar stimuli (63), thus a holistic approach to athlete monitoring may necessary to identify differences between athlete, especially in a team setting. Ultimately, attainment of a consistently high-level of performance requires effective training complemented by recovery sufficient to compensate for training and non-training stress.

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*Title:* Training Demands and Physiological Profile of Cross-Disciplined Collegiate Female Dancers.

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## ABSTRACT

Little is known about the physical demands of high-level dance training. Therefore, the purpose of this study was to investigate the physiological demands of a typical ballet and modern class via training load (TL), and to assess differences in TL between the two class types. Additionally, a physiological profile of cross-disciplined collegiate female dancers was determined. Seventeen college-aged female dancers were recruited and performed a battery of performance tests assessing body fat (%BF), lean body mass (LBM), vertical jump (VJ), peak power (PP), maximal oxygen consumption (VO<sub>2</sub>max), and ventilatory threshold (VT). Two ballet and modern dance classes were monitored for TL and exercise energy expenditure (EEE) using the Polar Team<sup>2</sup> Pro System. Performance testing results were: M<sub>%BF</sub>= 24.1±4.2 %, M<sub>LBM</sub>= 46.8±8.5 kg, M<sub>VO2max</sub>=  $42.9\pm4.3 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ ,  $M_{VT}=76.2\pm6.5$ % of  $VO_{2max}$ ,  $M_{VJ}=44.1\pm1.4$  cm, and  $M_{PP}=$  $519.1\pm177.5$  W. TL of  $41.0\pm17.0$  for ballet and  $44.8\pm27.4$  for modern dance were found, with an EEE of 394.0±111.9 kcal and 421.9±161.4 kcal, respectively. Time spent at or above VT was 1.2±2.6 min in ballet and 3.4±8.3 min in modern. Compared to other female power endurance athletes, the dancers accumulated a much lower TL during both class types. Low TL may inhibit typical adaptations seen in other athletes, which may explain why dancers in this study had lower aerobic and anaerobic capacities and higher body fat percentage than other collegiate female athletes. Also, it suggests that supplemental conditioning could be incorporated into a dancers training paradigm in order to optimize performance.

Key Words: Female Athletes, Artistic-Athletes, Body Composition, Training Load,

Aerobic Fitness, and Anaerobic Power

### INTRODUCTION

Despite the high physiological demand of top-level performers, dancers are often overlooked as athletes due to the artistic nature of their event. Artistically, choreography must be executed with precise technique to be aesthetically pleasing. Physiologically, choreography requires sufficient muscular strength, power, and endurance for satisfactory performance. Throughout a routine, dancers perform numerous leaps, kicks, turns, and lifts through extreme ranges of motion that may place extra stress on the body. Additionally, female ballerinas often perform en point (i.e. on the tips of the toes), which may further compound musculoskeletal strain (13). Another unique challenge to the artistic-athlete is the need to perform while maintaining an aesthetic appeal. Due to the demands of dance performance, there is a distinct need to study this population to optimize performance and minimize injury while maintaining ideal body composition.

Several studies have investigated the physiological characteristics of dancers, however they have focused on those who primarily train in a single discipline (i.e. ballet or contemporary/modern). Research in ballet dancers has shown that successful progression through a professional dance corps (e.g. apprentice to soloist) has been characterized by greater muscular power and strength (20). These muscular adaptations are required for the higher-ranking members of a dance company as they typically perform more difficult and physiologically demanding choreography. Similar to the differences in muscular strength found between professional ballet dancers and collegiate ballet dancers (31), greater muscular power has also been observed in professional contemporary dancers (5) compared to collegiate dancers (10). Aerobic capacity is also greater at the professional level in ballet and modern dance when compared with their collegiate counterparts, with a greater discrepancy found between professional and university modern dancers (4,31). Research also suggests that dancers are not as physically fit as more "traditional" athletes despite a high training volume (10). For example, collegiate female lacrosse players have a greater maximal aerobic capacity than professional female ballerinas (19,31). Given the fitness differences across different power-endurance sports and between levels of dancers, assessment of physical fitness should be used by dancers to identify specific components to be improved. Furthermore, the results of these assessments can be used by their support staff (e.g., choreographers, directors, athletic trainers, etc...) to identify any discrepancies that may increase the risk of injury or impair a dancer's ability to perform optimally.

Despite being a non-contact activity, injury rates amongst dancers have been reported to be as high as 97% across disciplines and skill levels, with overuse injuries being the most frequently reported injury (25). Allen et al. reported injury rates amongst 52 male and female ballet dancers, with 355 injuries in 1-year, and with an incidence rate of 4.4 injuries per 1,000 hours of dance exposure (2). Koutedakis and Sharp suggested that poor lower body strength and lower body strength imbalances in dancers are associated with lower body and back injuries, respectively (22). Overall, insufficient physical fitness appears to be a significant contributor to injury in dancers, whether injury is related to musculoskeletal, overuse, or both (25). Dancers may benefit from training load (TL) monitoring, as practitioners can identify training demands as well as the *responses* to those training demands in order to potentially alter the program prior to an injury occurring.

The concept of athlete monitoring and tracking TL is popular in sport, but has yet to be systematically applied to the athlete-artist. Heart rate monitoring (HRM) is popular in athletics as a means to monitor training and determine TL (8). HRM reflects the internal physiological training stress endured by the athlete which can be viewed as "effort" the individual puts forth to perform an activity. This enables coaches and practitioners to modify training programs to optimize performance and minimize the risk of injury (1). Several methods can be used to assess TL from heart rate including training impulse, summated heart rate zone score, and proprietary algorithms developed by manufacturers (1). Further, HRM can estimate exercise energy expenditure (EEE) during exercise in a field setting due to the relationship between HR and  $VO_2(1)$ . Thus, the incorporation of athlete characteristics (e.g. age, height, weight, sex, VO<sub>2max</sub>, HR<sub>max</sub> and Ventilatory Threshold) may augment the ability to assess an individual's response to the physical demands of training. Likewise, the individualized aspect of monitoring TL may enable dancers' and their support staff to make appropriate adjustments to maximize their training program goals (performance, recovery, maintenance).

Despite potential benefits related to both performance and health, we are currently unaware of any study investigating the TL of a collegiate dancer using HRM technology. Therefore, the purpose of this study was to investigate the physiological demands of a typical ballet and modern dance class via TL and to assess differences in TL between the two dance class types. Additionally, a physiological profile of cross-disciplined (e.g. those who simultaneously train in ballet and modern) collegiate female dancers was determined through performance and fitness assessments.

#### **METHODS**

### **Experimental Approach to the Problem**

A within-subjects design was used to determine the physiological training load of ballet and modern dance classes. First, subjects completed a battery of performance tests over a two-day span in order to individualize data collected from the HR monitoring system and establish physical capabilities and profiles. Subjects then wore HR monitors for two ballet classes and two modern classes. Each class lasted approximately 90 and 105 minutes, respectively. Average TL, EEE, and time spent above ventilatory threshold (VT) was calculated for each discipline.

## **Subjects**

Seventeen collegiate female dancers ( $M_{age}=19.9\pm1.3$  yrs;  $M_{height}=162.5\pm7.6$  cm;  $M_{weight}=59.8\pm6.6$  kg) enrolled in the Rutgers University Performing Arts program were recruited for study participation. Written informed consent was obtained from all subjects prior to the commencement of performance testing. All subjects had at least four years of formal dance training and were free of any major injuries or metabolic conditions. This research was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in accordance with the Declaration of Helsinki.

## Procedures

Subjects reported to the Rutgers University Center for Health & Human Performance for performance testing normally hydrated, at least two hours fasted, and without having trained for 24 hours prior to testing. All testing was conducted between 0900 and 1500h. Day 1 consisted of body composition and aerobic capacity (VO<sub>2max</sub>)

assessment. Body composition was assessed using air-displacement plethysmography (BOD POD; LMI, Concord, CA) (16). Subjects were tested wearing non-padded compression shorts and sports bra, and a swim cap. Total body weight, lean body mass (LBM), and %BF were all measured. The error of body volume reading is roughly 0.02%, which allows for calculation of %BF with only 0.01% error (15). Aerobic capacity testing was performed using a maximal graded exercise test (GXT) to assess VO<sub>2max</sub> and VT via direct gas exchange utilizing a modified Bruce protocol (TrueOne 2400, ParvoMedics, Sandy, UT). Rating of perceived exertion (RPE) was assessed at the end of each stage using Borg's 6-20 point scale (7). Heart rate was continuously monitored using the Polar S610 HR monitor (Polar Electro Co., Woodbury, NY, USA). The subjects performed each test until volitional exhaustion and VO<sub>2max</sub> was considered to have been achieved if subjects met at least 4 of the following criteria: HRmax within  $\pm 15$  beats min<sup>-1</sup> of age-predicted maximum HR, a HR that failed to increase with increased workload, respiratory exchange ratio > 1.15, RPE greater than 17, and a plateau of VO<sub>2</sub> (< 2.0 ml·kg-1·min-1) despite an increase in workload (28). The subject's VT was calculated using the point in which ventilation began to increase non-linearly with  $VO_2$  and was expressed as a percent of  $VO_{2max}(17)$ .

Subjects reported for Day 2 testing approximately 48 hours following Day 1. Day 2 consisted of a five-minute warm up followed by vertical jump testing and Wingate anaerobic testing (WAnT). Vertical jump (VJ) testing was performed using the Just Jump system (Probotics, Huntsville, AL, USA). Each dancer was allowed three attempts in good form, using a counter movement jump with arm swing. The best jump height was recorded and power was calculated as watts= weight (kg) \* jump height (m) (18). Next,

the subjects performed the WAnT on a Monark 894E Anaerobic Test Ergometer (Monark Exercise AB, Sweden). Peak power, average power, and fatigue index were calculated by associated computer software. Subjects were given a 5 sec familiarization trial, with 5-10 min rest, followed by the 30 sec test with a workload set at 7.5% of their body weight (kg). Subjects were instructed to pedal as fast as possible during the test, and the lab staff provided encouragement.

Ballet and modern dance classes were monitored two times each per subject using the Polar Team<sup>2</sup> system (Polar Electro Co., Woodbury, NY, USA). Monitoring occurred within 1-2 weeks following performance testing as the class schedule allowed. The quantification of an individual's workload was estimated by total Kcal expenditure (EEE) and training load (TL), the latter being calculated via an algorithm developed by Polar<sup>TM</sup> based on physiological attributes of the player obtained from laboratory testing and physical workload measured (12). Values obtained from the entirety of class activity (e.g. barre, center floor, and across the floor) were averaged across the two sessions for each type of class. Monitors were programmed with each subject's anthropometric and demographic data as well as values obtained during performance testing (e.g.,  $HR_{max}$ ) VO<sub>2max</sub>, VT) in order to improve accuracy of the estimated EEE and TL. Average heart rate (HR<sub>avg</sub>), time spent in HR zones 1-5 (55-64%, 65-74%, 75-84%, 85-94%, 95-100% HR<sub>max</sub>, respectively), and time above VT was also calculated by the Polar software. Rate of point accumulation (RPA) and rate of energy expenditure (REE) were calculated by dividing TL and EEE by session time, respectively. Ballet and modern classes were approximately 90 and 105 minutes long, and were taught by the same instructors, respectively.

## Statistical Analysis

All statistical analyses were completed using SPSS statistical software (SPSS version 23; IBM). Data are expressed as mean±SD, and statistical significance was set at  $p \le 0.05$ . A RM MANOVA with univariate follow-up was used to determine differences in exercise-load related variables (HR, TL, EEE, time above VT, RPA, and REE) between class types. For each univariate analysis, the Huynh-Feldt epsilon was calculated to test the assumption of sphericity. If this statistic was > .75, sphericity was assumed and the unadjusted statistic was used. If this statistic was < .75, then sphericity was considered to test significance. Microsoft Excel (Microsoft Corp, Redmond, WA) was used to calculate effect size (ES) for between-groups differences using Cohen's *d*. Using Cohen's conventions, *ds* of 0.20, 0.50, and 0.80 were considered indicative of small, medium and large-sized effects, respectively. Positive ES indicate greater effect in ballet. Descriptive statistics were calculated for the physiological profiles and time spent in HR zones.

#### RESULTS

The subjects' results from performance testing are given in Table 1. Their %BF was found to be  $24.1\pm4.2\%$ , with a LBM of  $46.8\pm8.5$  kg. The directly measured VO<sub>2</sub> max was found to be  $42.9\pm4.3$  ml•kg<sup>-1</sup>•min<sup>-1</sup>, with average VT found to occur at  $76.2\pm6.5\%$  of VO<sub>2max</sub>. VJ height was  $44.1\pm1.4$  cm, with  $26.3\pm2.9$  W of power produced. Peak power, average power, and fatigue index (FI%) were determined to be  $519.0\pm177.5$  W,  $365.6\pm77.8$  W, and  $57.4\pm13.7\%$  from WAnT, respectively.

Measures	Mean ± SD	
%BF	24.1 ± 4.2	
LBM (kg)	46.8 ± 8.5	
$VO_{2max}$ (ml•kg <sup>-1</sup> •min <sup>-1</sup> )	42.9 ± 4.3	
VT (% of VO <sub>2max</sub> )	76.2 ± 6.5	
VJ Height (cm)	44.1 ± 3.4	
VJ Power (W)	26.3 ± 2.9	
WAnTpp (W)	519.1 ± 177.5	
WAnTap (W)	365.6 ± 77.8	
F.I. (%)	57.4 ± 13.7	

Table 1: Physiological profile of cross-disciplined collegiate female dancers. Values are presented as mean±SD.

The comparison of the physiological demands of dance training and time spent in HR zones between dance class types are given in Table 2 and Table 3, respectively. There was a significant difference between ballet and modern dance class in  $HR_{avg}$  (P<0.05, ES= 0.61), but no significant differences for TL (41.0±17.0 vs. 44.8±27.4 a.u., P>0.05, ES= -0.16), EEE (400.0±116.2 vs. 421.5±149.8 kcal, P>0.05, ES=-0.19), and time spent above VT ( $1.3\pm2.7$  vs.  $3.8\pm149.8$  min, P>0.05, ES=-0.34). After adjusting for class duration, a trend toward significance for REE (P=0.10, ES=0.30) was found, but not RPA ( $0.45\pm0.19$  vs.  $0.43\pm0.26$  a.u.•min<sup>-1</sup>, P>0.05, ES=0.09).

Mean ± SD
120.4 ± 7.6 (B)*
114.6 ± 10.7 (M)*
41.0 ± 17.0 (B)
44.8 ± 27.4 (M)
0.45 ± 0.19 (B)
0.43 ± 0.26 (M)
394.0 ± 111.9 (B)
421.9 ± 161.4 (M)
4.30 ± 1.25 (B)
3.89 ± 1.47 (M)
1.23 ± 2.59 (B)
3.40 ± 8.26 (M)

Table 2: Training demands profile of collegiate female dancers, where (B) is Ballet and (M) is Modern. Values are presented as mean $\pm$ SD. \* denotes significant differences between dance forms p < 0.05.

HR Zone	Mean ± SD (min)	ES
1	66.4 ± 11.6 (B)	-1.16
	82.3 ± 15.0 (M)	
2	17.6 ± 6.2 (B)	0.31
	15.3 ± 8.1 (M)	
3	6.6 ± 4.2 (B)	-0.12
	7.2 ± 5.3 (M)	
4	1.0 ± 1.5 (B)	-0.73
	2.9 ± 3.3 (M)	
5	0.15 ± 0.5 (B)	-0.26
	$0.33 \pm 0.8$ (M)	

Table 3: Time spent in respective HR zone's during ballet (B) and modern (m) class. Values are presented as mean±SD. HR zones 1-5: 55-64%, 65-74%, 75-84%, 85-94%, 95-100% HR<sub>max</sub>, respectively.

## DISCUSSION

To the authors' knowledge, this is the first study to investigate the physiological demands of a typical collegiate ballet and modern dance class using TL assessed via HRM technology. Results of the current study indicate that there are only minor differences in physiological demands between a collegiate ballet and modern class. Compared to other female power-endurance athletes, the physiological stress incurred by the current dancers engaged in ballet and modern classes was lower in total with regards to TL (24). These findings may hold practical significance for this population, as it is possible that the low-intensity of dance class will inadequately prepare dancers for the physiological demands of staged performances (26).

Researchers have previously studied the cardiorespiratory response to ballet training and choreography performance in professional ballet dancers (14,26). Cohen et al. investigated the heart rate and oxygen consumption response during barre and center floor exercises using ECG and the Douglas bag technique during a 60-min ballet class (14). The researchers reported an average HR of 117±20 and 137±17 bpm and caloric expenditure of 3.96±1.0 and 4.86±1.1 kcal•min<sup>-1</sup> for barre (28 minutes) and center floor exercises (32 minutes), respectively. Despite using different monitoring instruments, a similar average heart rate and caloric expenditure to the current investigation was observed. When time is corrected for, nearly identical caloric expenditures are found in both the current study and Cohen et al. (399.6 vs. 394.0 kcal•90min<sup>-1</sup>) (14). Additionally, Twitchett et al. reported that ballet dancers spend a majority of their training day (~90%) at a moderate work intensity (3-6 METS) or less (< 3 METS) (30). This translates to a VO<sub>2</sub> of approximately 21 ml•kg<sup>-1</sup>•min<sup>-1</sup>, which is similar to values obtained by Cohen et al. (14,30). Furthermore, this corresponds to the time spent in low intensity heart rate zones observed in the present study, as 89.4% of class time was spent in HR zones 1 and 2 (55-74% of HRmax) contributing to the low TL found in this study.

The low-intensity of a typical dance class becomes more apparent when comparing TL of dance to that of other sports. Perrotta and associates reported a TL of 163 points during a 68 minute practice in elite female field hockey players (24). Compared to a dance class, the field hockey players accumulated approximately 4x more TL points during a 68-minute practice, with an equivalent TL accrued in just 20 minutes (24). Although time spent in HR zones was not reported, it can be reasonably assumed that the field hockey players had a greater time spent in HR zones 3-5 than the dancers. Accumulation of TL, or lack thereof, has implications for undertraining that can manifest in injury risk that may impair a dancer's ability to optimize their performance capabilities.

Athletes should utilize practice and training sessions in order to sufficiently prepare for the physiological demands required for performance. In dance preparation, it is typical for dancers to train with a high volume, in excess of 3 hours per day, and up to 6 days per week (3,22). These data demonstrate that much of this time is spent at a low-intensity, which is contradictory to the principles of training specificity given that staged performances have been found to have a greater physiological demand than classes. Schantz and Åstrand investigated the HR response to the performance of choreography during a final rehearsal for a show and reported that dancers worked at 80% of VO<sub>2max</sub> (40 ml•kg<sup>-1</sup>•min<sup>-1</sup>) and at a HR > 160 bpm (82% of HR<sub>max</sub>) during a staged performance (26). Compared to the HR observed by Schantz and Åstrand, the current dancers accumulated less than 10 min of activity in those respective HR zones, regardless of class type. The discrepancy in intensity between training and actual stage performance would suggest a sub-optimal approach to preparation.

Consistent with previous findings, the current study demonstrates that collegiate dancers have a lower aerobic capacity compared to other high-level athletes despite a high training volume (6,23). Female power-endurance athletes, such as elite field hockey players and Division III lacrosse starters, have recorded a  $VO_{2max}$  of 53.5±7.9 ml•kg<sup>-</sup>

<sup>1</sup>•min<sup>-1</sup> and 53.7±6.9 ml•kg<sup>-1</sup>•min<sup>-1</sup>, respectively (19,24). The results of the current study are consistent with Martyn-Stevens et al. (23), who reported a VO<sub>2max</sub> of 42.66±4.33 ml•kg<sup>-1</sup>•min<sup>-1</sup> in collegiate female modern dancers during their subjects' pre-season. Furthermore, similar aerobic capacities have been reported in other dancers that only train in ballet or modern. White et al. reported VO<sub>2max</sub> values of 40.8±1.6 ml•kg<sup>-1</sup>•min<sup>-1</sup> and 39.2±1.9 ml•kg<sup>-1</sup>•min<sup>-1</sup> in collegiate ballet and modern dancers, respectively (32). Interestingly, our subjects have a comparable VO<sub>2max</sub> to a group of professional American female ballet dancers (14), but both groups are less aerobically fit than professional Swedish ballet dancers (26).

Compared to the other collegiate athletes, our subjects performed notably lower for their age and weight for WAnT peak power (34). Zupan et al. investigated the performance of 64 Division I Female athletes on the WAnT, with a resistance of 7.5% of the subject's body weight, and reported a peak power output of 598±88 W, which is ~70W greater than the peak power produced by our subjects (34). Other studies investigating the anaerobic power of dancers have reported poor WAnT<sub>pp</sub> for collegiate modern dancers (23), and average WAnT<sub>pp</sub> for cross-disciplined collegiate dancers (10). In contrast, our subjects scored above average for vertical jump height compared to the general population and other power-endurance athletes (6,19). Notably, we found that our subjects jumped higher (44.1±3.4 cm) than other collegiate female dancers (37.1±6.0 cm), and Division III lacrosse players (38.4±5.6 cm) (11,19). Greater vertical jump heights in our population may occur due to specific neuromuscular adaptations that occur in dancers, as jumps and leaps are frequently performed in class. This is apparent when comparing outcomes from WAnT and vertical jump testing, as our subjects performed worse than other athletes on the WAnT, and better than others on a vertical jump. Some of the discrepancy in these power measurements observed in the literature may have occurred due to the time of year the testing was performed, as changes in caloric intake and expenditure occur as dancers prepare for staged performances (3). Also, researchers have used multiple techniques to assess vertical jump height in this population, which further contributes to the differences reported in the literature. Future research should consider utilizing jump protocols using dance-specific movements, such as sautés in first or second position that can be adapted for single- or multiple-jump efforts to measure power and fatigue index.

Dancers are typically expected to present with a low body fat percentage as part of an effort to maintain an "ideal" aesthetic (21). Traditionally, this has been viewed as a sylphlike aesthetic that encourages a drive for leanness (9). Unexpectedly, our subjects' %BF (24.1±4.2%) is categorized as average for their age group compared to normative values (6), and was higher compared to other professional ballet and modern dancers, and artistic-athletes (e.g., gymnasts) (4,31). One study used dual-energy x-ray absorptiometry (DEXA) to examine the %BF of 42 young, professional, and cross-disciplined dancers reported a mean %BF of 19.4±4.3%(33), which is notably lower than the values obtained in the current study. The difference between professional dancers and university students may occur due to perceived pressure on professional dancers to be as lean as possible (33). However, our body composition findings are comparable to other NCAA Division 1 female athletes (27). Also using DEXA, Trexler et al. reported a similar %BF (23.2±3.2%) to our subjects in 15 Division 1 NCAA female gymnasts (29). These data, in conjunction with our findings, suggests a deviation from a perceived "ideal" aesthetic in collegiate artistic-athletes. Furthermore, artistic-athletes may be prone to lower values of LBM due to a combination of training and nutritional practices. This may lead to the higher than expected %BF observed, even if athletes appear lean based on their bodyweight. For example, it was observed that 1/4 of the participants in the current study had potentially higher than ideal %BF (>25%), despite having a normal BMI (< 25 kg/m<sup>2</sup>). Research is needed in regard to strength training and sports nutrition roles in body composition in the artistic-athlete.

In summary, there are minimal differences in physiological demands between a collegiate ballet and modern class as determined by HRM. The subjects' also spent a similar amount of time in each HR zone during both class types. Additionally, we found our subjects' to be less fit aerobically than other power endurance athletes', but also have a higher vertical jump height. Our subjects' presented with a higher than expected %BF, however similar values have been found in other collegiate artistic-athletes. Thus, the low physiological demand of dance class likely plays a role in the observed body composition and aerobic fitness values. Participation in other dance classes, rehearsals, or supplemental training throughout the day was not accounted for as it was beyond the scope of this investigation. Future research is needed on the effects of supplemental training programs on aesthetic and performance variables in artistic-athletes.

#### PRACTICAL APPLICATIONS

Assessment of TL is a useful tool in tracking an athlete's physiological response to a training session. TL can also be used by dancers and their support staff to ensure a progressive overload in training that can help optimize performance and prevent injury because dancers are particularly susceptible to injury at the commencement of staged performances (25). This is likely due to an abrupt increase in TL caused by the greater intensity of performance, while still maintaining a regular training and rehearsal schedule. Furthermore, EEE can be used to help athletes meet energy intake needs and optimize athlete performance and health. The findings of this study would suggest that current training practices for collegiate dancers are likely suboptimal to induce meaningful performance-related adaptations in these artist-athletes.

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## **DISCLOSURE OF INTEREST**

The authors report no conflict of interest

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Title: The Effects of an 8-week Resistance Training Intervention on

Muscular Strength, Power, and Body Composition in Collegiate Female Dancers

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### ABSTRACT

Although weight training can enhance muscular strength, power, and body composition, outdated beliefs about muscular adaptations have limited its use in dancers. The purpose of this study was to investigate the effect of an 8-week progressive overload resistancetraining (RT) program on muscular strength, power and body composition in collegiate female dancers. Sixteen subjects were randomized into a dance-only control group (CON) or a RT + dance training group (EXP). EXP met 3x/week for RT. Body fat (%BF), lean body mass (LBM), girth measurements, vertical jump (VJ), muscular strength (1-RM squat (SQT) and 1-RM bench press (BP)), maximal oxygen consumption  $(\dot{V}O_{2max})$ , and ventilatory threshold (VT) were assessed pre- and post-study. Baseline performance characteristics across all subjects were: %BF=28.2±5.7%, VJ=33.4±5.4cm, SQT=57.5 $\pm$ 12.1kgs, BP=30.1 $\pm$ 7.6kgs,  $\dot{V}O_{2max}$ =40.6 $\pm$ 3.4 ml·kg<sup>-1</sup>·min<sup>-1</sup>, and VT=71.2 $\pm$ 3.4%VO<sub>2max</sub>. Strength improvements were significantly greater for EXP than CON (P<0.05). No significant differences were found between groups for %BF, LBM, girth measurements, VJ<sub>height</sub>, VO<sub>2max</sub>, or VT (P>0.05). However, a trend was observed for VJ<sub>power</sub> favoring EXP (P=0.07). EXP significantly improved strength, while no significant changes were observed in body composition. However, EXP improved their power-toweight ratio, which may be a positive performance adaptation. RT for dancers can improve strength and power, allowing enhanced muscular loading and fatigue-resistance, which may optimize performance and decrease injury risk.

**Key Words**: Female Athletes, Artistic-Athletes, Strength Training, Muscular Strength, and Muscular Power

### INTRODUCTION

Artistic-athletes (e.g., dancers, gymnasts, figure skaters, divers) are required to perform at a high-level while maintaining aesthetic appeal. Traditionally, dancers primarily focus their training on the creative and technical elements of their activity, while emphasis on fitness variables may be overlooked (Koutedakis & Jamurtas, 2004). Although the benefits of resistance training (RT) for athletic performance are well known (Suchomel, Nimphius, & Stone, 2016), RT is often not performed by dancers largely due to misunderstandings and outdated beliefs about muscular adaptations to lifting weights (e.g., muscle-bulking, decreased flexibility) (Koutedakis & Jamurtas, 2004). As a result, minimal time and resources are allocated for strength and conditioning at all levels of dance. However, due to the favorable impact RT has on athletic performance and body composition, there is a need to investigate its use and efficacy in dancers.

Despite the demands of choreography training and performances, Koutedakis et al. have reported that professional dancers are similar in strength and fitness to healthy, sedentary individuals (Koutedakis & Jamurtas, 2004). In comparison to other athletes, dancers have been shown to be less fit, typically exhibiting lower anaerobic power and cardiorespiratory fitness (i.e.  $VO_{2max}$ ) (Sanders, Walker, Prior, Poyssick, & Arent, 2019). The lower than expected physical fitness values may be attributed to the demands of a strict dance-only training regimen as seen in many collegiate dance programs. Previous research has demonstrated low training demands of college-level ballet and modern classes (Sanders et al., 2019). It is suggested that the workload from these classes is likely suboptimal to induce meaningful fitness-related adaptations (Sanders et al., 2019). Further, this may lead to the observation of higher than expected body fat percentage

(%BF) in conjunction with lower values of lean body mass (LBM) in dancers (van Marken Lichtenbelt, Fogelholm, Ottenheijm, & Westerterp, 1995; Yannakoulia, Keramopoulos, Tsakalakos, & Matalas, 2000). Typically, a lean appearance is a desired characteristic amongst dancers and thus, supplemental RT of sufficient volume and intensity may be beneficial in this population to improve body composition.

Additionally, aspects of fitness such as muscular strength and power are critical for successfully performing choreography that includes numerous leaps, kicks, turns, and lifts. Increased muscular strength via RT has well documented benefits on these general athletic skills (Suchomel et al., 2016), and one's ability to produce power (Newton & Kraemer, 1994). In dance, RT has been found to improve technical aspects of petit allegro including precision of movement (Stadler, Noble, & Wilkinson, 1990) and ability to point feet in the air (Brown, Wells, Schade, Smith, & Fehling, 2007), though this latter observation has been limited to subjective assessment. Despite the subjective evaluation of dance performance overall, RT has been shown to enhance neuromuscular control in this population. Additionally the subsequent increases in LBM (or muscular hypertrophy) from RT has been shown to augment performance by optimizing strength and power capabilities (Taber, Vigotsky, Nuckols, & Haun, 2019). However, more research regarding RT's effect on LBM in dancers is warranted.

Current research on RT in dancers is limited and often vague in its prescription. Koutedakis et al. reported a significant increase in quadriceps and hamstring strength due to a 12-week lower-body, RT program in professional ballet dancers typical of a "strength" training protocol (Koutedakis et al., 2007). The authors further demonstrated only lower-body strength improvements despite a full-body RT program in preprofessional modern dancers (Koutedakis et al., 2007). Similarly, Brown et al. reported significant increases in lower-body strength due to 6-weeks of high load ( $\geq$  80% 1RM), lower-body only RT in collegiate dancers (Brown et al., 2007). In the above studies, significant effects on LBM with RT have only been reported in professional ballet dancers (Koutedakis & Sharp, 2004). However, intervention length (Brown et al., 2007) and concurrent aerobic training (Koutedakis et al., 2007) may have impacted the lack of body composition changes in the other investigations. Thus, the results of high load RT on body composition changes in dancers remains equivocal.

Stalder et al. utilized a lower-body only protocol, (75% 1RM for 10 repetitions of leg press, calf raise, hip adduction and abduction) typical of "hypertrophy" training in collegiate ballet dancers (Stadler et al., 1990). A significant improvement in isometric hip adduction strength was reported with no changes in isometric leg extension strength as well as thigh and calf circumferences after 9-weeks of RT (Stadler et al., 1990). Additionally, the effect of the lower-body "hypertrophy" exercise prescription on LBM cannot be ascertained as it was not assessed from pre- to post-intervention (Stadler et al., 1990).

Of the aforementioned studies, only two of the interventions assessed muscular power and found conflicting results (Brown et al., 2007; Stadler et al., 1990). Stalder et al. found a significant increase in anaerobic power using the Margaria-Kalamen stair climb test (Stadler et al., 1990), while Brown et al. reported a non-significant increase in peak power from a Wingate anaerobic test (Brown et al., 2007). Interestingly, Brown et al. reported no change in standing or moving vertical jump height, but a significant improvement in subjective jump height while performing petit allegro (Brown et al., 2007). This finding suggests that a dance-specific vertical jump test may be more favorable for tracking changes in jump height and power production in dancers.

Several studies have reported lower-body muscular strength improvements; however, it remains uncertain as to whether the above exercise prescriptions were sufficient to elicit meaningful changes in muscular power or body composition. Additionally, most were limited to lower-body only training, which may not be conducive to overall modification of body composition variables. Therefore, the purpose of this study was to determine the effects of an 8-week high volume RT program targeting upper and lower body on muscular strength, power and body composition in collegiate dancers. It was hypothesized that RT would increase muscular strength and power while enhancing body composition to a greater extent than dance-training only.

#### **METHODS**

#### Design

A randomized intervention was used to determine the effects of RT on muscular strength, power and body composition in collegiate female dancers. Participants were randomly allocated to a dance training only control group (CON), or a dance plus RT experimental group (EXP). Both groups continued their required dance training as part of their academic curriculum, while EXP performed additional RT 3x/week for 8-weeks.

#### *Participants*

Sixteen collegiate female dancers enrolled in the Rutgers Performing Arts program were recruited for study participation at the beginning of the academic semester.

Informed consent and health history were obtained from all participants prior to participation. Participants were randomly assigned to CON (n = 8) or EXP (n = 8) following baseline testing. Participant descriptive data can be found in Table 1. All participants had at least five years of formal dance training and were void of any major injuries or metabolic conditions. Participants were excluded if they performed RT  $\geq$ 2x/week within the past 6-months. The Rutgers Institutional Review Board approved this research for the Protection of Human Subjects in accordance with the Declaration of Helsinki.

	EXP (N= 8)	CON (N= 8)
Age (y)	$19.3 \pm 1.3$	19.6 ± 1.3
Height (cm)	164.9±8.6	161.2±4.8
Weight (kg)	$65.9 \pm 9.5$	61.1 ± 2.9

Table 1: Subject characteristics at baseline. Values presented as mean ± SD.

## Performance Testing

A battery of performance tests was completed pre- and post-intervention, with post-testing occurring within 1 week of the last training session. Participants were instructed to report to the Rutgers Center for Health and Human Performance (CHHP) euhydrated and having refrained from eating and exercising 2-hours prior to testing sessions. Pre- and post-testing was standardized (± 1-hour) to control for diurnal variations. Upon arrival to the CHHP, participants' body composition was assessed via air-displacement plethysmography (BOD POD; Cosmed, Concord, CA) (Fields, Goran, & McCrory, 2002). Participants were tested wearing compression bottoms, a sports bra, and a swim cap. Total body weight, LBM, fat mass (FM), and %BF were all measured. The error of body volume reading is roughly 0.02%, which allows for the calculation of %BF with only 0.01% error (Dempster & Aitkens, 1995). Next, girth measurements were obtained at the chest, right upper arm, waist, hips, and right thigh with a flexible tape measure. One set of measurements were taken with a standard procedure (Thompson, Gordon, & Pescatello, 2000). The second set of measurements using the same standard procedure was taken in relevé with feet in second position (hip width apart and externally rotated), and with arms raised in high fifth position (overhead, elbows slightly bent) in order to measure girth while muscles are contracting in a dance specific position. Given the need for dancers to maintain both upper and lower body aesthetics, measuring body composition in a position similar to how they will be judged within a competition setting is important.

After a supervised, standardized dynamic warm-up, countermovement vertical jump (VJ) height was assessed (Just Jump Mat, Probotics, Inc.). Participants' were instructed to begin in first position on the mat, and then to perform a demi-plié (halfsquat) and immediately jump as high as possible off both feet keeping heels together, hips externally rotated, and feet pointed (a dance sauté). Three jumps were performed, non-consecutively. The highest jump height was recorded, and power was calculated as watts (W) = weight (kg) \* jump height (m) (Genuario & Dolgener, 1980). Muscular strength was assessed via 10-RM squat and bench press using NSCA guidelines. For both exercises, 2-3 warm-up sets were performed prior to the first 10-RM attempt. Participants were given 4-5 minutes of rest prior to each attempt and weight was increased until 10 repetitions executed in good form for each of the exercises were performed. Participants were given a maximum of 5 sets to perform a 10-RM. Estimates for 1-RM were made using a linear prediction equation [Epley's equation: 1-RM = rep<sub>wt</sub> [1 + (reps/30)] (Baechle, Earle, & Wathen, 2008).

After a 20-minute break, participants performed a maximal graded exercise test (GXT) on a treadmill to assess VO<sub>2max</sub> and Ventilatory Threshold (VT) via direct gas exchange (Cosmed Quark CPET, Concord, CA). The GXT utilized the standard Bruce treadmill protocol (Thompson et al., 2000) and rating of perceived exertion (RPE) was assessed at the end of each stage using Borg's 6-20 point scale (Borg, 1985). Heart rate (HR) was continuously monitored using the Polar H10 HR monitor (Polar Electro Co., Lake Success, NY, USA). Participants performed this test until volitional exhaustion.  $VO_{2max}$  was considered to have been achieved if at least 3 of the following criteria were met:  $HR_{max}$  within  $\pm 15$  beats·min<sup>-1</sup> of age-predicted  $HR_{max}$ , respiratory exchange ratio  $\geq$  1.10, RPE  $\geq$  17, a HR that fails to increase with increased workload, and a plateau of  $VO_2$  using the generated VE:VO<sub>2</sub> plot and was expressed as a percent of  $VO_{2max}$  (Gaskill et al., 2001).

#### Resistance Training Program.

All training sessions were held at the CHHP and were coached by a Certified Strength and Conditioning Specialist (CSCS). EXP engaged in RT on three non-consecutive days per week for 8-weeks. All training sessions began with a standardized, 5-minute dynamic warm up. Then, multi-joint exercises were performed for 3 sets of 10-12 repetitions per set. Next, single-joint and abdominal exercises were performed for 2 sets of 12-15 repetitions per set. Approximately 90-seconds of rest was given between each set. Each training session focused on either an upper- or lower-body routine, which was alternated throughout the intervention. On upper-body day the following exercises were performed in order: bench press, dumbbell row, seated dumbbell shoulder press, lat pull-down, biceps, triceps, and abdominal exercises. On lower-body day the following exercises were performed in order: back squat, barbell Romanian deadlift, barbell split squat, leg curl, leg extension, calf raise, and abdominal exercises.

This program followed principles of progressive overload, in which load was increased when subjects completed the prescribed exercise repetition range with proper form. The initial training load for squat and bench press exercises was set at 65% 1-RM, as determined from pre-testing. For untested exercises, load was determined by study personnel during the first training session through trial. Weight was appropriately adjusted until participants performed the required number of repetitions for that exercise. Load was increased when subjects performed 2 reps more than prescribed on the final set of that exercise. Weight increases were 1-2.5 kg and 2.5-4.5 kg for upper body and lower body exercises, respectively.

Statistical Analysis

Repeated measures (RM) ANOVAs were conducted to examine differences in the strength, body composition, and girth measures between groups over time. Univariate follow-ups using a simple effects model were performed to evaluate changes within each condition over time. For each univariate analysis, the Huynh-Feldt epsilon was calculated to test the assumption of sphericity. If this statistic was  $\geq$  .0.75, sphericity was assumed, and the unadjusted statistic was used. If this statistic was < 0.75, then sphericity was considered to have been violated and the Huynh-Feldt adjusted statistic was used to test significance. Cohen's d was calculated to determine the magnitude of the treatment effects. ES of 0.20, 0.50, and 0.80 were considered indicative of small, medium and large-sized effects, respectively (Cohen, 1992). Data are expressed as mean± SD, and statistical significance was set at P  $\leq$  0.05. SPSS statistical software was used for analyses (SPSS version 23; IBM).

# RESULTS

### Performance Testing

No significant differences were found between groups for any performance measures at baseline. There was a significant time by group interaction in 1-RM squat ( $\Delta_{EXP}$ = 39.2 ± 9.4 kg vs.  $\Delta_{CON}$ = 8.1 ± 5.5 kg; P < .001) and bench press ( $\Delta_{EXP}$ = 20.5 ± 8.3 kg vs.  $\Delta_{CON}$ = 2.7 ± 6.0 kg; P < .001) from pre- to post-intervention (see Figure 1). Also, a time by group trend toward significance was found in VJ power (P = .066). From pre to post testing, simple effects revealed significant increases in VJ height, VJ power, and 1RM squat in EXP and CON (P < .05). A significant increase was also found in EXP 1-RM bench press, but not in CON. No significant differences were found in VO<sub>2max</sub> or VT% in

EXP or CON; however, a large effect for increased  $VO_{2max}$  was observed in CON, but not EXP. Furthermore, EXP experienced a small effect for an increase in VT%, while CON exhibited a moderate decrease (Table 2).

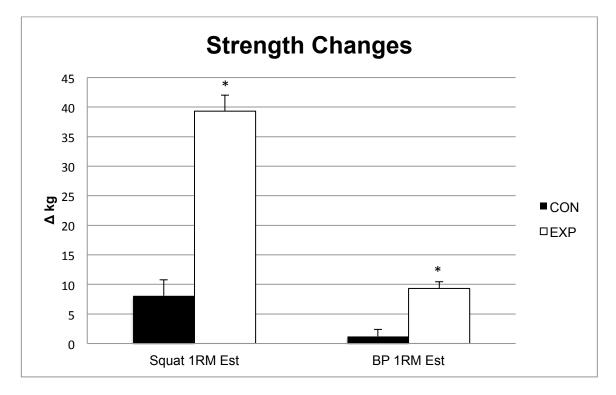


Figure 1: Changes in strength from pre- to post (mean  $\pm$  *SE*) for EXP and CON. \* Significantly different (p < 0.05) from CON.

Measure	Pre <sub>EXP</sub>	Post <sub>EXP</sub>	ES <sub>EXP</sub>	Pre <sub>CON</sub>	Post <sub>CON</sub>	ES <sub>CON</sub>
Weight (kg)	65.9 ± 9.5	66.1 ± 9.0	0.01	61.1 ± 2.9	59.8± 2.8*	-0.47
%BF	27.1 ± 6.0	25.3 ± 5.7*	-0.30	29.3 ± 5.6	26.3± 5.6*	-0.54
LBM (kg)	$47.5 \pm 6.3$	49.1 ± 6.5*	0.26	$43.2 \pm 3.6$	44.1±3.5	0.22
FM (kg)	18.4 ± 5.7	$16.9 \pm 5.2*$	-0.27	17.9 ± 3.6	15.7 ± 3.5*	-0.61
Sqt <sub>1RM</sub> (kg)	59.2 ± 13.5	98.5 ± 18.4*	2.92†	55.9 ± 11.2	64.0 ± 12.1*	0.72
BP <sub>1RM</sub> (kg)	$32.7 \pm 8.3$	$42.0 \pm 6.2^*$	1.12†	$27.5 \pm 6.4$	$28.7 \pm 4.8$	0.19

VJ (cm)	$34.6 \pm 6.3$	$40.4 \pm 7.2*$	0.90	$32.1 \pm 4.3$	35.8±4.7*	0.84
VJ (W)	22.3 ± 1.9	$26.3 \pm 4.4*$	2.12	$19.7 \pm 2.8$	21.4 ± 2.9*	0.61
VO <sub>2max</sub>	42.6 ± 3.0	42.5 ± 3.9	-0.02	38.6 ± 2.6	40.8± 5.0	0.87
VT%	$71.5 \pm 4.2$	73.1 ± 3.9	0.38	$70.9 \pm 2.5$	69.0± 3.8	-0.74

Table 2: Values presented as mean  $\pm$  SD. Effect size (ES) gain presented as variable x condition. %BF, body fat percentage; LBM, lean body mass; VJ, vertical jump; VT%, ventilatory threshold as % of VO<sub>2max</sub>. \* denotes significant differences from pre- to post-intervention P < 0.05. † denotes significant differences between groups P < 0.05.

# **Body Composition**

No significant differences were found between groups for all measures of body composition at baseline. Significant time main effects were found for LBM, FM, and %BF (P < .05). Follow-ups revealed significant changes over time across FM and %BF in both groups, for body weight in CON only, and LBM in EXP (P < .05; Table 2). A trend was observed for increased LBM in CON (P = .06). Despite the significant change in LBM for EXP only, the magnitude of effect was similar in for both groups (ES<sub>EXP</sub>= 0.26 vs. ES<sub>CON</sub>= 0.22). Additionally, EXP maintained weight (ES= 0.01), while CON lost weight (ES= -0.47), despite decreases in %BF for both groups (ES<sub>EXP</sub>= -0.30 vs. ES<sub>CON</sub>= -0.54). No significant differences were found between groups in girth measurements in the regular and relevé positions (Table 3). Time main effects were found in regular waist (P = .025) and hip (P = .034) girths only. Follow-up analyses showed a significant decrease in regular waist girth over time in CON (P < .05), but no significant changes in any other girth measurements for CON or EXP (Table 3).

Measure	Pre <sub>EXP</sub>	Post <sub>EXP</sub>	ES <sub>EXP</sub>	Pre <sub>CON</sub>	Post <sub>CON</sub>	ES <sub>CON</sub>
Chest <sub>reg</sub>	90.2 ± 8.7	89.8 ± 5.9	-0.04	86.4 ± 3.3	86.4 ± 3.5	0.00
Upper Arm <sub>reg</sub>	29.4 ± 2.8	$29.5 \pm 2.3$	0.06	28.1 ± 1.5	27.8 ± 1.4	-0.13
Waist <sub>reg</sub>	$75.2 \pm 6.9$	74.6 ± 6.1	-0.09	73.6 ± 3.0	71.9 ± 2.5*	-0.57
Hips <sub>reg</sub>	96.4 ± 6.1	98.7 ± 4.9	0.38	94.6 ± 3.9	96.1 ± 1.5	0.37
Thigh <sub>reg</sub>	56.7 ± 5.1	56.9 ± 4.7	0.04	55.4 ± 3.0	54.4 ± 3.1	-0.35
Chest <sub>rel</sub>	89.8 ± 8.3	89.1 ±7.1	-0.08	86.0 ± 3.1	85.6 ± 2.7	-0.11
Upper Arm <sub>rel</sub>	$29.2 \pm 2.7$	29.7 ± 2.6	0.19	29.1±1.6	27.8 ± 1.6	-0.79
Waist <sub>rel</sub>	74.4 ± 7.4	74.8 ± 8.3	0.06	$72.4 \pm 2.6$	71.7 ± 2.0	-0.25
Hips <sub>rel</sub>	95.8 ± 7.0	98.22 ± 6.0	0.34	$94.8 \pm 4.0$	95.8 ± 3.6	0.27
Thigh <sub>rel</sub>	$56.0 \pm 4.7$	56.4 ± 4.7	0.09	54.7 ± 2.7	$54.0 \pm 3.3$	-0.28

Table 3: Values were measured in cm and are presented as mean  $\pm$  SD. Effect size (ES) gain presented as variable x condition. Measurements were taken in the regular position (reg) and relevé position (rel). \* denotes significant differences from pre- to post-intervention P < 0.05.

# CONCLUSION

As hypothesized, increases in upper- and lower-body muscular strength were significantly greater in EXP than CON. Interestingly, the increases in strength observed in both upper and lower body measures in the current investigation were greater than previous investigations (Cadore et al., 2014; Koutedakis & Jamurtas, 2004; van Marken Lichtenbelt et al., 1995). Additionally, there were no differences between groups in either VJ height or power, however EXP experienced a greater magnitude of change in VJ power than CON. Although RT did produce a significant increase in LBM and decrease in %BF in EXP, the difference was not significantly greater than CON. Additionally, no significant changes in  $VO_{2max}$  or %VT were seen pre to post intervention.

The RT program implemented was a novel stimulus to the study subjects. Large increases in lower- and upper-body strength occurred in conjunction with a modest increase in LBM. These findings varied from previous strength training interventions in non-professional dancers, which reported strength increases with no change in body composition (Brown et al., 2017; Koutedakis et al., 2007; Stadler et al., 1990). Stalder et al. performed lower-body exercises for 3 sets of 10 reps at 10-RM, and found a significant improvement in isometric hip adduction strength only, along with a 40% increase in working weight for the leg press (Stadler et al., 1990). Similarly, Brown et al. performed lower-body exercises only for 3 sets of 6-8 reps in collegiate female dancers and reported a 32% increase in leg press 1-RM after 6-weeks of training (Brown et al., 2007). Using 5-6 sets of 8 reps or less in male and female modern dance students, Koutedakis et al. found only a 13% increase in lower body isometric strength after 3months of training (Koutedakis et al., 2007). In professional ballerinas, a 16% increase in lower body torque was reported using the same RT protocol (Koutedakis & Sharp, 2004). In comparison, the current intervention produced a 66% increase in squat 1-RM and a 28% increase in bench press 1-RM with an 8-week intervention. The large increase in strength observed suggests that the change was primarily driven by neuromuscular adaptation based on the intervention length and modest change in body composition (Gabriel, Kamen, & Frost, 2006).

Although all studies used different tests to determine maximal lower body strength, program design variables (e.g., exercise selection, exercise order, volume) may have contributed to the greater strength increases observed in the current study. Exercise selection and order were designed to optimize load throughout the training session (Baechle et al., 2008). Specifically, the inclusion of multiple compound exercises in the current study may have enhanced strength increases compared to other studies that used only one multi-joint exercise (Brown et al., 2007; Stadler et al., 1990). This study highlights an effective protocol that can be replicated in collegiate dancers. Additionally, the level of artistic-athlete recruited for study participation must be taken into consideration as differences in maximal physical capacity and/or initial fitness values may be observed between collegiate-level, pre-professional, and professional dancers.

Power production is an essential component of fitness for dancers, as it is needed to perform numerous jumps and leaps throughout a routine. The improvement in jump height observed in both groups suggests a sport-specific, neuromuscular adaption from practice of jumps during dance classes, as both groups continued their regular training. However, the larger increase in EXP jump power may be partially linked to the significant increase in strength, as body weight is taken into account when calculating power. The observed increase in strength may have facilitated EXP's ability to produce power (Newton & Kraemer, 1994), as they were able to jump higher despite a greater body weight. After taking body weight into account, this relative power measure is indicative of a positive performance adaptation for EXP, as the dancers' power-to-weight ratio was improved. Despite only a trend for between-group differences being observed for power, the greater magnitude of change observed in EXP may be of clinical relevance as increased lower body muscular power reduces injury risk as demonstrated by a decreased prevalence of injury with increased lower body muscular power in female contemporary dancers (Angioi, Metsios, Koutedakis, Twitchett, & Wyon, 2008).

Resistance training improved body composition, as EXP decreased %BF and increased LBM. However, contrary to our hypothesis, RT did not enhance body composition to a greater extent than dance-training only as no differences were found between groups in any measure, despite some interesting trends for EXP. The lack of changes found in thigh girth measurements (standard and on relevé) are consistent with previous investigations (Koutedakis & Sharp, 2004; Stadler et al., 1990). Small to medium effect sizes were found for decreases in %BF for EXP and CON, respectively. The greater decrease in %BF observed in CON may have occurred because of a slightly higher %BF at baseline in CON than EXP, even though the difference was not significant. Interestingly, EXP decreased %BF while maintaining weight from pre- to post-intervention. This outcome is driven by the significant increase in LBM and significant decrease in FM found in EXP. Even though the increase in LBM was not different between groups, RT provided a slight enhancement compared to dance-only training. This difference may be of physiological importance to the artistic-athlete who is training to optimize their power-to-weight ratio (Brown et al., 2017), which was reflected in the VJ power differences. The authors acknowledge that LBM and %BF changes might have become more pronounced if the intervention was continued beyond 8-weeks. However, limitations in a collegiate setting, such as university-imposed recess as well as academic and performances schedules, make implementation of long-term studies difficult. Though not examined in the current study, Cadore et al. have reported an improvement in muscle quality from strength training (i.e. decreased intramuscular

connective and adipose tissue) even with minimal changes in muscle size (Cadore et al., 2014). Therefore, the lack of girth changes observed with a concomitant decrease in %BF, increase in LBM, and weight maintenance in this study may suggest a modest trade-off between muscle tissue and adipose tissue. Future research would benefit from incorporating the use of ultrasound as changes in subcutaneous adipose tissue, muscle thickness and quality can be assessed regionally.

Aerobic fitness was tested in this study as part of a comprehensive performance testing battery. Consistent with previous investigations, the current participants had average aerobic capacity for their age group, but performed poorly when compared to other power endurance athletes (Hoffman et al., 2009). No significant changes occurred in  $VO_{2max}$  or %VT over 8-weeks in both groups, in agreement with other reports in collegiate and professional modern dancers from dance-only training (Koutedakis et al., 2007; Martyn-Stevens, Brown, Beam, & Wiersma, 2012). The lack of significant improvement in aerobic capacity demonstrates that the intensity of dance class is insufficient in eliciting substantial cardiovascular adaptations (Sanders et al., 2019).

The current investigation is not without limitations. First, only females were included, so our outcomes cannot be generalized to male dancers, especially with regard to girth and LBM. The length of the intervention may have been too short to elicit significant body composition changes compared to dance-only training, as only 12 sessions were performed for the upper- and lower-body, respectively. Additionally, no assessment of current dance training was completed, as it was beyond the scope of the current investigation. All participants were enrolled in their required dance curriculum; however, some participants may have been exposed to more rehearsal time than others due to selection for different performance pieces. Thus, varying recovery status and total energy expenditures may have impacted our findings. Further, dietary food logs were not collected throughout the study, so the influence of total calories and protein consumption cannot be determined. Based on this study as well as previous studies in other dance populations, it is possible that a short-term supplemental RT program alone is insufficient to produce notable body composition changes in these athletes without a concomitant nutritional intervention.

In summary, 8-weeks of high volume, moderate load RT elicited a significant increase in muscular strength compared to dance-only training. However, significant differences in power, LBM or %BF did not occur between groups. Both groups decreased their %BF over the 8-weeks, but EXP did so while maintaining their current weight and improving LBM. Finally, RT may also decrease injury risk by positively influencing power output and fatigue. Future research is needed to investigate the role of RT and nutrition in optimizing the health and performance of artistic-athletes in regards to strength, power, and body composition.

### CONCLUSION

By nature, dance is subjective. Thus, performance improvement is ultimately dependent upon those who are evaluating the dancer. However, results of this study show strength and conditioning can have a positive impact on the various components that comprise a successful performance, as muscular strength and power are improved. Meaningful changes in body composition may occur with adequate nutrition in conjunction with RT. Increases in LBM should be viewed positively by the artistic-

athlete, as this may have positive outcomes for health, performance and career success.

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Title: The Effects of a Semester of Vocational Dance Training on Biomarkers and

Performance Variables in Elite Adolescent Ballet Dancers

Submission Type: Original Investigation

Running Head: Biomarkers in Dance

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# Abstract

Energy expenditure (EE), performance and biomarker assessments are popular in athletics as they provide a comprehensive approach to athlete monitoring in order to optimize health and performance. Currently, research in elite, adolescent artistic-athletes (e.g. dancers) is limited, especially with regard to biomarkers. PURPOSE: To evaluate changes in performance and biomarker variables over the course of a semester in elite male and female adolescent ballet dancers and to compare differences between sexes. **METHODS:** Male (n= 10;  $M_{age}$ = 16.8±1.6 yrs;  $M_{height}$ = 173.7±7.8 cm) and female (n= 14;  $M_{age}$ = 15.4±1.3 yrs;  $M_{height}$ = 162.8±6.3 cm) ballet dancers were recruited from a vocational ballet school. Performance testing included body weight (BW), body fat percentage (%BF), lean body mass (LBM), vertical jump height (VJ) power (VJW) and a maximal graded exercise test to assess VO<sub>2max</sub> and ventilatory threshold (%VT) via indirect calorimetry. Menstrual status was assessed in females pre- and post-study via questionnaire. Biomarkers were collected at the beginning of the semester (T1), and every subsequent 4-weeks (T2-5). Athletes reported for blood draws fasted and euhydrated between 0700-0900h having refrained from activity. Thyroid-stimulating hormone (TSH), free and total triiodothyronine ( $T_{3F}$ ,  $T_{3T}$ ), free and total thyroxine ( $T_{4F}$ ,  $T_{4T}$ ), free and total cortisol (CORTF, CORTT), free and total testosterone (TESTF, TESTT), estradiol (E2), follicular-stimulating hormone (FSH), growth hormone (GH), insulin-like growth factor-1 (IGF1), creatine kinase (CK), TNF-α, omega-6:omega-3 ratio  $(O_{6:3})$ , vitamin d,25-OH (Vit-D), iron (Fe), iron binding capacity (IBC), and percent saturation (%Sat) were assessed. Performance testing was conducted at the beginning and end of semester. Total (TEE) and exercise (EEE) energy expenditure were collected over

a typical 7-day training epoch using a Polar M430 watch and H10 monitor. RM MANOVAs and univariate ANOVAs were used to identify differences between male and female dancers, and changes over time with significance set at p<0.05. **RESULTS:** Significant sex differences were found in EEE, TEE, BW, BM, VJ, VJW, VO<sub>2max</sub>, T<sub>3F</sub>, TESTF, TESTT, E2, FSH, GH, CK, and %Sat (p<0.05). Time main effects were found for BW, VJ, T<sub>3T</sub>, T<sub>4F</sub>, CORTF, CORTT, FSH, CK, TNF- $\alpha$ , Vit-D, Fe, and %Sat (p<0.05). In females, significant alterations in TSH, T<sub>4F</sub>, CORTT, TESTF, TESTT, FSH, IGF-1, TNF- $\alpha$ , O<sub>6:3</sub> Vit-D, Fe, and %Sat were found (p<0.05). No changes in E2 were found, but reported cases of amenorrhea increased. In males, significant alterations in T<sub>3T</sub>, CORTT, CORTF, TESTT, FSH, CK, TNF- $\alpha$ , Vit-D, and %Sat were found (p<0.05).

**CONCLUSIONS:** Biomarkers of iron status demonstrated differential responses between sexes, as adverse changes occurred in females only. Further, HPG-axis disruption is apparent in females, but not males. Adolescent male and female ballet dancers showed similar EE when BW and LBM are taken into account. Sex differences were observed in performance variables, and males had a greater increase in muscular power than females. **PRACTICAL APPLICATIONS:** Biomarkers, in conjunction with EE and performance testing, can be used to detect disruptions that may be negatively impacting health and performance, and individualize interventions to the artistic-athlete's specific needs in order to optimize and health and performance.

**Key Words**: Youth Athlete, Hormones, Energy Expenditure, Body Composition, Sex-Differences

# INTRODUCTION

Aspiring dancers face numerous challenges and demands in order to progress to the professional level. Youth athletes, and dancers in particular, often train with a high volume, and may specialize in sport at an early age (22,38). Young, elite dancers may also have the opportunity to attend vocational schools that requires up to 30 hours of dance training per week, in addition to academic requirements. Training stress, in conjunction with psycho-social stress, may lead to physiological maladaptations that impair aspects of growth, maturation, and overall health (29). Further, this may result in performance decrements and increase the risk for injury if stress is not continually met with adequate recovery (23). Therefore, athlete monitoring combined with periodic performance testing is important to optimize health and performance.

Systematic maximal performance testing (e.g. muscular power, aerobic capacity) and body composition assessments can be utilized to determine an individual athlete's capabilities, growth, and physiological adaptation to training and performance periods. Monitoring changes in these metrics may be indicative of athlete readiness to perform. However, frequent maximal testing may incur unnecessary fatigue and reduce training availability. Thus, monitoring techniques that determine physiological responses to training and minimize athlete stress should be implemented periodically throughout a training cycle.

Training load (TL) monitoring is popular in sport, but has yet to be systematically applied to the artistic-athlete. Heart rate monitoring (HRM) is a common technique to assess an athlete's internal load. Internal workload is considered an athlete's physiological response to training. (2). Importantly, HRM can estimate exercise energy expenditure (EEE) while training due to the relationship between HR and VO<sub>2</sub> (2). Such information can be used to address nutritional needs, as recent evidence has demonstrated dancers are at risk of energy deficiency (6). The aesthetic component of dance performance may lead to caloric restriction and energy deficiency in order to obtain a desired body composition (19,29). Further, chronically low energy intakes in association with high training volumes and EEEs increases an athletes' risk for low energy availability (LEA), an underlying cause of menstrual disturbances and relative energy deficiency in sport (RED-S) (29). Although HRM quantifies an athlete's workload, the use of biomarkers enables the comprehensive analysis of the physiological and biochemical response to all stressors (26).

Biomarker monitoring enables an objective assessment of athlete health, performance, and recovery status (41). Importantly, biomarkers can account for time when athletes are not being monitored by other techniques (26). Sex, stress, metabolic, inflammatory, nutritional, and hematological related markers have been used to assess athletes' response to training (5,11,21,26,41,42). Research evaluating biomarker responses to TL over time has been conducted in collegiate athletes, with a greater prevalence of this research conducted in male athletes (21,41). In one of the few studies evaluating the biomarker response of collegiate female soccer athletes during a competitive season, researchers found significant fluctuations in various biomarkers in conjunction with changes in training loads (41). Additionally, females have demonstrated dysfunction of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitarythyroidal (HPT) axis specifically related to LEA (4,40), while HPG axis dysfunction has been reported in male runners (20). In youth athletes, similar rates of anemia have been observed between males and females (36), however comprehensive research is limited in this population in regard to HPG- and HPT-axis function due to training. Given that elite, adolescent artistic-athletes endure high training volumes in addition to other social and academic pressure, more research is needed to determine the impact of these stressors on biomarkers, performance, and overall athlete health.

The purpose of this observational study was to evaluate the workload of elite, adolescent ballet dancers and assess changes in their body composition, performance variables, and blood-based biomarkers related to health throughout a semester of training and performing. Additionally, differences between male and female ballet dancers were evaluated. It was hypothesized there would be alterations in body composition, performance, and blood-based biomarkers over the course of the semester. Further, differences would be found between sexes in regards to energy expenditure, performance, and biomarker responses.

#### METHODS

#### Experimental Approach to the Problem

This observational study sought to evaluate the semester-long effect of training on body composition, performance, and various biomarkers in a real-world setting using high-level male and female ballet dancers from an elite vocational ballet school. A battery of performance tests were conducted at the beginning of the semester and 5months later during the final week of the semester. Training load variables and daily energy expenditure were monitored during a 7-day period of training. Biomarkers were assessed at the beginning of the semester (T1) and every 4 weeks thereafter (T2-T5) to evaluate the effects of accumulated stress of training on biomarkers representing general health, performance, growth and maturation, and metabolism.

## **Subjects**

Male (N=10; Age=16.8 $\pm$ 1.8 years; Height= 173.2 $\pm$ 7.9 cm) and female (N=14; Age=15.43 $\pm$ 1.3 years; Height= 162.8 $\pm$ 6.3 cm) ballet dancers were recruited from an elite vocational ballet school for this study. Descriptive and performance data are presented in Table 2. For participants under the age of 18, participant assent and parental consent was obtained. All subjects had at least 4 years of dance training having reached an advanced level, and were free of any major injuries or metabolic conditions. All subjects received clearance by the medical staff before baseline testing. One female began hormonal contraceptive use during the study, and was excluded from biomarker analysis. This research was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects before the commencement of the study. **Procedures** 

*Performance Testing.* Athletes reported to the Rutgers Center for Health and Human Performance (CHHP) at the beginning of the semester (PT1) and 5-months later at the end of the semester (PT2) to complete a battery of performance testing. Participants reported to the CHHP rested and euhydrated, and having refrained from eating  $\geq$  2-hours and exercise >24 hours prior to testing sessions. Pre- and post-testing was standardized (± 1-hour) to control for diurnal variations.

Upon arrival to the CHHP, participants' body composition was assessed via airdisplacement plethysmography (BOD POD; Cosmed, Concord, CA). Participants were tested in accordance with manufacturer guidelines for body weight (BW), lean body mass (LBM), and body fat percentage (%BF). The Brozek equation was used to determine %BF. The error of body volume reading is roughly 0.02%, which allows for the calculation of %BF with only 0.01% error (13). After a supervised, standardized dynamic warm-up, vertical jump (VJ) height was assessed (Just Jump Mat, Probotics, Inc.). VJ height was assessed using a standard countermovement jump with hands on hips (25). Three jumps were performed non-consecutively, and the highest jump height was recorded. Power was calculated as watts (W) = weight (kg) \* jump height (m) (17). Relative power was assessed by dividing by LBM (VJW<sub>LBM</sub>). After this, participants performed a maximal graded exercise test (GXT) on a treadmill to assess VO<sub>2max</sub> and Ventilatory Threshold (VT) via direct gas exchange (Cosmed Quark CPET, Concord, CA) using a standard Bruce treadmill protocol. Participants performed this test with encouragement from the lab staff until volitional fatigue. VO<sub>2max</sub> was considered to have been achieved if at least 3 of the following criteria were met:  $HR_{max}$  within  $\pm 15$ beats  $\cdot$  min<sup>-1</sup> of age-predicted HR<sub>max</sub>, respiratory exchange ratio  $\ge 1.10$ , RPE  $\ge 17$ , a HR that fails to increase with increased workload, and a plateau of VO<sub>2</sub> (< 2.0 ml·kg-1·min-1) despite an increase in workload. Heart rate (HR) was continuously monitored using the Polar H10 HR monitor (Polar Electro Co., Lake Success, NY, USA). VT was calculated as the point at which ventilation (VE) began to increase non-linearly with VO<sub>2</sub> using the generated VE:VO<sub>2</sub> plot and was expressed as a percent of VO<sub>2max</sub> (16). Additionally, females completed a menstrual history questionnaire to determine current menstrual

status, which included questions such as age of first menses, date of last period, length of cycle and contraceptive use.

*Energy Expenditure Assessment.* Athletes' activity was monitored over a 7-day period using a Polar M430 watch and associated H10 heart rate monitor (Polar Electro Co.). Monitoring occurred during a typical week of classes. The quantification of an individual's workload was determined by exercise energy expenditure (EEE) (10). Values obtained from multiple dance classes were summed per day to obtain an average EEE. Monitors were programmed with each subject's values obtained during performance testing (e.g., HRmax, VO2max, and VT) to improve accuracy of the estimated EEE. Non-exercise energy expenditure (NEEE) was obtained from wrist-based HR assessment via Polar M430. Athletes were instructed to wear the device at all times outside of dance class. Total energy expenditure (TEE) was determined (TEE= NEEE + EEE). Relative energy expenditure variables were calculated by dividing the absolute measure (e.g. TEE) by BW (kg).

*Sample Collection and Analysis.* Athletes reported for blood draws 5 times throughout the semester. Initial blood draw was conducted at first availability upon semester commencement (T1); subsequent blood draws were conducted every 4 weeks in the morning following (approximately 12 hours after) their most recent training session (T2-5). Athletes arrived for testing between 0700 and 0900 hours following an overnight fast. Blood samples were centrifuged for 10 minutes at 4,750 rpm (Allegra x-15R, Beckman Coulter, Brea, CA, USA) and were shipped to Quest Diagnostics for analysis via LC-MS/MS-based assays. Samples were run in duplicate and the coefficient of variation (CV) was between 0.5-7.5 % for all biomarkers. Blood biomarkers in the

analysis included thyroid-stimulating hormone (TSH), free and total triiodothyronine  $(T_{3F}, T_{3T})$ , free and total thyroxine  $(T_{4F}, T_{4T})$ , free and total cortisol (CORTF, CORTT), free and total testosterone (TESTF, TESTT), estradiol (E2), follicular-stimulating hormone (FSH), growth hormone (GH), insulin-like growth factor-1 (IGF1), creatine kinase (CK), tumor necrosis factor alpha (TNF alpha), omega-3 (n-3FA), omega 6/omega 3 ratio (O<sub>6:3</sub>), vitamin d,25-OH (Vit-D), iron (Fe), iron binding capacity (IBC), and percent saturation (%Sat).

### Statistical Analysis

Energy expenditure and related variables were evaluated using one-way ANOVAs to compare differences between sexes. Body composition and performance changes were analyzed using a 2 x 2 (Sex x Time) RM MANOVA. The multiple imputation method was used to adjust for missing biomarker data from laboratory tests not performed due to insufficient quantities of blood serum/plasma. Values for missing data were obtained from the aggregation of the outcomes from 10 imputations (18). Biomarkers were assessed with imputed data utilizing a 2 x 5 (Sex x Time) RM MANOVA. Univariate follow-ups using a simple effects model were performed to evaluate changes from baseline at each timepoint within each sex. For each univariate analysis, the Huynh-Feldt epsilon was calculated to test the assumption of sphericity. If this statistic was  $\geq .0.75$ , sphericity was assumed, and the unadjusted statistic was used. If this statistic was < 0.75, then sphericity was considered to have been violated and the Huynh-Feldt adjusted statistic was used to test significance. Cohen's d was calculated to determine within sex magnitude of change, with positive effect sizes (ES) indicating an increase in value from baseline. ES of 0.20, 0.50, and 0.80 were considered indicative of small, medium and

large-sized effects, respectively (12). Data are expressed as mean $\pm$  SD, and statistical significance was set at P  $\leq$  0.05. SPSS statistical software was used for analyses (SPSS version 26; IBM, Armonk, NY, USA).

# Results

# **Energy Expenditure**

Energy expenditure variables can be found in Table 1. Males had a significantly greater TEE, EEE, and NEEE compared to their female counterparts (p < 0.001). There were no differences between sexes in any of these variables after adjusting for body size (P > 0.1).

		Mean ± SD
755 (here))	м	3129 ± 511.5″
TEE (kcal)	F	2318 ± 334.3
	м	51 ± 3.7
TEE <sub>kg</sub> (kcal·kg <sup>-1</sup> )	F	49 ± 7.5
	м	1982 ± 236.5″
NEEE (kcal)	F	1506 ± 243.5
NEEE (keel.keel)	м	33 ± 7.5
NEEE <sub>kg</sub> (kcal·kg <sup>-1</sup> )	F	32 ± 5.6
FFF (here)	м	1306 ± 286.2"
EEE (kcal)	F	852 ± 174.9
555 (head heat)	м	20 ± 3.3
EEE <sub>kg</sub> (kcal·kg <sup>-1</sup> )	F	18 ± 3.4

Table 1: Values presented as mean  $\pm$  SD. TEE, total energy expenditure; TEEkg, total energy expenditure per kg body weight; NEEE, non-exercise energy expenditure; NEEEkg, non-exercise energy expenditure per kg body weight; EEE, exercise energy expenditure; EEEkg, exercise energy expenditure per kg body weight. # denotes significant difference between sexes (P < 0.01)

#### Performance

Body composition and performance variables can be found in Table 2. Males had a significantly greater weight, %BF, VJ, VJW, VJW<sub>LBM</sub>, and VO2max , while females had a significantly greater LBM (p < 0.05). Significant time main effects were found for

weight, VJ, VJW, and VJW<sub>LBM</sub> (p < 0.05), and a trend in %BF (p=0.061). From pre- to post-testing, simple effects revealed significant increases in weight and VJW (p < 0.05), while trends were observed for increased LBM (p=0.069) and VJW<sub>LBM</sub> (p=0.059) in females. In males, simple effects revealed significant increases in weight, VJW, and VJW<sub>LBM</sub> (p < 0.05), while a trend was observed for VJ (p=0.071).

		PT 1	PT 2	ES
Maight (kg)	M#	62.23 ± 11.10	63.39 ± 10.32 <sup>\$</sup>	0.10
Weight (kg)	F	47.86 ± 4.71	49.55 ± 4.46 <sup>s</sup>	0.36
	M#	57.57 ± 10.54	58.02 ± 9.12	0.04
LBM (kg)	F	40.75 ± 4.39	41.41 ± 3.70	0.15
%BF	м	7.54 ± 3.22	8.42 ± 2.79	0.27
70 DF	F#	14.71 ± 6.23	16.17 ± 5.65	0.23
\// (ame)*	M#	53.31 ± 5.12	56.36 ± 4.71	0.68
VJ (cm)*	F	38.08 ± 4.55	39.25 ± 4.26	0.10
VJW*	M#	33.80 ± 7.50	36.10 ± 6.66 <sup>\$</sup>	0.40
VJVV	F	17.94 ± 2.30	19.15 ± 1.80 <sup>\$</sup>	0.30
V/DA/ *	M#	$0.57 \pm 0.05$	0.61 ± 0.06 <sup>\$</sup>	0.83
VJW <sub>LBM</sub> *	F	$0.45 \pm 0.05$	0.47 ± 0.03	0.23
VO2max	M#	59.83 ± 4.35	59.23 ± 3.92	-0.13
(ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	F	50.80 ± 4.28	49.23 ± 4.03	-0.23
VT (% VO2max)	м	71.33 ± 3.46	70.33 ± 3.57	-0.19
VT (% VO2max)	F	73.91 ± 4.99	70.09 ± 7.31	-0.59

Table 2: Values presented as mean  $\pm$  SD. Effect size (ES) gain presented as variable x condition. LBM, lean body mass; %BF, body fat percentage; VJ, vertical jump height; VJW, vertical jump power; VJWLBM, vertical jump power per kg LBM; VT%, ventilatory threshold as % of VO2max. # denotes significant difference between sexes (P < 0.05). \* denotes time main effect (P < 0.05). \$ denotes significant within group change (P < 0.05).

# **Biomarker Responses**

All hormonal data can be found in Table 3 and 5. Males had significantly greater values for  $T_{3F}$ , TestF, and TestT, while females had significantly greater values for E2, FSH, and GH (p < 0.05). There was a significant time by sex interaction in CortF (p <

0.05), and a trend was observed for FSH (p = 0.051). Significant time main effects were found for  $T_{3T}$ ,  $T_{4F}$ , CortF, CortT, and FSH (p < 0.05).

		T1	T2	Т3	Т4	Т5	Ref. Range
TSH	М	2.2 ± 1.14	2.2 ± 0.79	1.9 ± 0.82	2.1 ± 0.84	2.1 ± 0.88	0540
(mIU·L⁻¹)	F	2.1 ± 1.24	2.1 ± 1.20	$2.1 \pm 1.06$	1.9 ± 1.03	1.9 ± 0.75	0.5-4.3
<b>T</b> (marked)	M#	5.7 ± 0.65	5.8 ± 0.50	$5.8 \pm 0.45$	5.8 ± 0.42	5.7 ± 0.76	4.6-7.2
T <sub>3F</sub> (pmol·L <sup>-1</sup> )	F	5.0 ± 0.93	4.9 ± 0.74	5.1 ± 0.72	5.2 ± 0.76	5.3 ± 0.82	3.1-7.2
T <sub>3T</sub>	М	1.8 ± 0.30	1.8 ± 0.30	$1.8 \pm 0.28$	1.8 ± 0.28	1.6 ± 0.19#	1220
(nmol·L <sup>-1</sup> )*	F	1.6 ± 0.40	1.6 ± 0.33	1.6 ± 0.29	1.6 ± 0.28	1.5 ± 0.32	1.3-3.0
T <sub>4F</sub>	М	16.7 ± 3.03	16.5 ± 2.63	16.2 ± 1.84	16.1 ± 1.63	16.2 ± 2.37	
(pmol·L <sup>-1</sup> )*	F	15.9 ± 2.32	15.0 ± 2.06	14.9 ± 1.71	14.2 ± 1.39 <sup>\$</sup>	15.3 ± 1.44	10.3-18.0
T₄T (nmol·L-1)	М	95.5 ± 16.79	96.0 ± 13.47	94.7 ± 11.98	92.0 ± 12.86	92.4 ± 11.51	65.6-132.6
	F	94.1 ± 12.28	91.4 ± 13.63	94.9 ± 9.70	86.5 ± 14.20	90.7 ± 13.59	68.2-150.6

Table 3: Values presented as mean  $\pm$  SD. TSH, thyroid stimulating hormone; T3F, free triiodothryonine; T3T, total triiodothryonine; T4F, free thyroxine; T4T, total thyroxine. # denotes significant difference between sexes (P < 0.05). \* denotes time main effect (P < 0.05). \$ denotes significant within group change (P < 0.05).

In females, there was a significant decrease in  $T_{4F}$  (ES= -0.76) at T4 (p < 0.05), while a trend for decreased  $T_{4T}$  (p= 0.073; ES= -0.62) at T4 was found. No other changes were observed in thyroid hormones. A trend for decreased CortF (p= 0.063; ES=-0.41) and CortT was found at T3 (p = 0.051; ES= -0.42), and then increased through T5 (ES= 0.14, ES= 0.05, respectively). TestF and TestT significantly increased at T2 (p < 0.05; ES= 0.86, ES= 0.43, respectively), and at T5 (p < 0.05; ES= 0.40, ES= 0.44, respectively). No significant changes in E2 were found despite a large increase at T2 (ES= 0.90) and a moderate increase at T5 (ES= 0.61). Interestingly, there was a significant increase in FSH from T1-T2 (p < 0.05; ES= 0.84), and a significant decrease in FSH from T2-T5 (p < 0.05; ES= -1.36). Despite no changes in E2, there was an increase in the number of reported cases of amenorrhea (Table 4). No changes were found in GH, while there was an increasing trend in IGF-1 from T1-T2 (p=0.058; ES= 0.44) that returned to baseline at T3.

Menstrual Status	PT 1	PT 2
1° or 2° Amenorrhea	5	7
Oligomenorrhea	5	2
Regular	3	4

Table 4: Reported menstrual status at the beginning of study (performance testing 1, PT1) and end (performance testing 2, PT2).

In men, there was a significant decrease in  $T_{3T}$  at T5 (p < 0.05; ES= -0.72), while no other changes were observed in thyroid hormones. CortF and CortT significantly decreased from T1-T3 (p < 0.05; ES= -0.76, ES= -1.09, respectively), and remained suppressed through T5 (p < 0.05; ES= -0.97, ES= -1.19, respectively). No changes in TestF (p > 0.05) were found, while TestT significantly increased at T4 (p < 0.05; ES= 0.47). Interestingly, there was a significant increase in FSH at T4 (p < 0.05; ES= 0.24) that occurred in conjunction with the highest observed TestT values. No changes in GH or IGF-1 were found.

		Т1	Т2	Т3	Т4	Т5	Ref. Range
CORTF	М	28.5 ± 11.27	22.5 ± 8.65	20.0 ± 9.61 <sup>\$</sup>	21.2 ± 9.00	17.6 ± 9.66 <sup>\$</sup>	1.9-25.7
(nmol·L <sup>-1</sup> )^*	F	$20.0 \pm 11.30$	$16.8 \pm 9.28$	$15.4 \pm 7.57$	$15.1 \pm 8.76$	21.5 ± 12.30	1.9-25.7
CORTT (nmol·L-1)*	М	473.5 ± 90.76	$417.3\pm134.48$	374.3 ± 95.49 <sup>\$</sup>	415.6 ± 113.33	365.1 ± 112.79 <sup>\$</sup>	127-569
CORT (IIIIOPE -)	F	415.6 ± 150.17	391.5 ± 133.08	351.8 ± 125.10	352.5 ± 98.29	423.8 ± 167.67	127-569
TECTE (non al 1-1)	M#	242.0 ± 68.92	281.4 ± 54.13	267.3 ± 64.01	267.6 ± 56.09	291.7 ± 95.06	160-777
TESTF (pmol·L <sup>-1</sup> )	F	5.6 ± 3.35	8.5 ± 5.46 <sup>\$</sup>	5.2 ± 2.27	5.8 ± 2.45	7.0 ± 4.68 <sup>\$</sup>	≤ 12.5
TECTT (	M"	19.0 ± 5.48	$21.1 \pm 4.45$	20.7 ± 4.23	21.6 ± 3.50 <sup>\$</sup>	$21.0 \pm 3.90$	8.7-38.2
TESTT (nmol·L <sup>-1</sup> )	F	$0.9 \pm 0.44$	1.1 ± 0.49 <sup>\$</sup>	$0.7 \pm 0.18$	$0.8 \pm 0.26$	1.1 ± 0.49 <sup>\$</sup>	≤ 1.6
5 (	М	83.3 ± 41.38	77.8 ± 32.31	76.3 ± 24.02	81.6 ± 24.55	68.3 ± 33.02	≤ 106
E <sub>2</sub> (pmol·L <sup>-1</sup> )	F″	163.5 ± 109.89	262.3 ± 314.67	209.2 ± 263.38	171.2 ± 87.89	230.1 ± 159.76	≤ 1039
ECU (multi multi)*	М	$3.8 \pm 1.70$	$4.1 \pm 2.10$	$3.9 \pm 1.63$	4.2 ± 2.01 <sup>\$</sup>	4.1 ± 2.03	1.6-8.0
FSH (mIU·mL <sup>-1</sup> )*	F″	7.1 ± 3.23	9.8 ± 2.28 <sup>\$</sup>	7.8 ± 3.37 <sup>≒</sup>	7.7 ± 2.92 <sup>⊨</sup>	6.7 ± 2.51 <sup>5</sup>	1.5-10.2
CH (us 1-1)	М	$1.1 \pm 1.25$	0.6 ± 0.59	$1.2 \pm 1.59$	$2.3 \pm 4.94$	2.6 ± 3.19	< 10.1
GH (µg·L⁻¹)	F″	5.6 ± 6.58	2.7 ± 3.56	6.3 ± 4.53	4.0 ± 5.43	6.8 ± 5.31	≤ 10.1
IGE 1 (ug.1-1)	М	309.7 ± 89.85	278.3 ± 41.39	284.7 ± 72.19	297.8 ± 59.52	278.2 ± 68.92	108-548
IGF-1 (µg·L <sup>-1</sup> )	F	$268.8 \pm 100.71$	313.4 ± 84.85	273.0 ± 45.50	269.5 ± 72.35	273.1 ± 68.56	185-551

Table 5: Values presented as mean  $\pm$  SD. CORTF, free cortisol; CORTT, total cortisol; TESTF, free testosterone; TESTT, total testosterone; E2, estradiol; FSH, follicular-stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1. # denotes significant difference between sexes (P < 0.05). \* denotes time main effect (P < 0.05). \$ denotes significant within group change (P < 0.05). \$ denotes significant difference from T2 (P<0.05)

Muscle damage, inflammatory and nutritional markers can be found in Table 6.

Males had significantly greater CK, while females had significantly greater n-3FA (p <

0.05). A trend was found for greater TNF alpha (p= 0.059) was observed in males. A time

x sex interaction was found for  $O_{6:3}$  (p < 0.05). Time main effects were found for CK,

TNF alpha, and Vit-D (p < 0.05).

		T1	T2	Т3	T4	Т5	Ref. Range
CV (111-1)*	M	516.6 ± 268.14	411.7 ± 157.94	417.0 ± 143.02	408.2 ± 164.29 <sup>\$</sup>	335.8 ± 246.00	82-1083
CK (U·L <sup>-1</sup> )*	F	261.7 ± 118.09	253.9 ± 185.56	246.8 ± 117.23	222.8 ± 129.00	198.9 ± 164.74	47-513
TNF α	М	$1.3 \pm 0.42$	1.0 ± 0.18 <sup>\$</sup>	1.0 ± 0.21 <sup>\$</sup>	1.0 ± 0.19 <sup>\$</sup>	1.0 ± 0.22 <sup>\$</sup>	
(pg·mL <sup>-1</sup> )*	F	$1.1 \pm 0.22$	$0.9 \pm 0.17$	0.9 ± 0.20 <sup>\$</sup>	0.8 ± 0.15 <sup>\$</sup>	0.9 ± 0.13 <sup>\$</sup>	0.56-1.4
	М	1.7 ± 0.46	$1.7 \pm 0.42$	$2.1 \pm 0.85$	$2.5 \pm 0.92$	2.5 ± 0.83	
n3-FA (%)	F″	$2.4 \pm 1.01$	$2.7 \pm 1.05$	$2.1 \pm 0.85$	2.5 ± 0.92	2.5 ± 0.83	1.4-4.9
	М	13.5 ± 2.86	13.5 ± 3.53	12.6 ± 3.10	12.6 ± 2.70	13.1 ± 3.55	
O <sub>6:3</sub> (%)^	F	11.2 ± 3.64	9.3 ± 3.78 <sup>\$</sup>	12.0 ± 4.05	10.5 ± 3.65	10.8 ± 3.13	5.7-21.3
Vit-D	М	24.4 ± 10.35	21.7 ± 9.38 <sup>\$</sup>	24.2 ± 10.97	27.3 ± 11.28	25.9 ± 9.12	
(ng·mL <sup>-1</sup> )*	F	24.7 ± 6.97	23.1 ± 6.99	23.0 ± 9.69	24.3 ± 8.38	24.3 ± 7.15	30-100

Table 6: Values presented as mean  $\pm$  SD. CK, creatine kinase; TNF  $\alpha$ , tumor necrosis factor alpha; omega-3, n-3FA; O6:3, omega6/omega3 ratio; Vit-D, vitamin d,25-OH. # denotes significant difference between sexes (P < 0.05). \* denotes time main effect (P < 0.05). \$ denotes significant within group change (P < 0.05).

For CK, no change was observed in females, while males significantly decreased at T4 (p < 0.05; ES= -0.40) and remained suppressed through T5 (p < 0.05; ES= -0.67). TNF alpha significantly decreased from T1-T2 (p < 0.05) and remained depressed through T5 (p < 0.05) in males (ES= -0.81, ES= -0.78, respectively), and there was a significant decrease from T1-T3 (p < 0.05), which remained depressed through T5 in females (ES= -0.67, ES= -1.09, respectively). An increasing trend was found for males in n-3FA (p= 0.064; ES= 0.049) at T3, while no changes occurred in females. O<sub>6:3</sub> significantly decreased from T1-T2 in females (p < 0.05; ES= -0.49) then returned to baseline, while no changes were observed in males. At T2, VitD significantly decreased in males (p < 0.05; ES= -0.26), and trend was observed in females (p= 0.064; ES= -0.23) then returned to baseline. Vit-D was below reference range values at all time points in both sexes. Hematological markers can be found in Table 7. Males had a significantly greater %Sat than females (p < 0.05). No time x sex interactions were found, but time main effects were seen for Fe and %Sat (p < 0.05)

		T1	T2	Т3	T4	T5	Ref. Range
Fe	Μ	18.7 ± 7.26	24.3 ± 8.87	21.6 ± 5.44	18.4 ± 7.17	19.9 ± 8.16	5-29
(µmol·L⁻¹)*	F	19.6 ± 7.47	21.2 ± 9.10	15.0 ± 6.35 <sup>\$</sup>	14.2 ± 5.46 <sup>\$</sup>	16.1 ± 8.24	5-29
IBC	М	59.6 ± 4.22	60.1 ± 5.58	57.4 ± 4.91	61.1 ± 4.17	59.5 ± 3.70	40.00
(µmol·L <sup>-1</sup> )	F	63.0 ± 8.55	63.6 ± 11.58	63.3 ± 7.38	66.2 ± 9.13	64.9 ± 8.65	49-80
0/Cat (0/)*	M#	31.2 ± 11.21	39.9 ± 13.19 <sup>\$</sup>	37.4 ± 8.10 <sup>\$</sup>	30.3 ± 12.29	33.6 ± 13.54	16-48
%Sat (%)*	F	31.5 ± 11.53	33.5 ± 12.53	24.4 ± 10.94 <sup>\$</sup>	23.0 ± 11.03 <sup>\$</sup>	24.9 ± 12.22	15-45

Table 7: Values presented as mean  $\pm$  SD. Fe, iron; IBC, iron binding capacity; %Sat, percent saturation. # denotes significant difference between sexes (P < 0.05). \* denotes time main effect (P < 0.05). \$ denotes significant within group change (P < 0.05).

In females, Fe significantly decreased from T1-T3 (p < 0.05; ES= -0.61) and remained suppressed at T4 (ES= -0.72), while a trend for increased Fe was found in males at T2 (p= 0.053; ES= 0.77) then returned to baseline values. A similar response was found for %Sat. Females significantly decreased from T1-T3 (p < 0.05; ES= -0.62) and remained suppressed through T4 (ES= -0.74). Males significantly increased at T2 (p < 0.05; ES= 0.78), and remained elevated at T3 (p < 0.05; ES= 0.55) before returning to baseline.

#### DISCUSSION

To the authors' knowledge, this is the first study to comprehensively investigate biomarker changes in adolescent dancers throughout 5-months of training in conjunction with assessment of performance and EE variables. Results of this study indicate that pituitary hormones and hematological biomarkers demonstrated differential responses between adolescent male and female ballet dancers. Sex differences were observed in all performance variables. Males demonstrated a greater physiological capacity and showed a greater increase in muscular power over the course of the study than females. Additionally, males had a lower %BF and greater LBM than their female counterparts. Lastly, male ballet dancers expend more calories than their female counterparts. However, this difference is mitigated when BW and LBM are taken into account. These findings provide valuable insights into physiological and hormonal changes of elite adolescent male and female athletes over a period of training, as adolescent males and females may require individualized interventions to optimize health and performance.

### Energy Expenditure and Performance

Researchers have previously investigated energy expenditure in female ballet and contemporary dancers, however data in men are lacking. Expectedly, we found that males' absolute caloric expenditure was significantly greater than that of females. However, after adjusting for BW, EE was similar between sexes. Compared to other studies, the females in the current study had a greater caloric expenditure relative to their body weight (49.9 vs. 43.9; 42.7; 42.3; 38.3 kcal/kg/day (5,6,11,24)), which is also consistent with the current dancers having a greater EEE than previously reported (11,24). The type of dance being performed and time spent training must also be taken into account, as it has been recently shown that ballet has a greater rate of energy expenditure than contemporary modern (34). Heart rate monitoring is a useful tool to assess EE that may be used to educate the artistic-athletes about the energy requirements needed to meet the demands of training.

Despite differences in performance metrics at baseline, changes from pre- to posttesting followed a similar trend between groups. Interestingly, a significant increase in VJ

metrics occurred, while a small decrease was found in  $VO_{2max}$ . This may be a specific adaptation to dance training due to the greater anaerobic power implications for performance and/or training intensity being too low to increase aerobic capacities (34). Further, the observed VO<sub>2max</sub> is approximately 20% greater than what has been found to be achieved during a choreographed dance performance (35), so these athletes' natural aerobic capacity may be sufficient for staged performance. The elite level of this adolescent cohort is demonstrated through their physiological profile. Their body composition, jump height, and aerobic capacity are similar to those of professional adult dancers, and more favorable than those of other high-level and collegiate level dancers (34,39). Although most changes were statistically similar between groups, ES revealed a greater increase in VJ metrics for males than females, especially relative to LBM. This outcome may have occurred from a combination of males performing more demanding jumps during training and performing, and differences in mechanical stiffness increases of the lower limb tendons between sexes that effect the rate of force development (31). Power production may be of clinical relevance as increased lower body muscular power reduces injury risk as demonstrated by a decreased prevalence of injury with greater lower body muscular power in female contemporary dancers (3). Thus, monitoring changes in performance gives valuable information about an athlete's physiological adaptation to training.

#### Biomarkers

In conjunction with periodic performance testing, the addition of biomarker monitoring can provide additional insights into the athletes physiological state (41).

Dancers are at risk of hormonal disruption due to a drive for thinness inherent to the aesthetic nature of their sport. Hormonal changes may impact performance outcomes as well as result in short- and long-term health consequences. Specifically, female dancers are at high risk of menstrual dysfunction (1), disordered eating, and low bone mineral density (19). As such, down regulation of the reproductive system may occur in order to maintain other cellular functions necessary for immediate survival (14). Our findings suggest such an occurrence as 69% of our subjects reported menstrual dysfunction, which is in agreement with previous reports (1). Also, the present cases of amenorrhea increased from pre- to post-study (38% vs. 54%). Interestingly, this shift occurred without expected changes in E2 occurring. Potentially, the decreasing levels of FSH from T2 through T5, and suppressed TSH and  $T_{4F}$  at T4 are due to a decreasing EA, leading to the increase in cases. This is alarming for adolescent female athletes as menstrual irregularities are occurring despite sex hormones being within clinical reference ranges. Menstrual irregularities and amenorrhea are considered hallmark symptoms of RED-S. Other outcomes, including low bone mineral density, can result in long-term diminished bone health leading to increased risk of stress fractures and osteoporosis. (29).

In contrast, HPG axis function in the males appears normal throughout the study. Testosterone levels increased from baseline levels, reaching significance at T4, in conjunction with a significant increase in FSH. Further, testosterone levels increased, while cortisol values decreased as well. This dynamic interaction between the HPA- and HPG-axis was observed in females as well at T2. The increase in testosterone at T2 occurred in conjunction with the highest observed values of FSH and E2, and decreased cortisol in females. Thus, this may suggest that adolescent females are more sensitive to acute changes in EA than their male counterparts. More research is needed in young athletes to further elucidate these relationships.

The thyroid hormones are important mediators of growth and metabolism (14), and HPT-axis status has been associated with correlates of body weight and energy expenditure (30). All thyroid hormones measured were found to be within reference ranges throughout the study, though T<sub>3T</sub> values were close to the low end of the reference range. Although these markers were within "normal" range, it is plausible that these values are inadequate for adolescents, and females in particular, as the current athletes'  $T_{3T}$  values approached values found by Loucks et al. in amenorrheic athletes (1.53 vs. 1.50 nmol/L) (27). Despite this occurrence, the other thyroid biomarkers are more reflective of the previously observed cycling athletes and sedentary controls (27). Females had a significant decrease in TSH and  $T_{4T}$  at T4, while the males had a significant decrease in  $T_{3T}$  at T5. Potentially, there are differing compensation mechanisms between sexes in order to preserve adequate levels of the more metabolically active T<sub>3F</sub> to facilitate the expected growth that occurs during teenage years. Finally, higher T<sub>3F</sub> and T<sub>4F</sub> values found in males could potentially be due to greater energy intake. However, this is difficult to ascertain without sufficient dietary information, a recognized limitation of the study.

Stress and HPA axis function may be assessed through cortisol. Cortisol secretion is typically increased during times of high training loads and stress because of its catabolic nature (26), and ability to stimulate gluconeogenesis, lipolysis and proteolysis. Further, cortisol may inhibit recovery from training because it interferes with testosterone binding to its receptor, ultimately down-regulating muscle protein synthesis (26).

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Interestingly, higher levels of stress (cortisol), muscle damage (CK), and inflammation (TNF alpha) were found at T1 than T2 and T3, despite an accumulation of training time. The authors speculate that this occurred due to winter performances and other auditions occurring immediately prior to the start of the dancers' semester and the commencement of this study. Walker et al. reported an increase in cortisol values after a preseason in collegiate female soccer players that was sustained through the end of their season (41). In contrast, the diminished cortisol values observed in both sexes through T4 suggest that adolescent dancers are able to recover after a period of increased stress, while training is continued. Lastly, the increase in cortisol from T4-T5 (ES= 0.55) in females, compared to the decrease in males (ES= -0.43), may be contributing to the observed menstrual irregularities, as cortisol has been found to be elevated in cases of LEA (14).

Growth hormone and IGF-1 were also assessed to further evaluate the anabolic status of both males and females. Both markers are involved in muscle protein synthesis and muscle mass regulation (26). GH is critical in female athletes as it is the primary anabolic hormone and has been shown to correlate with training volume and intensity (26). As such, females had greater GH values than the males. Further, despite menstrual irregularities indicating the presence of LEA, GH values in females appear undisrupted. Increases in GH, with a concomitant decrease in IGF-1, have been reported in exercising women with LEA (28). Because of this relationship, GH should be assessed in conjunction with IGF-1. Interestingly, there was a trend for increase in IGF-1 in conjunction with the lowest GH values at T2 in females, which may be indicative of an acute increase in EA at T2. GH and IGF-1 values did not significantly change in males

over the course of the study. This outcome is expected, as testosterone increased in males, which acts as the primary anabolic hormone.

Nutritional markers such as Vit-D, n3-FA, and  $O_{6:3}$  can provide additional information as to an athlete's nutritional health, independent of use of diet logs. Both markers have been found to impact health and performance in regard to bone maintenance, protein synthesis, and inflammation (26). Omega fatty acid values were maintained within reference range, and were fairly consistent throughout the study. A non-significant increase in n3-FA at T2 was likely driving significant decrease in O<sub>6:3</sub> observed in females. Potentially, this may be indicative of an increase in EA due to decreased energy expenditure at T2, as dietary compensation may yield greater n-3FA values. Although omega fatty acids were consistent, dancers may benefit from Omega-3FA supplementation as lower  $O_{6:3}$  (1:1 or 2:1) may enhance recovery and improve motor skills (15). Vit-D deficiency was observed in both sexes over the course of the semester, which is consistent with observations from other dancers who practice at latitudes with reduced daily sunlight during winter months (11,42). Assessments in this study began in the winter and were conducted through the spring in the northeast region of the United States. Low Vit-D may increase risk of injury, specifically stress fractures, and has been associated with greater injury rates during winter months in professional adult ballet dancers (42). Greater injury risk is related to Vit-D's role in calcium absorption and bone mineralization as well as positively affecting muscle repair after exercise (33). Thus, dancers may benefit from increasing Vit-D levels through diet or supplementation to reduce injury risk, especially during the winter months. Light

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exposure in conjunction with vitamin  $D_3$  supplementation has been shown to optimize improvement in Vit-D status (9).

Iron is a mineral required for aerobic metabolism because of its role of transporting oxygen and facilitating oxidative phosphorylation (26). The complimentary measures of total IBC and %Sat reflect the total number of Fe binding sites on transferrin, and the ratio of Fe to IBC, respectively (26). Collectively these markers can be used to assess aerobic performance decrements that are associated with anemia. Previous investigations suggests high prevalence of inadequate iron stores in adolescent male and female athletes (5,7,36). However, our findings were within reference ranges at all time points, particularly in males. Walker et al. recently demonstrated training induced irondeficiency over the course of a competitive season in high-level female soccer players (41).In the current study, females showed significantly reduced values from baseline, although the magnitude of change was less severe than reported by Walker et al. (ES= -0.72 vs. -1.1, respectively) (41). The observed differences may be due to the age of the studied athletes, but they are more likely due to differing demands of aerobic metabolism during regular training (37). Interestingly, opposing hematological adaptations were revealed between sexes in the current study. The minimal changes observed in the males are consistent with outcomes established previously in male soccer players (32). Females' Fe and %Sat significantly decreased from baseline at T3 and remained suppressed while the males' maintained fairly stable measures close to baseline values. Although values remained within clinical reference ranges, the significant decrease from baseline in females may not be optimal for athletic performance. It is important to note, iron is an essential component for aerobic performance. Monitoring changes in iron status may

provide an opportunity to intervene before suboptimal iron stores are reported. Thus, monitoring of hematological markers is warranted in female artistic-athletes as significant disruptions occur over the course of training that may impair performance.

The current investigation is not without limitations. First, one dance company was included in this study leading to a small sample size. However, selection criteria for this company are rigorous, and represent a population of elite level ballet dancers. Additionally, for comparison purposes, it was important that males and females had similar dance training requirements throughout the entirety of the study. Participants' training was consistently scheduled from week to week, thus it was determined that a 7day sampling period provided a fair representation of typical training demands. All participants attended their required classes; however, some participants may have been exposed to more demanding training than others due to selection for different parts in performance pieces. Previous research has demonstrated differences in exercise intensity between dancer ranking (e.g. corps, soloist, principal) (38). Although these dancers were not separated by rank for class, distinct performance roles may cause variations in total EEE between participants over the course of the semester. Further, menstrual cycle was not strictly controlled for in our female athletes, but samples were taken every 28-days in order to account for a typical cycle. However, in this study it is important to note that amenorrhea was reported to occur in upwards of 69% of dancers (1), so attempting to control for "clinically normal" was deemed to not be feasible for this population. In addition to tracking menstrual status, accounting for diet intakes potentially would have been desirable in this population. However, obtaining nutritional intake for youth and adolescent athletes is a challenging task over the course of a semester. In addition to

feasibility and adherence, accuracy of self-reported dietary intakes is limited as research has shown that self-reported dietary measures can be highly unreliable when used by free-living athletes (8). Thus, periodic biomarker assessment may enable an accurate and feasible method for indirectly assessing an athlete's nutritional status.

Overall, this study provides a comprehensive evaluation of performance and biomarker changes in elite male and female dancers throughout a semester of training and performing at a vocational ballet school. Differential responses in biomarkers were observed, specifically in the hematological markers, between males and females. In addition, a high rate of menstrual irregularities was found in this elite population of female dancers. The lack of a menstrual cycle in adolescent female artistic-athletes may lead to negative long-term health outcomes. In regards to performance outcomes, males had a greater increase in muscular power than their female counterparts, thus females may benefit from additional specific training to address this deficit. Future research is needed to investigate the efficacy of supplemental training and individualized nutrition programming in optimizing the health and performance of artistic-athletes.

# PRACTICAL APPLICATIONS

This study provides much needed observational data on elite-level adolescent athletes in a real-world setting. When comparing male and female athletes performing similar training at the same vocational school, our study found that adolescent males and females experience different fluctuations in biomarkers over the course of a 5-month training and performing period. As such, biomarkers appear to change more in adolescent female than males. Biomarker monitoring may be used to provide valuable insight into the nutritional status of athletes. Questionnaires that determine menstrual status should be used to assess overall health of young female athletes throughout a training cycle. Changes in menstrual status may require additional nutritional assessments in conjunction with EEE monitoring to determine if the athlete may be in LEA. Sports medicine practitioners should be aware of the physiological, as well as the psychological, consequences associated with menstrual dysfunction and LEA. Biomarkers, in conjunction with HRM and performance testing, can be used to detect disruptions that may be negatively impacting health and performance, and individualize interventions to the artistic-athlete's specific needs in order to optimize and health and performance.

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# **DISCLOSURE OF INTEREST**

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