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Effects of Sports Nutrition Education on Athletic Performance and Iron Status in High School-Aged Youth Athletes

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ABSTRACT

The purpose of this study was to examine the effects of an online sports nutrition curriculum on athletic performance and iron status in high school-aged male and female athletes. A repeated-measures design evaluated forty-three males (n=18) and females (n=25). Athletic performance and biomarkers of iron status were measured before and after participating in an eight-week online sports nutrition curriculum. Performance tests included vertical jump height and power (VJ_{PP} and VJ_{H}, respectively), broad jump (BJ), pro-agility (PA), L-cone (LC), 20-yard-dash (20YD), and push up strength and power (PPU_{F} and PPU_{PP}, respectively). Concentrations of ferritin, soluble transferrin receptor (sTfR), and hemoglobin (Hb) were reported from capillary blood samples. Dietary recalls were collected as part of the first curriculum lesson. There were no changes in any measurement from pre- to post-curriculum (p=0.070 – 0.977). As expected, males were greater than females for VJ_{H}, VJ_{H}·kg^{-1}, VJ_{PP}, VJ_{PP}·kg^{-1}, BJ, BJ·kg^{-1}, PA, LC, 20YD, and ferritin concentrations (p<0.001 – 0.039), but there were no sex differences for PA·kg^{-1}, LC·kg^{-1}, 20YD·kg^{-1}, PPU_{F}, PPU_{F}·kg^{-1}, PPU_{P}, PPU_{P}·kg^{-1}, Hb, Hb·kg fat-free mass (FFM)$^{-1}$, ferritin·kg FFM^{-1}, sTfR, and sTfR·kg FFM^{-1}(p=0.075 – 0.952). While males met most recommendations, females were below recommendations for energy, carbohydrate, protein, and iron intakes. Although the sports nutrition education did not directly enhance athletic performance or iron status, high school-aged female athletes may benefit from sports nutrition education to encourage dietary intakes that fall within recommended ranges.

Keywords: Performance, Sports nutrition, Education, Youth athletes, Iron

Introduction

Training demands for athletic performance requires adequate calories, macronutrients, and micronutrients to not only optimize performance, but to also ensure the health and well-being of the athlete. In particular, adolescent athletes may have greater nutritional needs to meet requirements for training and to also support growth and development [1]. Nutritional needs for growth encompass energy for growing tissues [2] such as skeletal muscle. In addition tothe stimulus of training for sports, healthy growth of skeletal muscle during childhood and adolescence requires additional energy [1,2] and protein requirements [1,3,4] to replace losses from exercise, sustain a net protein balance, and support normal growth and development [3,5]. Depending on the volume of exercise training, carbohydrate intake may need to be enhanced to meet energy requirements of training and restore muscle glycogen stores between training sessions [6]. Consequently, recommendations for adolescent athletes vary from 5 - 7 g·kg^{-1}·d^{-1} to upwards of 10 g·kg^{-1}·d^{-1}, depending on training volume [6]. Educating young athletes on these basic needs and the competing influences of sport and growth demands has become paramount.

In addition to macronutrient needs, adequate micronutrient intake is essential for performance. Iron, in particular, is an essential

micronutrient for performance with roles such as production of red blood cells, oxygen transport, and transport of electrons during oxidative phosphorylation [7–10]. Young athletes have greater dietary iron needs corresponding to high growth rates of bone and muscle, onset of menarche in females, and insufficient dietary iron intake [11–14]. In addition to these maturity-related influences is the increased risk of iron losses associated with training demands [15,16], suggesting that youth athletes may show a heightened demand for dietary iron. Since many youth athletes are iron deficient [14,17–19], highlighting this important nutrient within a sports nutrition-focused education curriculum may attenuate or prevent the development of poor iron status in at-risk youth athletes to enhance athletic performance and overall health.

Improvements in self-esteem, nutrition knowledge, self-efficacy and self-reported dietary intakes have been reported in previous studies examining nutrition education interventions [20–25]. For example, nutrition education administered to college female athletes have aimed to improve nutrition knowledge and self-efficacy towards making healthful dietary choices and improving dietary intake [21]. Nutritional knowledge and self-efficacy for the treatment group improved, with no changes in the control group. However, there were no specific improvements in self-reported dietary intakes [21]. Nutrition education coupled with supervised resistance training in male college athletes has also enhanced nutritional knowledge, as well as improved measurements of dietary intake, body composition, and performance [25]. However, little is known regarding the effects of a nutrition education intervention alone on quantitative outcomes of athletic performance and nutritional status, such as biomarkers of iron status.

An online educational curriculum may be an easily accessible, appealing method to teach young athletes about sports nutrition. Quantitative outcomes such as improved athletic performance scores and iron biomarker concentrations would demonstrate definitive, positive adaptations through sports nutrition education. Therefore, the purpose of this study was to examine the effects of an online sports nutrition education curriculum on athletic performance and iron status in high school male and female athletes. We hypothesized that athletic performance scores and biomarkers of iron status would improve after participating in the eight-week sports nutrition-focused curriculum.

Materials and Methods

Study design

A repeated measures design was used to evaluate changes in iron status biomarker concentrations and athletic performance before and after participating in a sports nutrition education curriculum. Participants were tested twice, before (pre-) and after (post-) completion of the curriculum. This study was conducted at two separate rural high schools in the Midwest, and testing took place in the school gymnasiums. Equipment was set-up by the investigators in the same manner, and participants were tested at the same time of day (±1 hour) during the pre- and post-curriculum testing. Baseline anthropometrics included standing and seated height, body mass, skinfolds at three sites, and circumferences at two sites. Each testing session measured vertical jump (VJ), broad jump (BJ), pro-agility (PA), L-cone (LC), 20-yard dash (20YD), and power push-up (PPU). Capillary blood samples were also taken for measurements of hemoglobin (Hb), ferritin, and soluble transferrin receptor (sTfR) concentrations. Independent variables included time (pre- and post-curriculum) and sex.

Participants

Male (n=66) and female (n=73) high school students volunteered to participate in this study. All participants were high school students ranging from 14 – 18 years old. To be included in this per protocol analysis, each participant must have (a) been actively participating in school-or club-sponsored sports with regular practices, (b) provided capillary blood samples at the pre- and post-curriculum assessments, (c) participated in all anthropometric and athletic performance testing at the pre- and post-curriculum assessments, and (d) actively participated in the 8-week sports nutrition curriculum. To determine athletic status, an exercise and sport history questionnaire was administered during the introductory section of the curriculum. Twenty-eight students (males, n=15; females, n=12) did not complete the questionnaire, and 22 students (males, n=6; females, n=16) were not actively involved in any school- or club-sponsored sports; subsequently, these participants were excluded in this analysis. Of the remaining 89 participants, only 53 (males, n=25; females, n=28) provided capillary blood samples at both testing sessions. Of these, only 43 participants (males, n=18; females, n=25) completed all athletic performance testing as well as the 8-week curriculum; subsequently, only data from these participants were analyzed and reported in the present study. This study was approved by the University of Nebraska-Lincoln Institutional Review Board for the protection of human subjects (IRB # 20180117682EP, Title: Sports Nutrition and Performance in High School Athletes, approval date: January 4, 2018). Each participant signed an approved youth assent form, and at least one parent or legal guardian of each participant signed an approved informed consent document. All participants also completed The Physical Activity Readiness Questionnaire for Everyone(PAR-Q+) [26] and answered affirmatively for participation in the athletic performance assessments.

Anthropometrics

Height (cm) and body mass (kg) were measured using a beam scale with attached stadiometer (Seca gmbh & co. kg, Hamburg, Germany). Seated height was measured for calculating maturity offset to predict age at peak height velocity (PHV) [27]. Measurements of body composition included percent body fat (BF%), fat-free mass (FFM), arm estimated cross-sectional area (eCSA), and thigh eCSA. To calculate BF%, skinfold measurements were taken with a Lange caliper (Model 68902, Cambridge Scientific Industries, Inc., Cambridge, MD, USA). Three-site skinfold measurements were taken on the right side of the body and were recorded to the nearest 0.5 mm [28] to be entered into equations established by Housh et al. [29] and Brozek et al. [30] to estimate body density and BF%, respectively. Arm and thigh circumferences were measured with a Gulick measurement tape (Baseline® measurement tape with Gulick attachment, Fabrication Enterprises, White Plains, NY) and recorded to the nearest 0.1 cm. Arm circumference and triceps skinfold were used to calculate arm eCSA, while thigh circumference and thigh skinfold were used to calculate thigh eCSA as described previously [31].

Dietary intake

A total of 40 participants (males, n=16; females, n=24) completed

a 24-hour dietary recall at baseline. Only 3 participants (males, n=2; female, n=1) failed to complete the dietary recall. The recall was administered online using the Automated Self-Administered 24-hour (ASA24®) Dietary Recall System. Participants were prompted with detailed questions regarding food intake, including serving sizes and composition of food choices. Total energy ($kcal \cdot d^{-1}$), carbohydrate ($g \cdot d^{-1}$), protein ($g \cdot d^{-1}$), fat ($g \cdot d^{-1}$), and iron ($mg \cdot d^{-1}$) intakes were quantified and reported from the ASA24®.

Athletic performance

Athletic performance testing was conducted in a manner similar to the National Football league (NFL) scouting combine as described previously [32]. The VJ test assessed peak vertical power output and maximal jump height (VJ_{PP} and VJ_{H}, respectively). Vertical ground reaction forces were sampled with force plates (PASCO PS-2142, PASCO Scientific, Roseville, CA) located under the participants' feet while they performed counter-movement vertical jumps. Participants were instructed to begin by standing in an upright position with their feet in the middle of the force plates and their knees and hips extended. Then, participants rapidly descended into an eccentric counter-movement of self-selected depth, followed by a maximal, explosive, concentric vertical jump. VJ_{H} was measured with a standard, free-standing jump height device (Sports Imports, Freestanding Vertec Jump Trainer, Hilliard, OH, USA) and was calculated as the difference between a two-handed standing reach (cm) and the highest jump height achieved (cm). VJ_{PP} was calculated using the sum of the left- and right-foot vertical ground reaction forces with previously-described methods [33]. The BJ assessed horizontal jumping performance, recorded as the distance between the starting line and the heel of the participant closest to the starting line (cm) after the participant jumped forward. Agility performance was assessed as described previously [32] by the PA and LC tests, while the 20YD tested linear speed. These performance tests were measured in seconds (s) using digital, laser beam-actuated timing gates with motion start sensors (Brower Timing Systems, Brower TC Motion Start Timer, Knoxville, TN, USA). Splits were recorded at the 5- and 10-yard markers during the 20YD.

Finally, the PPU test was performed to examine upper body strength (PPU_{F}) and power (PPU_{PP}). Ground reaction forces were collected using force plates (PASCO-PS-2142, PASCO Scientific, Roseville, CA). The raw force data were stored on a personal computer and analyzed offline using custom written software (LabVIEW,v. 170.0 National Instruments, Austin, TX, USA). PPU_{F}(N) and PPU_{PP}(W) were calculated based on methods described in detail elsewhere [34]. Based on the recommendations of De Ste Croix [35], all athletic performance variables were expressed and analyzed in absolute (original units) and relative (original units per kilogram of body mass) units.

Iron status biomarkers

Capillary blood samples of 400 µL were collected in microvettes (Microvette® 200 µL, K3 EDTA, violet US code; 10.8 mm x 46.6 mm) to analyze ferritin ($\mu g \cdot L^{-1}$) and sTfR ($nmol \cdot L^{-1}$). Plasma from the capillary samples was stored at -80°C after centrifugation until analysis. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to assess concentrations of ferritin (Ramco Laboratories, Inc., Houston, Texas) and sTfR (R&D Systems Inc., Minneapolis, Minnesota). ELISAs were performed per manufacturer instructions, and all samples were analyzed in duplicate. Limits of detection for ferritin and sTfR were 0.59 $\mu g \cdot L^{-1}$ and 0.5 $nmol \cdot L^{-1}$, respectively. In a previous study performed by this laboratory involving similar participants [36], none of the participant's blood samples met the threshold for human alpha 1-acid glycoprotein (AGP) of > 1 $g \cdot L^{-1}$ [37], indicating that correcting these iron biomarkers for inflammation was unnecessary. Hb concentration ($g \cdot L^{-1}$) was assessed on site during the performance testing with a handheld hemoanalyzer (AimStrip® *Hb* Hemoglobin meter, Germaine Laboratories, Inc.). Two cutoffs for ferritin concentrations were utilized in this study: < 30 $\mu g \cdot mL^{-1}$ for *iron depletion* and < 15 $\mu g \cdot mL^{-1}$ for *iron deficiency* [38,39]. Low tissue iron levels were determined by sTfR concentrations > 21 $nmol \cdot L^{-1}$ [40]. Anemia was classified with Hb concentrations <120 $g \cdot L^{-1}$ for males and females 12 - 15 years and <130 $g \cdot L^{-1}$ for males older than 15 years [41]. All biomarkers of iron status were expressed in absolute (original units) and relative (original unit per kilogram of FFM) units.

Sports nutrition curriculum

A basic sports nutrition education curriculum was developed to be accessible online through a learning management system (Instructure, Inc. 2019, Salt Lake City, UT, USA).The curriculum was modeled from a sports nutrition curriculum developed at Michigan State University, (http://spartanperf.com/) and consisted of seven total lessons emphasizing athletic performance test protocols, macronutrient and micronutrient intake, building a performance plate, energy balance, timing of intake, and dietary supplements (canvas.instructure.com/courses/sportsnutrition). After approval by both school's administrative leadership, the young student-athletes enrolled into the curriculum. The introductory lesson showed video clips of the athletic performance test procedures, emphasizing correct techniques. The introductory lesson also contained a link and instructions to complete a 24-hour dietary recall administered online using the Automated Self-Administered 24-hour (ASA24®) Dietary Recall System. The introductory lesson was completed by all participants prior to the pre-curriculum assessments. Once the pre-curriculum assessments were complete, the participants were able to continue to the second lesson in the online curriculum. Completion of all lesson activities was required before the student could proceed to the next lesson. Lesson activities included watching each lesson's introductory video, short lecture display with voiceover, infographic resources, homework assignments, and a short quiz. Athletes completed one lesson per week on average and completed the post-curriculum assessments during the week after the final curriculum lesson was completed.

Statistical analyses

Data were evaluated for distributional normality with the Shapiro-Wilk test. Variables exhibiting non-normal distributions were subsequently analyzed after log transformation [42] or with a nonparametric rank transform procedure [43]. One-way ANOVAs were used to compare the mean values of males versus females at baseline (Table 1). Mixed factorial ANCOVAs (time x sex) compared the corrected means for absolute and relative values of athletic performance and biomarkers of iron status (Table 2). Anthropometric variables that were significantly different between males and females at baseline (height, arm eCSA, and thigh eCSA, $p<0.001$-0.006) were used as covariates in the ANCOVA models. Statistical analyses

Table 1: Means ± standard deviations for baseline anthropometrics and dietary intakes of the male and female high school athletes.

	Composite (n=43)	Males (n=18)	Females (n=25)	*p-value*
Age (y)	16.3±1.0	16.6±1.1	16.1±1.0	0.141
Maturity Offset (y)	2.3±0.9	1.7±1.0	2.8±0.6	<0.001*
Height (cm)	168.84±9.38	176.72±7.09	163.17±6.19	<0.001*
Body Mass (kg)	67.13±15.84	71.47±15.13	64.01±15.90	0.129
Percent Body Fat (%)	22.18±10.08	16.44±8.67	26.24±9.11	0.001*
Fat Free Mass (kg)	51.99±10.32	59.61±10.07	46.28±5.96	<0.001*
Arm Estimated Cross-sectional Area (cm^2)	19.10±10.82	28.88±7.30	12.17±6.75	<0.001*
Thigh Estimated Cross-sectional Area (cm^2)	119.31±35.61	136.94±32.40	106.83±32.91	0.006*
Energy Intake ($kcal \cdot d^{-1}$)†	2238±1140	3017±1304	1719±627	0.001*
Carbohydrate ($g \cdot d^{-1}$)†	266±138	350±158	210±89	0.007*
Protein ($g \cdot d^{-1}$)†	86±48	118±58	65±24	0.001*
Fat ($g \cdot d^{-1}$)†	95±56	131±65	72±32	0.003*
Iron ($mg \cdot d^{-1}$)†	15±9	21±10	12±7	0.001*

*Indicates a significant difference between males and females at baseline ($p \leq 0.05$).
†Included three less participants (composite, n=40; males, n=16; females, n=24).

Table 2: Means (unadjusted) ± standard deviations for pre- and post-curriculum measurements of athletic performance and biomarkers of iron status for the male and female high school athletes.

	Composite (n=43)		Males (n=18)		Females (n=25)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Vertical Jump Height (cm)*	45.41±11.61	45.37±14.43	55.61±8.93	55.53±12.12	38.07±6.73	38.05±11.28
Vertical Jump Height ($cm \cdot kg^{-1}$)*	0.70±0.19	0.73±0.21	0.81±0.17	0.81±0.21	0.63±0.18	0.67±0.18
Vertical Jump Peak Power (W)*	1978.84±922.98	2994.80±1153.22	2734.85±806.19	3722.05±1027.04	1434.52±541.43	2316.03±811.07
Vertical Jump Peak Power ($W \cdot kg^{-1}$)*	29.46±12.01	42.86±13.17	39.04±11.17	52.41±11.04	22.56±6.75	33.92±7.53
Broad Jump (cm)*	175.35±37.42	182.21±36.20	206.45±31.44	216.39±22.71	152.96±22.42	157.58±20.47
Broad Jump ($cm \cdot kg^{-1}$)*	2.73±0.75	2.76±0.70	3.04±0.74	3.06±0.72	2.50±0.68	2.55±0.61
Pro-agility (s)*	5.85±0.75	5.93±0.69	5.20±0.36	5.39±0.50	6.33±0.57	6.32±0.52
Pro-agility ($s \cdot kg^{-1}$)	0.09±0.02	0.091±0.02	0.08±0.2	0.08±0.02	0.10±0.02	0.10±0.02
L-cone (s)*	10.36±1.59	10.24±1.40	9.25±0.98	9.15±0.95	11.28±1.40	11.03±1.13
L-cone ($s \cdot kg^{-1}$)	0.16±0.06	0.16±0.04	0.14±0.04	0.13±0.03	0.18±0.06	0.18±0.04
20-yard Dash (s)*	3.71±0.45	3.72±0.49	3.36±0.24	3.41±0.47	3.97±0.40	3.94±0.37
20-yard Dash ($s \cdot kg^{-1}$)	0.06±0.02	0.06±0.02	0.05±0.01	0.05±0.02	0.06±0.02	0.06±0.01
Power Push Up Force (N)	232.16±115.52	259.10±119.84	307.41±115.91	321.24±148.03	173.26±74.99	213.18±65.84
Power Push Up Force ($N \cdot kg^{-1}$)	3.41±1.48	3.72±1.42	4.29±1.50	4.29±1.81	2.72±1.05	3.30±0.88
Power Push Up Peak Power (W)	580.07±440.99	851.25±579.20	693.65±495.73	899.14±662.51	491.18±380.61	815.86±521.91
Power Push Up Peak Power ($W \cdot kg^{-1}$)	8.66±7.02	12.45±8.50	9.54±7.00	11.92±8.16	7.97±7.11	12.85±8.91
Ferritin ($\mu g \cdot L^{-1}$)	30.85±28.69	27.43±19.97	40.39±30.90	35.95±24.92	23.97±25.43	20.98±12.66
Ferritin ($\mu g \cdot L^{-1} \cdot kg\ FFM^{-1}$)*	0.61±0.55	0.54±0.35	0.71±0.52	0.62±0.40	0.54±0.56	0.48±0.31
Prevalence of *Iron Deficiency*	12 (28.0%)	15 (34.9%)	3 (16.7%)	4 (22.2%)	9 (36.0%)	11 (44.0%)
Prevalence of *Iron Depletion*	27 (63.0%)	30 (69.8%)	7 (39.0%)	9 (50.0%)	20 (80.0%)	21 (84.0%)
Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	16.74±7.06	19.77±8.71	16.99±5.56	18.50±5.31	16.56±8.07	20.69±10.51
Soluble Transferrin Receptor ($nmol \cdot L^{-1} \cdot kg\ FFM^{-1}$)	0.33±0.15	0.40±0.20	0.29±0.09	0.32±0.10	0.36±0.18	0.46±0.24
Prevalence of *Low Tissue Iron*	8 (18.6%)	11 (25.6%)	4 (22.2%)	5 (27.8%)	4 (16.0%)	6 (24.0%)
Hemoglobin ($g \cdot L^{-1}$)	137.5±17.1	140.0±20.3	140.9±18.3	147.1±21.6	135.1±16.1	134.9±18.0
Hemoglobin ($g \cdot L^{-1} \cdot kg\ FFM^{-1}$)	2.8±0.6	2.8±0.6	2.5±0.6	2.5±0.6	3.0±0.5	3.0±0.6
Prevalence of Anemia	6 (14.0%)	7 (17.3%)	3 (16.7%)	3 (16.7%)	3 (12.0%)	4 (16.0%)

*Indicates a main effect for sex ($p \leq 0.05$) in which males performed better and had higher concentrations than females, collapsed across time, when covarying for height, arm eCSA, and thigh eCSA.

were performed using IBM SPSS Statistics, Version 25 (IBM Corp., Chicago, IL, USA). An alpha of $p \leq 0.05$ was considered statistically significant for all comparisons.

Results

Means ± standard deviations for baseline anthropometrics and dietary intakes are reported in Table 1. Pre- and post-curriculum performance measurements and biomarkers of iron status are reported as unadjusted means ± standard deviations in Table 2. The Shapiro-Wilk test indicated non-normal distributions for all baseline dietary intakes, as well as pre- and post-curriculum ferritin and sTfR concentrations ($p<0.001$ – 0.019). Values were log-transformed and re-evaluated for normality. Only sTfR concentrations remained non-normal ($p<0.001$); thus, sTfR concentrations were rank-transformed and statistical analyses were performed on the ranks. All other log-transformed variables were normally distributed ($p>0.05$) and were subsequently used for all statistical analyses.

One way-ANOVAs indicated significant sex differences at baseline for maturity offset, height, BF%, FFM, arm eCSA, thigh eCSA, energy intake, protein, carbohydrates, fat, and iron ($p<0.001$ – 0.007) (Table 1). There were no baseline differences between males and females for body mass or age ($p=0.129$ and $p=0.141$, respectively). The mixed factorial ANCOVAs indicated no time x sex interactions and no main effects for time ($p=0.070$ – 0.977). There were, however, significant main effects for sex, indicating that males were greater than females for VJ_H, $VJ_H \cdot kg^{-1}$, VJ_{PP}, $VJ_{PP} \cdot kg^{-1}$, BJ, $BJ \cdot kg^{-1}$, PA, LC, 20YD, and ferritin concentrations ($p<0.001$ – 0.039) collapsed across time (Table 2). There were no sex differences (collapsed across time) for $PA \cdot kg^{-1}$, $LC \cdot kg^{-1}$, $20YD \cdot kg^{-1}$, PPU_F, $PPU_F \cdot kg^{-1}$, PPU_P, $PPU_P \cdot kg^{-1}$, Hb, $Hb \cdot kg\ FFM^{-1}$, $ferritin \cdot kg\ FFM^{-1}$, sTfR, and $sTfR \cdot kg\ FFM^{-1}$ ($p=0.075$ – 0.952).

Individual changes occurred from pre- to post-curriculum for the classifications of iron status biomarkers. One female improved from iron deficient to iron depleted, one male improved from iron deficient to normal, and four athletes (males, n=2; females, n=2) improved from iron depleted to normal when examining individual ferritin concentrations. Four athletes decreased from iron depleted to iron deficient (males, n=2; females, n=2), while seven athletes with normal ferritin concentrations became iron depleted (males, n=4; females, n=3). Therefore, 14% (6 out of 43) improved their ferritin status, while 26% (11 out of 43) exhibited decreases in ferritin status. One male and three females improved their iron tissue levels (9%, 4 out of 43), while two males and five females changed from normal to low tissue iron levels (16%, 7 out of 43) when examining individual sTfR concentrations from pre- to post-curriculum. Three males and one female were no longer anemic (9%, 4 out of 43); however, three males and two females became anemic (12%, 5 out of 43) when tallying individual Hb responses over time.

Discussion

Our primary findings indicated that the eight-week online sports nutrition education curriculum used in the present study did not improve athletic performance or iron status in male or female high school athletes. These findings suggest that online education alone is not sufficient to improve athletic ability or iron status in this population. While an online nutrition education curriculum emphasizing sports nutrition concepts is convenient, easily accessible, and appealing for teachers and coaches of young athletes, such an approach may not manifest in quantitative improvements in performance or iron status.

Previous studies have examined education programs focusing on sports nutrition in athletes, but few have translated into measurable improvements in performance [20,21,25]. For example, Curry et al. [20] reported that athletes enrolled in a performance enhancement educational course exhibited higher scores for hope, self-esteem, and sports confidence than athletes in the control group, but no data quantifying performance were included. Abood et al. [21] showed improvements in nutrition knowledge and self-efficacy in college female athletes, with no improvements in a control group after an educational intervention. However, the authors reported no changes in dietary intake after the nutrition education program [21]. Rossi et al. [25] showed that a sports nutrition education intervention in NCAA Division I male baseball players improved nutritional knowledge and dietary intake. Furthermore, when coupled with supervised resistance training, the authors' program also improved body composition and performance [25]. Collectively, the results of the present study in high school athletes, in conjunction with previous studies [20–24], suggest that sports nutrition education interventions alone are insufficient for improving physical performance or nutritional status outcomes.

Anthropometric, body composition, and performance differences between the males and females were observed at baseline in the present study. It has long been known that adolescent males and females differ in muscle CSA, fat mass, weight, and height [44–46]. However, even after controlling for sex differences in anthropometry and body composition variables in the present study, sex-related differences in most of the athletic performance tests were still present at pre- and post-curriculum (Table 2). Specifically, males performed 20 - 91% better in unadjusted absolute and relative lower-body strength and power (VJ_H, VJ_{PP}, and BJ). Males also performed 15 -28% better in agility (PA and LC) and 13-17% better in linear speed (20YD) than females, but the sex differences in agility and speed (PA, LC, and 20YD) disappeared when expressing values relative to body mass (Table 2). Despite covarying for height, arm eCSA, and thigh eCSA, sex differences between the adjusted means were still present for VJ_H, VJ_{PP}, and BJ, regardless if they were expressed as absolute or relative terms (Table 2). Our findings regarding lower-body strength and power were consistent with O'Brien et al. [47] who demonstrated that sex differences in strength can only be eliminated when expressed per unit of muscle cross-sectional area. In contrast, our findings suggested that both male and female adolescents can be compared on the same scale for speed and agility, but only when expressing scores relative to body mass.

As expected, males performed 10 – 77% better than females in absolute measures of upper-body strength and power (PPU_F or PPU_{PP}) (Table 2). However, there were no differences between males and females for PPU_F or PPU_{PP} when normalizing for body mass and covarying for height, arm eCSA, and thigh eCSA (Table 2). While sex differences in strength and power are usually more profound for the upper-body versus lower-body in adolescents after the onset of puberty [48,49], muscle mass accounting for the variance in strength measurements aligns with previous studies [48,50–52]. For example, Wood et al. [52] reported that sex differences in elbow flexion and extension were eliminated after accounting for differences in muscle size. Hosler and Morrow [48] reported that sex only accounted for 1% and 2% of the variance in muscle strength in the upper-body

and lower-body, respectively, after differences in body size and composition were accounted for. The results of the present study, in conjunction with previous findings [48–52], suggest that adjusting for muscle mass may account for sex differences in upper-body strength in adolescents.

On average, the females in the present study were 1.1 years more mature than the males, despite being equal in chronological age (Table 1). However, the females in the present study were also 8% shorter, exhibited the same body mass (statistically), 10% greater percent body fat, 22% less fat free mass, 58% less eCSA for the arm, and 22% less eCSA for the thigh on average than the males. Therefore, despite the females being more mature, anthropometric differences in the present sample appeared to be most prominent in muscle mass, body fat, and stature. It is known that females typically reach peak height velocity at a younger age than males [53]. Males, however, experience greater muscle hypertrophy and begin to athletically outperform females at pubertal onset [54,55] which coincides with PHV [56–58]. These sex differences in maturity offset are thought to coincide with hormonal responses, particularly the testosterone increase in males [54,59,60]. Although not collected in the present study, higher concentrations of testosterone in males often results in greater skeletal muscle hypertrophy [54,61]. Since muscle mass differences in males and females are usually a consequence of physical maturity differences, our findings suggest that the greater maturity offset in the females could not overcome the influence of muscle mass, body composition, and stature when comparing absolute performance scores.

Males and females in the present study also exhibited baseline differences in recorded dietary intake. Specifically, females showed 43% less energy intake, 40 – 45% less carbohydrate, protein, and fat intake, and 43% less iron intake than the males (Table 1). Males showed energy intakes that fell within the general recommendations of 2,804 – 3,799 kcalskcal·d^{-1} for 14 – 18-year-old males who are active to highly active [62]. Conversely, energy intake in the females fell below the 2,309 – 2,833 kcal·d^{-1} recommendations [62], with an average intake of 1,719 kcal·d^{-1}. Carbohydrate intake in the males and females was 46% (4.7 g·kg·d^{-1}) and 49% (3.3 g·kg·d^{-1}) of total energy intake, respectively. Both males and females fell below carbohydrate recommendations for athletes of 5 - 7g·kg^{-1}·d^{-1} [6,63]. Protein intakes of 1.2 – 1.8 g·kg^{-1}·d^{-1} are recommended for adolescent athletes [63,64], and the males were within this range with an average protein intake of 1.65 g·kg^{-1}·d^{-1}. However, the females were below this recommendation with an average intake of 1.02 g·kg^{-1}·d^{-1} (Table 1). In contrast, fat intake for both males and females was higher than recommendations of 25 – 35% of total energy intake [62,65]. While iron intake exceeded recommendations for males, females were below the recommended daily allowance (RDA) of 15 mg·d^{-1} [11] (Table 1). Overall, these baseline dietary intakes suggested that the adolescent female athletes of the present study were not meeting recommendations for energy intake, carbohydrate intake, protein intake, or iron intake. The males fell below guidelines for only carbohydrates. Thus, replacing fat intake for both male and female adolescent athletes with healthy carbohydrate choices, such as whole grains, fruits, and vegetables, may resolve some insufficiencies. However, increasing protein intakes that also provide iron, such as beef, in female athletes may address the most glaring deficiencies.

With the exception of a decrease in ferritin concentrations relative to fat-free mass, there were no differences in biomarkers of iron status from pre- to post-curriculum. Previous studies examining iron status have also found no change or decreasing values in biomarker concentrations [66–68]. For example, Auersperger et al. [66] showed that female runners exhibited lower hepcidin and hemoglobin concentrations and higher sTfR concentrations after eight weeks of exercise training [66]. However, in studies administering iron supplementation as an intervention in adolescents and adults, improvements in iron status [69,70], as well as performance [71–73], have been reported. Food interventions may also be an effective method by which to improve iron status. Lyle et al. [74] reported that beef supplements improved serum ferritin, iron, iron-binding capacity, and hemoglobin in exercising women, even more effectively than iron supplementation [74]. Future studies should examine the effects of beef supplementation on iron status and athletic performance in adolescent athletes.

A strength of this study is the uniqueness in examining measurable, quantitative outcomes such as athletic performance and iron status, in response to an online sports nutrition education. Adolescents are a protected population, making a research study providing multiple assessments of athletic performance and three different biomarkers of iron status pre- and post-intervention contributory to research. However, there are certain limitations. A one-day dietary recall was obtained from participants at the beginning of the study, yet was unable to be obtained at the end of the study. Another limitation is the lack of data on sports nutrition knowledge and attitudes from the young athletes. Quizzes were completed at the end of each lesson, with average quiz scores for each lesson at 76% or higher, indicating that much of the information was retained. However, we did not perform pre- and post-nutrition knowledge questionnaires. The purpose of the study was to measure athletic performance and iron status, rather than qualitative assessments of knowledge. Since there is much literature available examining nutrition knowledge changes, this study emphasized quantitative, applied physiological outcomes in response to the curriculum.

For this introduction to implementing online sports nutrition education curricula into high schools, we allowed the teachers to deliver the curriculum in a method most conducive to their particular classroom. This reduced the amount of control researchers had on implementation and compliance, possibly influencing the lack of improvement in athletic performance and iron status. A longer period of time for the intervention may also be necessary in order to see improvements.

In conclusion, an eight-week online sports nutrition education curriculum did not improve athletic performance or iron status in the male and female high school athletes. As expected, males performed better than females when examining absolute scores (Table 2). However, performance was equated for speed and agility, as well as upper body strength and power, when scores were expressed relative to body mass (Table 2). Furthermore, dietary records suggested that young female athletes do not meet recommendations [6,11,62–65] for energy intake, carbohydrate intake, protein intake, or iron intake, whereas young male athletes do (Table 1). Thus, despite no improvements in athletic performance or biomarkers of iron status, high school-aged female athletes, specifically, may need continual sports nutrition education to promote dietary intakes to fall within recommended ranges [6,11,62–65]. Future studies are needed to study combined education- and exercise-related interventions to improve performance and iron biomarkers, as well as the effects of online sports nutrition education on dietary intake and records.

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Author Contributions

All authors were involved in the study. MES, ZMG, BDM, JAF, CH, NAB, KK, and JTC contributed to the data collection and analysis. MES and JTC prepared and wrote the manuscript. ZMG, BDM, NAB, SMG contributed edits and critiques for the manuscript. All Authors read and approved the manuscript.

Disclosure Statement

All authors have declared no competing interests or financial interests regarding this study.

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