Acute Soy Supplementation Improves 20-km Time Trial Performance, Power, and Speed

AFTON D. SEELEY[1], KEVIN A. JACOBS[1], and JOSEPH F. SIGNORILE[2]

[1]Laboratory of Clinical and Applied Physiology, University of Miami, Coral Gables, FL; and [2]Laboratory of Neuromuscular Research and Active Aging, University of Miami, Coral Gables, FL

ABSTRACT

SEELEY, A. D., K. A. JACOBS, and J. F. SIGNORILE. Acute Soy Supplementation Improves 20-km Time Trial Performance, Power, and Speed. *Med. Sci. Sports Exerc.*, Vol. 52, No. 1, pp. 170–177, 2020. **Introduction:** Isoflavones, a chemical class of phytoestrogens found in soybeans and soy products, may have biological functions similar to estradiol. After binding with ER_β or perhaps independently of estrogen receptors, isoflavones may augment vascular endothelial relaxation, contributing to improved limb blood flow. **Purpose:** To determine if acute fermented soy extract supplementation influences 20-km time trial cycling performance and cardiac hemodynamics compared with a placebo. **Methods:** Subjects included 25 cyclists and triathletes (31 ± 8 yr, $\dot{V}O_{2peak}$: 55.1 ± 8.4 $mL \cdot kg^{-1} \cdot min^{-1}$). Each subject completed a $\dot{V}O_{2peak}$ assessment, familiarization, and two 20-km time trials in randomized order after ingestion of a fermented soy extract supplement or placebo. The fermented soy extract consisted of 30 g powdered supplement in 16 fl. ounces of water. The placebo contained the same quantities of organic cocoa powder and water. Each trial consisted of 60 min of rest, 30 min at 55% W_{peak}, and a self-paced 20-km time trial. **Results:** Soy supplementation elicited a faster time to 20-km completion (−0.22 ± 0.51 min; −13 s), lower average HR (−5 ± 7 bpm), and significantly greater power (7 ± 3 W) and speed (0.42 ± 0.16 $km \cdot h^{-1}$) during the last 5 km of the time trial compared with placebo. Analysis of the results by relative fitness level (<57 vs ≥ 57 $mL \cdot kg^{-1} \cdot min^{-1}$) indicated that those with a higher level of fitness reaped the largest performance improvement alongside a reduced HR (−5 ± 7 bpm). **Conclusions:** Ingestion of a fermented soy extract supplement improved sprint-distance performance through improvements in both power and speed. For those with great aerobic fitness, soy supplementation may help to decrease cardiac demand alongside performance improvement. **Key Words:** ISOFLAVONES, CYCLING, IMPEDANCE CARDIOGRAPHY, NITRIC OXIDE

Phytoestrogens, common to nearly all plant foods and structurally similar to estradiol, have received a surge of attention due to their potential to improve vascular function and prevent cardiovascular disease. Isoflavones, a chemical class of phytoestrogens, can be found predominately in soybeans and equivalent soy products in the form of genistein and daidzein, which may be capable of biological functions similar to estradiol (1). Although these particular "active-form" (aglycone) isoflavones predominate, soy products can contain other "precursor-form" (glucoside) isoflavones that can be metabolized in the gut to their active forms. Soy products containing isoflavones are often fermented to facilitate greater digestive-based conversion of glucoside isoflavones to aglycone isoflavones (2,3). While these isoflavones function as antioxidants in plants, in mammalian tissue these natural molecules act as agonists, or partial agonists, of estrogens (1). This agonistic behavior is accomplished primarily through competitive binding of isoflavones, significantly genistein, to β-type estrogen receptors (ER_β), the most abundant form in the endothelial tissue of arteries (4). After binding with ER_β or perhaps even independently of estrogen receptors (5), isoflavones may act to initiate endothelial nitric oxide (NO)-dependent signaling cascades that could potentially alter vascular reactivity (6). Nitric oxide is a potent endothelium-derived relaxing factor that is biosynthesized endogenously from NO synthase (NOS) enzymes. The vascular endothelium uses NO to signal relaxation of the smooth muscle of the vasculature, resulting in vasodilation (7).

Research regarding isoflavone supplementation has largely focused on postmenopausal females as a potential auxiliary for hormone replacement therapy; however, changes in endothelial activity have been noted. Squadrito et al. (7) investigated the effects of 6 months of genistein supplementation on endothelial function in ovariectomized rats compared with a placebo. The authors demonstrated significant augmentation of NO products, nitrite, and nitrate, after the isoflavone supplementation. Si and Liu (8) deviated from a postmenopausal female focus and conducted a mixed-sex rat study investigating the use of 6 wk of genistein supplementation at varying levels (0, 0.2, 0.5, or 2.0 $g \cdot kg^{-1}$) on spontaneous hypertension *in vivo*. The authors demonstrated that at physiologically achievable concentrations, genistein was able to significantly activate endothelial NOS (eNOS) transcription leading to eNOS synthesis and NO production.

Address for correspondence: Afton D. Seeley, M.S., Department of Kinesiology and Sport Science, University of Miami, Room 315, 5202 University Dr., Merrick Bldg, Coral Gables, FL 33146; E-mail: ads129@miami.edu.
Submitted for publication April 2019.
Accepted for publication July 2019.

0195-9131/20/5201-0170/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000002102

In human investigations, NO also seems to be implicated as the vascular biomarker of interest after soy isoflavone exposure. Walker et al. (9) examined the effects of brachial artery dose-progressive genistein infusion in males age 20 to 51 yr on forearm blood flow as measured by strain-gauge plethysmography. Genistein infusion resulted in a dose-dependent increase in forearm blood flow in men nearly identical to equimolar concentrations of 17β-estradiol. The influence of both genistein and 17β-estradiol on forearm blood flow were inhibited similarly with administration of the NO synthase inhibitor N^G-monomethyl-L-arginine, indicating that the isoflavone genistein is capable, at appropriate blood concentrations, of inducing L-arginine/NO-dependent vasodilation in human forearm vasculature. The vascular endothelium uses NO to signal relaxation of the smooth muscle of the vasculature, resulting in vasodilation (7). The resulting increase in blood flow, if elicited during an exercise bout, could augment oxygen delivery to skeletal muscle lending to an improved muscular work capacity alongside attenuated cardiac stress. Nitric oxide levels at rest and during exercise have been found to be highest in individuals who are of greater fitness level, and the rate of NO production has been established to positively correlate with exercise performance in humans (10,11).

Negligible research has been conducted regarding implementation of acute isoflavone supplementation specifically in human males, although introductory studies imparting daily 40-mg isoflavone supplementation for 2 months to healthy males demonstrated no negative effect of the supplementation on reproductive health or viability (12). Even less information is available regarding its use as an acute performance enhancer in trained cyclists; however, the potential for vascular endothelial manipulation via the activities of NO may be applicable to this population. Therefore, the purpose of this study was to determine if acute fermented soy extract supplementation with Fermalife could improve 20-km time trial performance in trained male cyclists. Secondarily, the cardiovascular influence of this supplementation was investigated using quantification of cardiac hemodynamics.

METHODS

The University of Miami Institutional Review Board approved the study which was performed in accordance with the standard set by the Declaration of Helsinki. All participants provided written informed consent before participation.

Twenty-five recreationally trained male cyclists and triathletes were recruited for this investigation. Twenty-five subjects were determined sufficient to detect a small difference in outcome variables over time between trials, with an alpha level of 0.5 and a desired power of 0.95 (13). Considering limited empirical data regarding effect size as it pertains specifically to the influence of fermented soy extract supplementation on cycling performance, a small effect size (0.3) was utilized to allow for detection of even a small effect. Demographic information for the sample is presented in Table 1. Participation consisted of four data collection sessions, including a $\dot{V}O_{2peak}$ assessment, familiarization, and two time trials completed after ingestion of either the fermented soy extract supplement or the placebo. $\dot{V}O_{2peak}$ was assessed during baseline screening using a continuous linear ramp exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Velotron Dyna-Fit Pro; RacerMate, Seattle, WA). Seat height, handlebar height, and distance from saddle post to handlebars were adjusted to the subjective comfort of each participant, and these values were recorded and maintained for use during each data collection session. Each subject was encouraged to bring personal clip-in pedals to allow maximal conversion of muscular power to mechanical power. Subjects began cycling at 100 W and intensity was increased by 50 W continuously across 2-min stages. Once each subject reached 250 W, each subsequent stage increased continuously by 30 W until exhaustion. Two subjects identified themselves as endurance athletes, as they were primarily volume-trained as runners. To allow their participation and to account for the biomechanical lack of familiarity with distance cycling, a less aggressive $\dot{V}O_{2peak}$ testing protocol was implemented. These two subjects began cycling at 80 W, held 80 W for the first 2 min of the assessment, and then continuously increased intensity by 40 W for 2-min stages until volitional fatigue. Power at peak oxygen consumption (W_{peak}) was identified as the power output associated with the greatest measured oxygen consumption ($\dot{V}O_{2peak}$). Expired respiratory gases were collected continuously and analyzed with an online open-circuit metabolic cart (Sensormedics Vmax 229; Viasys Healthcare, Palm Springs, CA). HR, stroke volume, and cardiac output were measured continuously with a noninvasive impedance cardiography device (PhysioFlow PF05 L1; Manitec Biomedical, Macharen, France).

Next, subjects randomly completed two exercise trials (placebo and supplement) with at least a 48-h recovery between trials. The fermented soy extract supplement (Fermalife) consisted of 30 g (CHO, 6 g; PRO, 9 g; FAT, 4.5 g) of powdered supplement combined with 16 fluid ounces of water. The placebo, as a taste and texture match, contained the same quantities of organic cocoa powder (CHO, 15 g; PRO, 5 g;

TABLE 1. Subject demographics (mean ± SD).

	All Subjects (N = 25)	Moderate Fitness (n = 14)	High Fitness (n = 11)
Age (yr)	31 ± 8 (19–43)	29 ± 7 (19–41)	33 ± 8 (21–43)
Height (cm)	177.3 ± 5.9 (165.1–190.0)	176.6 ± 4.8 (169.0–183.0)	178.1 ± 7.1 (165.1–190.0)
Weight (kg)	78.3 ± 8.5 (62.2–94.1)	79.7 ± 9.8 (62.2–94.1)	76.5 ± 6.4 (68.2–86.8)
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	55.1 ± 8.4 (41.5–71.1)	48.9 ± 3.9 (41.5–55.8)	63.1 ± 4.9* (57.1–71.1)
$\dot{V}O_{2peak}$ (L·min^{-1})	4.3 ± 0.7 (3.0–5.6)	3.9 ± 0.5 (3.0–4.9)	4.8 ± 0.5* (4.1–5.6)
Peak power output (W)	315 ± 42 (247–400)	288 ± 24 (247–332)	350 ± 33* (298–400)

Moderate fitness: <57 mL·kg^{-1}·min^{-1}; High fitness: ≥57 mL·kg^{-1}·min^{-1}.
*Significantly different than moderate fitness group.

FAT, 2.5 g) and water. Each trial included 60 min of rest to allow digestion/absorption, 30 min of steady-state exercise at 55% of W_{peak}, a 5-min delay, and a 20-km time trial.

Subjects were asked to refrain from alcohol consumption and exercise for 36 h before testing and not to consume caffeine on the day of testing. Subjects arrived at the laboratory fully hydrated, having consumed an additional 16 ounces of water, mixed with the supplement or placebo powder, 1 h before testing. Subjects were asked to abstain from food for at least 1.5 h before each experimental trial. Additionally, subjects were asked to refrain from ingesting foods high in soy (such as edamame, miso, soy milk, tofu, and meat alternatives) for the duration of the study. This was further verified with the performance of a 24-h food log upon each arrival to the laboratory. Trial completion order was double-blind, randomized, and counterbalanced. An independent investigator prepared the placebo and supplement making the conditions indistinguishable to both subjects and investigators. After ingestion of the supplement or placebo and the rest period, subjects were seated on an electromagnetically braked cycle ergometer while physioflow electrodes were placed on the neck and chest to allow continuous measurement of cardiac output, HR, and stroke volume. A cooling fan was positioned in front of the cycle ergometer and set to "low" for the duration of each experimental bout to mimic convective heat loss with overground cycling. The workload on the ergometer was progressively ramped over a period of 2 min until the subject reached 55% of W_{peak}. This power output was held for 30 min at a self-selected cadence. HR, stroke volume, and cardiac output were recorded continuously and averaged every 10 s by the PhysioFlow. Subsequently, the time trial was performed on an electromagnetically braked cycle ergometer with adjustable road cycle geometry and a gearing switch that allowed the subjects to alter the resistance on the flywheel. Therefore, subjects' speed and power output were a function of their self-selected resistance and pedaling cadence. Subjects completed the time trial in the fastest time possible and received no verbal encouragement and no physiological feedback other than the distance covered at 16, 18, 18.5, 19, and 20 km. HR, stroke volume, and cardiac output were recorded continuously as previously described. The ergometer software continuously recorded power output, speed, pedaling cadence, and time to completion to the nearest second.

Statistical analysis. Data were analyzed using SPSS version 24.0 (IBM Corporation, Chicago, IL) statistical software. Time to completion (min), along with average power output (W), speed ($km \cdot h^{-1}$) cadence (rpm), stroke volume (mL per beat), HR (bpm), and cardiac output ($L \cdot min^{-1}$) were collected and analyzed for each time trial. Normality of dependent variables was determined using a Shapiro–Wilk test while homogeneity of variance was determined using a Levene's test. Paired samples *t* tests were utilized to analyze means collected during both the supplementation and placebo time trials for all 25 subjects. An alpha level of 0.05 was set *a priori*. Further paired-samples analysis by relative fitness level (<57 mL·kg^{-1}·min^{-1} vs ≥ 57 mL·kg^{-1}·min^{-1}) were conducted for each variable. Additionally, power output and speed were averaged for each time trial to determine representative samples for each 5 km of distance completed. Average power output and speed for 5, 10, 15, and 20 km were statistically analyzed using a repeated-measures (distance covered (4) × supplement type (2)) ANOVA. *A priori* simple main effects were examined with a least significant differences correction for multiple planned comparisons, when applicable. All data values are reported as mean ± standard deviation or mean difference ± standard deviation.

RESULTS

Age (yr), height (cm), and weight (kg) were not significantly different between the moderate fitness and high fitness groups (Table 1). Relative $\dot{V}O_{2peak}$ (MD, 12.12 ± 1.98; $P < 0.001$ [8.06–16.19]), absolute $\dot{V}O_{2peak}$ (MD: 0.78 ± 0.20, $P < 0.001$ [0.38–1.19]), and maximal power output (MD: 53 ± 12, $P < 0.001$, [28–78]) were significantly greater in the high fitness group compared with the moderate fitness group.

During the 30-min steady state warm-up conducted at 55% W_{peak} (172 ± 24 W), subjects ($n = 25$) exhibited a significantly reduced average HR after fermented soy supplementation (−4 ± 7 bpm, $P = 0.010$, [1–7]) when compared with placebo while stroke volume and cardiac output were relatively unchanged. Further analysis revealed a similar reduction in HR for the high fitness group (−5 ± 5 bpm, $P = 0.011$, [1–9]; 191 ± 20 W) while hemodynamics within the moderate fitness group (158 ± 15 W) were statistically unchanged.

Considering all subjects ($n = 25$), paired samples *t*-tests demonstrated a significantly faster time to completion for soy supplementation compared with placebo, with soy supplementation eliciting on average a 0.22-min (−0.22 ± 0.51, $P = 0.040$ [−0.43 to −0.01], or 13 s) faster completion time (0.62% improvement; Table 2). This was supported by an

TABLE 2. Mean ± SD for performance and cardiac hemodynamic variables across time trials ($n = 25$).

	Fermented Soy Extract	Placebo	Mean Difference	*P*
Performance				
Time to completion (min)	35.31 ± 3.11	35.53 ± 3.09	**–0.22**	**0.040**
Power output (W)	219 ± 47	216 ± 46	**3.6**	**0.015**
Speed ($km \cdot h^{-1}$)	34.3 ± 2.9	34.1 ± 2.9	0.2	0.172
Cadence (rpm)	98 ± 9	99 ± 9	–0.40	0.642
Cardiac hemodynamics				
Stroke volume (mL per beat)	122 ± 23	120 ± 22	1.5	0.567
HR (bpm)	155 ± 13	160 ± 14	**–5**	**0.001**
Cardiac output ($L \cdot min^{-1}$)	18.9 ± 3.3	19.2 ± 3.7	–0.38	0.417

Values in bold emphasis indicate significant mean difference between fermented soy extract and placebo.

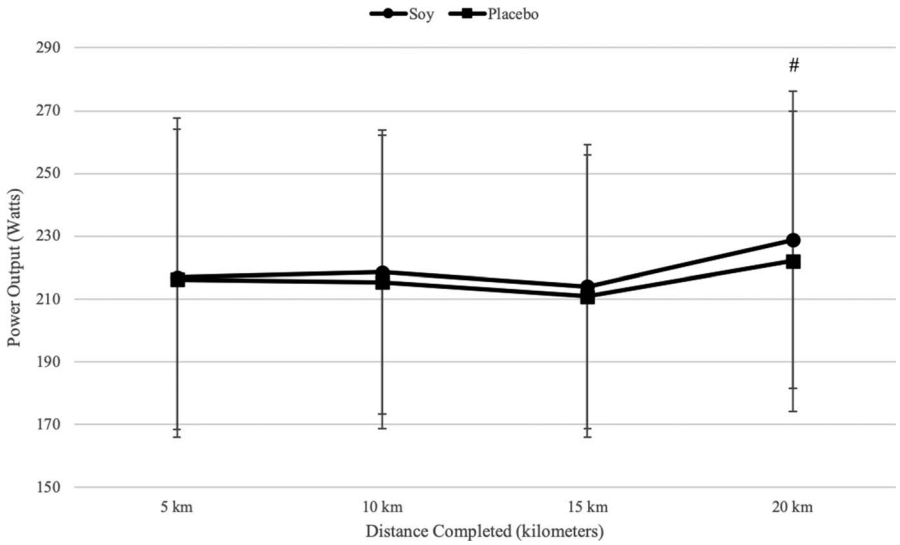

FIGURE 1—Power output (W) at 5, 10, 15, and 20 km for fermented soy extract supplementation and placebo time trials (mean ± SD; *n* = 25). Each time marker is representative of power output (W) during the previous 5 km. #*P* < 0.05 between soy and placebo.

approximately 4 W (4 ± 7, $P = 0.015$ [0.77–6]) greater average self-selected power output for the soy supplementation trials. Average HR during the soy supplementation time trial was also significantly lower compared with the placebo trial by approximately 5 bpm (−5 ± 7, $P = 0.001$ [−8 to −2)]). Both power output (7 ± 3 W, $P = 0.012$ [2–12]) and speed (0.42 ± 0.16 km·h^{-1}, $P = 0.010$ [0.11–0.74]) were significantly greater at 20 km, although not at 5, 10, or 15 km, for the fermented soy extract supplement time trial when compared with the placebo time trial (Figs. 1 and 2).

In the high fitness group, soy supplementation was able to demonstrate a significant and proportionally larger improvement in completion time compared with the entire sample size (−0.24 ± 0.35, $P = 0.047$ [−0.47 to −0.004]), 0.24 min or 14 s faster; 0.72% improvement; Table 3). Average power output was significantly increased by approximately 5 W (5 ± 6 W, $P = 0.027$ [1–9]) supported by a significant increase in speed of approximately 0.26 km·h^{-1} (0.26 ± 0.34, $P = 0.028$ [0.04–0.49]). The significant improvement in performance variables occurred alongside dampened cardiac demand, as illustrated by the significantly lower HR (−5 ± 7 bpm, $P = 0.039$, [−10 to −0.30]; Table 3).

In the moderate fitness group, fermented soy supplementation was able to demonstrate an improvement, albeit not statistically significant, in completion time (0.21 min or 13 s faster; 0.56% improvement; Table 4). However, the moderate fitness category was able to demonstrate a significantly lower average time trial HR (−5 ± 7 bpm, $P = 0.019$ [−8 to −0.89]) compared with placebo (Table 4).

DISCUSSION

This investigation sought to determine whether acute fermented soy extract supplementation with Fermalife is

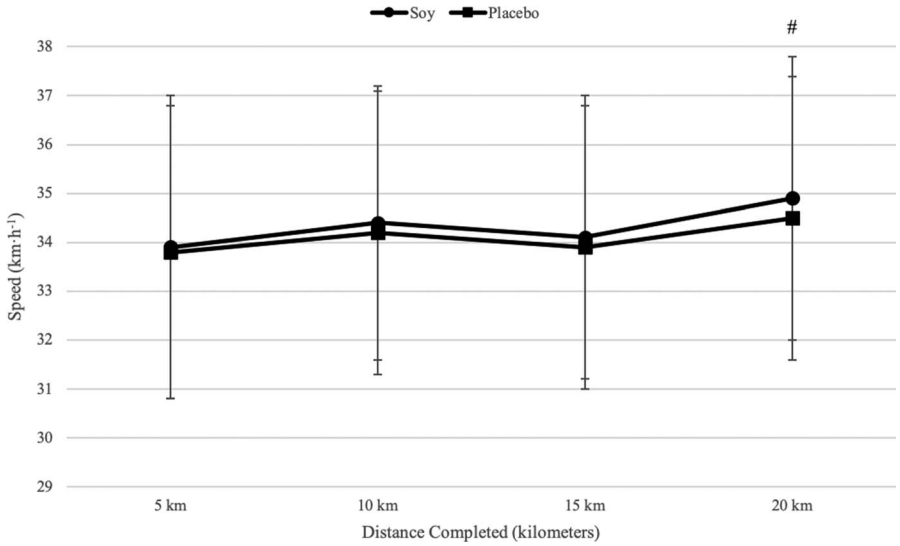

FIGURE 2—Speed (km·h^{-1}) at 5, 10, 15, and 20 km for fermented soy extract supplementation and placebo time trials (mean ± SD; *n* = 25). Each time marker is representative of the average speed (km·h^{-1}) during the previous 5 km. #*P* < 0.05 between soy and placebo.

TABLE 3. Mean ± SD for performance and cardiac hemodynamic variables across time trials for high fitness category (n = 11).

	Fermented Soy Extract	Placebo	Mean Difference	P
Performance				
Time to completion (min)	32.89 ± 1.50	33.13 ± 1.46	**–0.24**	**0.047**
Power output (W)	258 ± 29	253 ± 29	**4.9**	**0.027**
Speed (km·h^{-1})	36.62 ± 1.61	36.36 ± 1.57	**0.26**	**0.028**
Cadence (rpm)	99 ± 7	100 ± 8	–0.64	0.641
Cardiac hemodynamics				
Stroke volume (mL per beat)	127 ± 24	129 ± 25	–2.6	0.333
HR (bpm)	160 ± 14	165 ± 13	**–5.1**	**0.039**
Cardiac output (L·min^{-1})	20.1 ± 2.7	21.2 ± 3.8	–1.2	0.066

High fitness: ≥57 mL·kg^{-1}·min^{-1}.
Values in bold emphasis indicate significant mean difference between fermented soy extract and placebo.

capable of improving 20-km time trial performance in trained male cyclists. The influence of supplementation was further investigated using quantification of cardiac hemodynamics. The novelty of this investigation lies in both the acute nature of supplementation and the trained male population in which it was administered. When considering the entire sample (n = 25), supplementation with fermented soy extract significantly improved 20-km performance time compared with placebo as indicated by improvements in average time trial power output at a lower average chronotropic demand. In addition, 20-km average power output and speed were most improved during the last 5-km sprint. The effects of fermented soy extract supplementation appeared to be most meaningful for those with higher relative fitness levels ($\dot{V}O_{2peak} \geq 57$ mL·kg^{-1}·min^{-1}). Male cyclists and triathletes in the higher relative fitness group demonstrated significantly augmented 20-km time trial performance as demonstrated by improved average power output and speed at a significantly lower average time trial HR. Evaluating participants within the moderate fitness group (<57 mL·kg^{-1}·min^{-1}), statistical significance indicated only a dampened HR response with fermented soy extract supplementation. Lack of significance with regards to performance variables, specifically 20-km performance time, within the moderate fitness group may be at least partially explained by lesser performance experience and therefore greater performance heterogeneity within the group (20-km time to completion; moderate fitness, 32.81 to 42.23 min; SD, 2.70 min vs high fitness, 31.18–36.00; SD, 1.50 min). Performance times among the moderate fitness subjects differed by as much as approximately 10 min while performance times among the high fitness group differed only by as much as approximately 5 min.

Isoflavones, the chemically "active" components of soy, have been linked to two relatively well-researched mechanisms of physiological action. First, isoflavones may act to initiate endothelial NO-dependent signaling cascades that could potentially alter vascular reactivity (6). Nitric oxide acts as a potent endothelium-derived relaxing factor that is biosynthesized endogenously from NOS (7). Regulation of blood flow during exercise in any tissue is governed largely by variation in vascular resistance modulated by changes in vascular smooth muscle tone. Vascular tone modulation within skeletal muscle during exercise is the product of two interwoven influences: local vascular control mechanisms and central cardiovascular control mechanisms (14). According to a metabolic hypothesis of vascular control, local tissue metabolic requirements are complimented and facilitated by arterial vascular tone. Local accumulation and diffusion of metabolites from the contracting muscle to the interstitial fluid and finally to capillaries and small arterioles initiates propagation of vasodilation throughout the vasculature. In response to these biochemical stimuli, in conjunction with intravascular shear stress and vessel stretch signals, the vascular endothelium is able to release vasodilatory substances including NO. Consequently, a greater blood flow to the working skeletal muscle can provide essential metabolically relevant oxygen as well as clearance of potentially deleterious metabolic byproducts, such as carbon dioxide. Oral supplementation with fermented soy extract, if capable of inducing an earlier increase in systemic NO production within the vasculature, may elicit improved metabolic clearance during exercise through augmentation of skeletal muscle blood flow. This could explain the improved 20-km performance, especially increases seen in speed and power output within the last

TABLE 4. Mean ± SD for performance and cardiac hemodynamic variables across time trials for moderate fitness category (n = 14).

	Fermented Soy Extract	Placebo	Mean Difference	P
Performance				
Time to completion (min)	37.21 ± 2.69	37.42 ± 2.70	–0.21	0.229
Power output (W)	189 ± 34	187 ± 34	2.6	0.213
Speed (km·h^{-1})	32.4 ± 2.3	32.3 ± 2.4	0.06	0.706
Cadence (rpm)	97 ± 10	97 ± 11	–0.21	0.855
Cardiac hemodynamics				
Stroke volume (mL per beat)	118 ± 23	113 ± 17	4.7	0.265
HR (bpm)	152 ± 11	156 ± 14	**–4.6**	**0.019**
Cardiac output (L·min^{-1})	17.9 ± 3.6	17.7 ± 2.9	0.24	0.719

Moderate fitness: <57 mL·kg^{-1}·min^{-1}.
Values in bold emphasis indicate significant mean difference between fermented soy extract and placebo.

5-km sprint of the performance, when fast glycolytic processes maximally contribute to ATP production. The physiological counterpart to local control of vascular resistance, central cardiovascular control mechanisms, couple local metabolic tissue need with hemodynamic coordination of cardiac work. Group III and IV afferents in local skeletal muscle relay a "metabolic and mechanical message" to medullary brain centers, effectively modulating neural innervation to the heart. Chronotropic influence to the heart is then modulated to increase the volume of blood leaving the heart each minute (15). If, as mentioned previously, earlier metabolic clearance is possible with improved blood flow attributable to fermented soy extract supplementation, a reduced reliance on these cardiovascular ramping mechanisms may follow.

Among the high fitness subjects (≥ 57 mL·kg^{-1}·min^{-1}), significant improvements in performance, including 20-km completion time, power output, and speed were demonstrated after acute fermented soy extract supplementation. As mentioned earlier, this may be explained by improved skeletal muscle blood flow lending to augmented metabolic clearance. Also present in this group after supplementation was a substantially reduced cardiac demand as manifested as a decrease in both HR (−5 bpm) and cardiac output (−1.2 L·min^{-1}). Considering the small but meaningful increase in physical work (5 W) capable of improving 20-km time trial performance, along with maintained or potentially even increased oxygen consumption, a concurrent decrease in cardiac demand is surprising. This mismatch may highlight an influence of acute fermented soy extract supplement on arteriovenous difference and peripheral oxygen offloading at the working skeletal muscle, facilitated by improvements in oxygen delivery relative to augmented blood flow.

Although a measure of vascular resistance, clinically evaluated as arterial blood pressure, was omitted as it was both cumbersome and distracting to assess during time trial performance, consideration of arterial blood pressure homeostasis in the context of soy extract supplementation may be justified. Facilitated NO-mediated vasodilation due to acute fermented soy extract supplementation, paired with attenuated cardiac hemodynamics, may implicate exercise blood pressure attenuation as a consequential cardiovascular effector variable for further future assessment. Enhanced vasodilation may translate into a decrease in systemic peripheral resistance, and paired with a lower cardiac demand could mean a decrease in mean arterial pressure, if further compensatory vasoconstriction of nonprimary tissues is not possible. A previous meta-analysis including 14 randomized controlled trials further implicates blood pressure as an effector for soy supplementation, with indications that daily ingestion of 25 to 375 mg soy isoflavones for 2 to 24 wk is capable of attenuating resting systolic blood pressure by approximately 2 mm Hg when compared with placebo (16). Additionally, particular soy bioactive peptide sequences have been studied extensively for their potential antihypertensive effect. These peptides act by inhibiting angiotensin-converting enzyme, which typically functions to convert angiotensin I into the potent vasoconstrictor, angiotensin II, thus decreasing blood pressure (17,18). Although these sequences have not been directly confirmed in the supplement used within this investigation, the relatively significant peptide composition (17.8 g/100 g) and presence of many associated antihypertensive individual peptide constituents (aspartic acid, leucine, proline, and glycine) (19) within the Fermalife fermented soy extract further indicts blood pressure as a physiological marker of interest in need of further investigation.

Among the moderate fitness subjects (<57 mL·kg^{-1}·min^{-1}), a nonsignificant improvement in 20-km time to completion and power output may be explained by the lack of group homogeneity contributing to a higher statistical variation around the mean. Similar to the high fitness group, HR was significantly dampened despite only a small improvement in work output, but there was no attenuation in overall cardiac output. It may be speculated that within this less vascularly adapted faction (20), the increase in skeletal muscle blood flow contributing to improved metabolic clearance and lesser afferent feedback to higher brain centers may be balanced by nonceilinged improvements in stroke volume caused by augmented muscle pump facilitated venous return. Although the improvements in performance are lacking in statistical confidence and application plausibility, the significant attenuation of HR may be meaningful to decrease performance-related perception of effort. The positive linear relationship between HR and perception of effort (RPE) is well supported in the literature and has contributed to the development of many subjective RPE measurement scales still in use today (21). As such, due to the extraordinary influence perception of effort can have on mental efficacy and downstream physical performance in all modalities, fermented soy extract supplementation may still improve performance of those at a moderate fitness level.

Further, isoflavones contained within soy-based products have demonstrated significant antioxidant properties. Oxidative stress is caused by excessive production and accumulation of reactive oxygen species (ROS) inherent to mitochondrial activity. Reactive oxygen species can be deleterious to both cellular structure and function if antioxidant compounds are not present. Several clinical trials, ranging in length from 30 d to 12 months, indicate that isoflavones genistein and daidzein significantly increased production of antioxidant enzymes in both animal and human models (22–24). Although the acute nature of this study compared with chronic supplementation studies may engender some questions regarding the importance of this mechanism of action to this particular investigation, it cannot be discounted as potentially influential.

There is little literature evaluating the effects of acute fermented soy extract and/or isoflavone supplementation on exercise performance, making a contextual comparison of the results of this study difficult. The majority of existing literature centers around exercise endurance (time to exhaustion) rather than exercise performance (time trial completion) and is presented through the lens of investigators interested in soy's role in macronutrient-focused supplement timing and antioxidative mechanisms. Ghosh et al. (25) induced a 37% increase in high-intensity endurance, as assessed by a

time to exhaustion ride at 90% $\dot{V}O_{2max}$, in male recreational cyclists after a sago (starch)–soy supplementation strategy. Supplementation was provided at 0, 20, and 40 min during a preliminary 60 min at 60% $\dot{V}O_{2max}$. Both plasma glucose and plasma insulin were elevated in the sago-only and sago–soy groups compared with placebo and therefore availability of carbohydrate did not provide a meaningful explanation for improvements in endurance capacity. Similarly, although plasma was not collected for analysis, because the supplementation in our study was done at least 60 min before the start of any exercise and 90 min before time trial performance, the effect of supplement-manipulated insulin and plasma glucose on performance is assumed to be minimal. Additionally, despite the larger absolute carbohydrate content of the placebo (15 g vs 6 g with fermented soy), greater improvements in performance variables were demonstrated after fermented soy supplementation rather than the placebo. As such, it appears carbohydrate availability does not credibly explain the performance time discrepancy between trials. The results of our study, indicating the plausibility of blood flow modulation with fermented soy extract supplementation in those capable of the highest performance intensities, may assist in further interpreting the improvements in high-intensity endurance capacity seen with the addition of soy supplementation in the work of Ghosh et al. (25).

Most other exercise-relevant investigations of soy supplementation have been chronic in nature, ranging in length from weeks to months. Chronic supplementation strategies, due to their logistic difficulty, have utilized rodent models, making investigation of the antioxidant properties of soy more feasible. Chen et al. (26) administered 598 $mg \cdot kg^{-1}$ of diet of genistein daily for 4 wk to male rats. After supplementation, the rats demonstrated an increase in treadmill running time to exhaustion; but were not capable of defending their liver and skeletal muscle against oxidative stress, despite an accumulation of genistein in these same tissues. The authors speculated that the amount of supplement utilized may be excessive; and therefore, may have contributed to a prooxidant rather than antioxidant state within the tissues in which it had accumulated. Ren et al. (27) also used a rat model to investigate a daily soy–whey (100 mg soy, 100 mg whey) supplementation strategy during 7 wk of resistance training that included loaded swimming and grasping exercises. When compared with controls, the rats that were soy–whey supplemented demonstrated a 1.5-fold increase in time to exhaustion during a loaded swimming trial, an increase in the force/weight ratio produced during grasping exercise, and an increase in the activity of lactate dehydrogenase and superoxide dismutase, alongside a decrease in malondialdehyde. The authors hypothesized that a potential advantage of the soy–whey protein combination is the provision of necessary branched-chain amino acids (BCAA) to stimulate skeletal muscle protein synthesis without a concomitant high level of amino acid oxidation. This is beneficial as a growing body of evidence exists that suggests exercise-induced protein oxidation can generate ROS, resulting in muscle fatigue. That said, as a stimulus for muscle protein synthesis and/or carbohydrate depletion were not present in our acute investigation, the contribution of ROS modulation from amino acid oxidation is most probably minimally influential. An attempt within our investigation to replicate dietary intake before each repeated-measures trial and conduct each session at the same time of day should have also minimized the influence of pretrial substrate deficiencies.

The results of this investigation are limited by the exclusive use of a male population and the necessity that all subjects have previous cycling experience. It is speculated that the significant preexisting estrogen and estrogen receptor presence within the vasculature of women, known to upregulate eNOS activity (28) independent of soy ingestion, may confound the recreation of our results within a similarly trained female population. However, the assumption of such a ceiling effect may be difficult to completely elucidate as the largest performance enhancement and physiological consequence of fermented soy supplementation was demonstrated within the high fitness group. Members of the high fitness group may arguably have a larger propensity for training-induced increases in eNOS content and eNOS/NADP(H) oxidase protein ratio indicative of an enhanced vasodilatory capacity (29). Further research should be conducted to explore the proposed eNOS/NO-mediated mechanism of isoflavone activity, particularly within populations other than healthy recreationally trained male cyclists and triathletes. Conclusive cardiovascular interpretation is also limited by the decision to prioritize time trial performance in lieu of cumbersome arterial blood pressure measurement. Future evaluation of arterial blood pressure throughout time trial performance is warranted. In an effort to evaluate the effects of fermented soy extract supplementation on peripheral oxygen offloading, the inclusion of oxygen consumption measurement using a metabolic system during time trial performance may also be feasible. Finally, the short sprint-type distance of the 20-km performance evaluation limits the scope of the results of this study. It is unclear whether the same performance benefits would have been evidenced for a longer 50-km or 100-km endurance cycling event.

Together, these results indicate that fermented soy extract supplementation with Fermalife administered 90 min before the start of a 20-km time trial was able to elicit a small but significant increase in both power production and time trial performance at a lower cardiac contractile frequency. These benefits were greatest in male cyclists and triathletes with greater fitness level ($\geq$57 $mL \cdot kg^{-1} \cdot min^{-1}$). We conclude that acute fermented soy extract supplementation may induce meaningful improvements in sprint distance cycling performance in males.

The authors would like to thank Aska Cheung, Natasha Cruz, Saul Brenman, and Brittany Castoro for their assistance with data collection. The authors disclose that funding was received from BESO Biological Research, Inc. Diamond Bar, CA 91765 to support this investigation.

Disclosures: The authors declare that the results of the study are presented clearly, honestly, and without fabrication, or inappropriate data manipulation in any way to intentionally portray anything but those outcomes that were empirically observed. The authors do not have any conflicts of interest to report. The results of the study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1. Anderson JJ, Anthony M, Messina M, Garne SC. Effects of phytooestrogens on tissues. *Nutr Res Rev*. 1999;12:75–116.
2. Cheng C, Tsai S, Chiu CP, Pan T, Tsai T. The effect of probiotic-fermented soy milk on enhancing the NO-mediated vascular relaxation factors. *J Sci Food Agric*. 2013;93:1219–25.
3. Setchell KDR. The history and basic science development of soy isoflavones. *Menopause*. 2017;24(12):1338–50.
4. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemical and phytoestrogens with estrogen receptor beta. *Endocrinology*. 1998;139(10):4252–63.
5. Sirotkin AV, Harrath AH. Phytoestrogens and their effects. *Eur J Pharmacol*. 2014;741:230–6.
6. Beavers DP, Beavers KM, Miller M, Stamey J, Messina MJ. Exposure to isoflavone-containing soy products and endothelial function: a Bayesian meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis*. 2012;22:182–91.
7. Squadrito F, Altavilla D, Morabito N, et al. The effect of phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. *Atherosclerosis*. 2002;163:339–47.
8. Si H, Liu D. Genistein, a soy phytoestrogen, upregulated the expression of human endothelial nitric oxide synthase and lowers blood pressure in spontaneously hypertensive rats. *J Nutr*. 2008;138(2):297–304.
9. Walker HA, Dean TS, Sanders TA, Jackson G, Ritter JM, Chowienczyk PJ. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17β-estradiol. *Circulation*. 2001;103:258–62.
10. Allen JD, Cobb FR, Gow AJ. Regional and whole-body markers of nitric oxide production following hyperemic stimuli. *Free Radic Biol Med*. 2005;38(9):1164–9.
11. Dreibigacker U, Wendt M, Wittke T, Tsikas D, Maassen N. Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men. *Nitric Oxide*. 2010;23(2):128–35.
12. Mitchell JH, Cawood E, Kinniburgh D, Provan A, Collins AR, Irvine DS. Effect of a phytoestrogen food supplement on reproductive health in normal males. *Clin Sci*. 2001;100(6):613–8.
13. Faul F, Erdfelder E, Lang A, Buchner A. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–91.
14. Laughlin MH, Korthuis RJ, Duncker DJ, Bache RJ. Handbook of physiology, exercise: regulation and integration of multiple systems. *Compr Physiol*. 2011;(29 Suppl):705–69.
15. Amann M. Significance of group III and IV muscle afferents for the endurance exercising human. *Clin Exp Pharmacol Physiol*. 2012;39(9):831–5.
16. Taku K, Lin N, Cai D, et al. Effects of soy isoflavone extract supplements on blood pressure in adult humans: systematic review and meta-analysis of randomized placebo-controlled trials. *J Hypertens*. 2010;28:1971–82.
17. Wang W, Gonzalez de Meijia E. A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. *Compre Rev Sci Food Saf*. 2005;4:63–78.
18. Singh BP, Vij S, Hati S. Functional significance of bioactive peptides derived from soybean. *Peptides*. 2014;54:171–9.
19. Wu J, Ding X. Characterization of inhibition and stability of soy–protein-derived angiotensin I-converting enzyme inhibitory peptides. *Food Res Int*. 2002;35:367–75.
20. Laughlin MH, McAllister RM, Delp MD. *Physical Activity and the Microcirculation in Cardiac and Skeletal Muscle*. Champaign, IL: Human Kinetics Publishes, Inc; 1994.
21. Borg G, Hassmén P, Lagerstrom M. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Eur J Appl Physiol*. 1987;65:679–85.
22. Cai Q, Wei H. Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. *Nutr Cancer*. 1996;25:1–7.
23. Pusparini DR, Dharma R, Suyatna FD, Mansyur M, Hidajat A. Effect of soy isoflavone supplementation on vascular endothelial function and oxidative stress in postmenopausal women: a community randomized controlled trial. *Asia Pac J Clin Nutr*. 2013;22:357–64.
24. Xiao Y, Zhang S, Tong H, Shi S. Comprehensive evaluation of the role of soy and isoflavone supplementation in humans and animals over the past two decades. *Phytother Res*. 2018;32:384–94.
25. Ghosh AK, Rahaman AA, Singh R. Combination of sago and soy–protein supplementation during endurance cycling exercise and subsequent high-intensity endurance capacity. *Int J Sport Nutr Exerc Metab*. 2010;20:216–23.
26. Chen CY, Holtzman GI, Bakhit RM. High-genistin isoflavone supplementation modulated erythrocyte antioxidant enzymes and increased running endurance in rats undergoing on session of exhausting exercise—a pilot study. *Pak J Nutr*. 2002;1(1):1–7.
27. Ren G, Yi S, Zhang H, Wang J. Ingestion of soy–whey blended protein augments sports performance and ameliorates exercise-induced fatigue in a rat exercise model. *Food Funct*. 2017;8:670–9.
28. Sader MA, Celermajer DS. Endothelial function, vascular reactivity and gender differences in the cardiovascular system. *Cardiovasc Res*. 2002;53(3):597–604.
29. Cocks M, Wagenmakers AJ. The effect of different training modes on skeletal muscle microvascular density and endothelial enzymes controlling NO availability. *J Physiol*. 2016;594(8):2245–57.