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Cortitrol supplementation reduces serum cortisol responses to physical stress

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Abstract

The supplement Cortitrol was formulated to mitigate the cortisol response to physiological and psychological stress. Therefore, the purpose of this study was to examine the effects of Cortitrol on serum cortisol concentrations before, during, and after a high-intensity resistance exercise protocol (EX) and a resting control day (REST). We used a matched, balanced, randomized, double-blind, placebo-controlled, cross-over design. Blood samples were obtained at matching time points during EX and REST. Cortitrol significantly ($P < .05$) reduced cortisol area under the curve concentrations during REST. During EX, Cortitrol reduced cortisol concentrations at 20, 10, and 0 minutes pre-exercise, at mid-exercise, immediately post-exercise, and at 5 minutes post-exercise. In addition, serum cortisol and plasma adrenocorticotropin hormone area under the curve concentrations during EX were significantly lower after Cortitrol than placebo. Furthermore, Cortitrol significantly reduced free radical production. This was indicated by significantly lower plasma malondialdehyde concentrations at the 65-minute post-exercise time point during REST, and at pre-exercise, immediate post-exercise, and 65 minutes post-exercise during EX. Serum total testosterone, free testosterone, dehydroepiandrosterone, and growth hormone showed exercise-induced increases but no treatment effects. These data demonstrate that Cortitrol was effective in modulating the physiological stress responses of exercise from the anticipatory rises before physical stress and into early recovery by reducing cortisol and associated free radical production.

1. Introduction

When human beings are confronted with physiological and/or psychological stress the adrenal gland secretes cortisol. This response increases glucose and fatty acid concentrations in the blood and stimulates gluconeogenesis to prepare the body for "fight or flight." Although cortisol is necessary for normal physiological function, chronic elevations have a negative impact on muscle and immune cell function and bone metabolism. Nutritional interventions aimed at partially attenuating the cortisol response would prove valuable for those faced with, for example, intense physical training, labor in the work place, or chronic physiological stress.

There are 2 components of the supplement regimen tested. The first component is a general vitamin/mineral combination of pantothenic acid (30 mg), pyridoxine (10 mg), riboflavin (8.5 mg), thiamine (7.5 mg), vitamin C (250 mg), calcium (100 mg), and magnesium (100 mg). B vitamins (pantothenic acid, pyridoxine, riboflavin, and thiamine) were added to prevent deficiencies common in active individuals [1], which have been shown to decrease $\dot{V}O_2$max, onset of blood lactate accumulation, peak power, and mean power [2]. Vitamin C supplementation has the potential to reduce blood pressure, cortisol, and subjective responses to acute psychological stress in healthy subjects [3], as well as exercise stress responses in competitive weightlifters [4]. Calcium intake is essential for minimizing bone loss and osteoporosis [5], a disease common among individuals with persistently elevated cortisol levels [6].

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Finally, chronic stress may exacerbate preexisting magnesium deficiencies, which can lead to impaired energy metabolism and decreased physical work capacity [7].

The second component of the supplement is Cortitrol, a proprietary herbal anticortisol blend containing magnolia bark extract (*Magnolia officialis*), L-theanine (from *Camillia sinensis*), *Epimedium* extract (*Epimedium koreanum*), phophatidylserine (soy derived), and β-sitosterol. Limited data exist on the effectiveness of these herbal supplements; however, preliminary studies show promising results. Magnolol, a phenolic constituent of magnolia bark, has been shown to suppress cortical serotonin (5-hydroxytryptamine) release [8], which may be advantageous because serotonin plays a role in stress and anxiety–related disorders [9]. L-theanine, commonly found in tea, may prime blood T cells and provide natural resistance to infection [10] and promote brain α-wave [11] and suppress β-wave activity [12]. *Epimedium* has been shown to lower cortisol levels in animal models [13]. Monteleone et al [14,15] showed that phosphatidylserine supplementation attenuated the cortisol response to physical stress in healthy men. Finally, subjects supplementing with β-sitosterol before competing in a marathon had a decreased inflammatory response, cortisol/dehydroepiandrosterone (DHEA) ratio, and immune suppression during the postmarathon recovery period [16].

Although cortisol is necessary to respond to physiological stress, chronic elevation of cortisol may have negative effects on a host of target tissues, such as reduced immune cell function, protein wasting in muscle, and suboptimal bone metabolism. However, complete elimination of the overt cortisol response to stress would be physiologically inappropriate for normal human health. Thus, Cortitrol was designed and formulated to reduce the magnitude of the overt stress response and absolute cortisol concentrations.

Intense resistance exercise and training has been shown to cause dramatic increases in cortisol levels [17,18]. Furthermore, athletes show elevated cortisol levels in anticipation of intense physical challenge [19]. Therefore, high-intensity resistance exercise provides an ideal forum to test the purported benefits of Cortitrol. The purpose of this investigation was to determine the efficacy of Cortitrol to influence cortisol responses to an intense physical exercise stress model in human beings known to dramatically increase cortisol concentrations in the blood. We hypothesized that

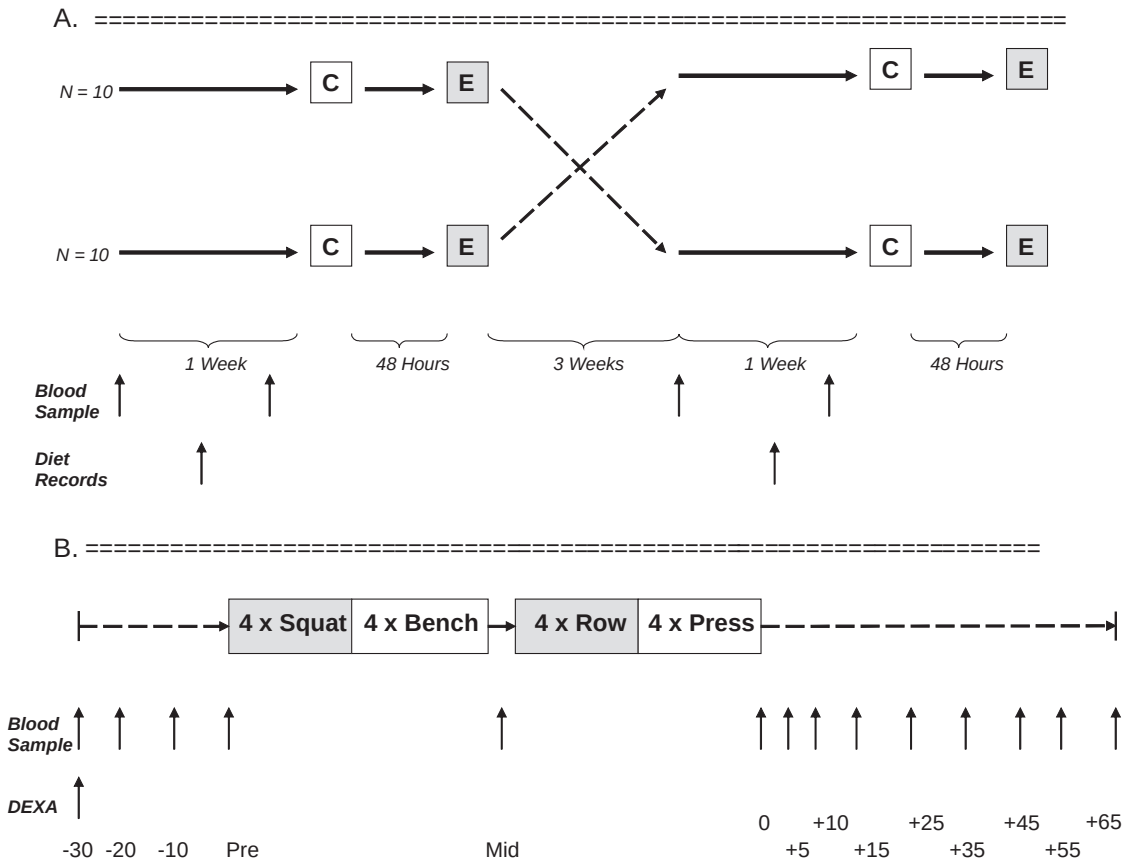

Fig. 1. A, Experimental design. A balanced, randomized, double-blind, placebo-controlled, cross-over design was used to determine the effects of Cortitrol. C indicates control day; E, exercise day. B, Testing sequence during exercise testing days. Blood samples were obtained at matching time points during resting control days. DEXA indicates dual-energy x-ray absorptiometry.

supplementation would result in decreased cortisol concentrations before, during, and after the exercise challenge.

2. Methods

2.1. Subjects

The Institutional Review Board for use of Human Subjects in Research at the University of Connecticut approved all study procedures before its initiation. Twenty-one men volunteered to participate and gave written consent after being informed of the risks associated with the study. All subjects acted as their own controls with the within-group design used in this study. Subjects were healthy, college-aged men with resistance training experience of 4 years or more. Mean (±SD) age and height were 21.1 ± 1.2 years and 180.5 ± 7.0 cm, respectively. Each subject was specifically instructed to maintain their individual exercise routine (frequency, duration, and intensity) during the study, which was verified through evaluation of physical activity diaries. Each subject was tested for individual 1 repetition maximum (RM) using previously described methods [20]. One RM of the back squat, bench press, bent over row, and shoulder press exercises were 130.8 ± 27.4, 112.9 ± 21.2, 90.2 ± 17.5, and 72.1 ± 11.3 kg, respectively.

2.2. Experimental design

A matched, balanced, randomized, double-blind, placebo-controlled, cross-over design was used to determine the effects of Cortitrol on serum cortisol concentrations (see Fig. 1). Subjects were matched according to age, body size, and training experience. All subjects used physical activity diaries and food diaries to replicate lifestyle patterns for the 3 days before each testing protocol. All subjects participated in several familiarization sessions before exercise testing to reduce the experimental variance from learning effects and to expose subjects to the physical challenge of the protocol.

Resting baseline measures were obtained before (V-1) and after (V-2) 1 week of supplementation following the placebo and Cortitrol conditions to determine measurement stability of the dependent variables. Subjects were then asked to report to the laboratory on 2 separate occasions for each condition: once for a resistance training exercise challenge (EX) and once for a resting control day (REST). Treatment order was balanced and randomized; REST proceeded EX by 48 hours.

The EX protocol consisted of 4 sets of 10 RM using 4 different exercises. A 10RM (the amount of weight that allowed only 10 repetitions to be performed) was determined during familiarization sessions and the resistances used for each set of every exercise were established with ICC *R*'s of more than 0.98 for 2 consecutive exercise sessions. The goal of the familiarizations was to create a reliable workout that could be highly replicated to produce a similar if not identical physiological stress response [18]. During the experimental workout, resistances were reduced to allow only 10 repetitions to be performed but both experimental workouts used very similar if not identical resistances in the workout sequences because of careful familiarization and practices of the experimental protocol [18,21]. To maximally stimulate all major muscle groups, the back squat, bench press, bent over row, and shoulder press exercises were used. Two minutes of rest was allowed between each set and exercise. This protocol has been found to produce high physiological stress, as evidenced by cortisol (>400 nmol/L) and lactate concentrations (>14 mmol/L) [18]. Blood was collected at 30 minutes (−30), 20 minutes (−20), and 10 minutes (−10) pre-exercise, immediately before exercise (PRE), at the midpoint of the exercise protocol (MID), immediately post-exercise (IP), and at 5 minutes (+5), 10 minutes (+10), 15 minutes (+15), 25 minutes (+25), 35 minutes (+35), 45 minutes (+45), 55 minutes (+55), and 65 minutes (+65) after exercise. Testing procedures for REST (including duration, timing and number of blood draws, and time of day) were identical. All treatment sessions were performed within the same identical time of day window (8:00 to 10:30 AM) to reduce the influence of normal circadian variations, which can add to experimental variance. After completion of EX and REST protocols, subjects were crossed-over (into either the treatment or placebo group) and the study protocol was repeated.

2.3. Anthropometry and body composition

Whole body composition was assessed using fan-beam dual-energy x-ray absorptiometry (Prodigy, Lunar Corporation, Madison, Wis). Subjects were positioned on the dual-energy x-ray absorptiometry according to the manufacturer's guidelines having removed all metallic objects from their body. Whole body analysis of fat tissue, lean tissue, and bone mineral content was assessed according to anatomical landmarks by the same technician using computer algorithms (enCORE version 6.00.270). Coefficients of variation for lean body mass and fat mass on repeat scans with repositioning within a group of male subjects in our laboratory were 0.4% and 1.4%, respectively. The same experienced technician performed all measurements throughout the study period. Body mass was measured to the nearest 0.01 kg using an electronic scale (American Business Equipment Company, Inc, New Holland, Pa). Scans were performed on all subjects at 4 time

Table 1
Pre- and post-supplementation body composition and bone characteristics

	Placebo	Cortitrol
Pre-supplementation		
Mass (kg)	87.20 ± 9.10	87.67 ± 9.12
Body fat (%)	14.88 ± 4.91	15.29 ± 4.41
BMC (g)	3.72 ± 0.40	3.72 ± 0.42
Post-supplementation		
Mass (kg)	87.46 ± 9.57	88.94 ± 8.43
Body fat (%)	15.05 ± 5.01	15.44 ± 4.42
BMC (g)	3.69 ± 0.41	3.75 ± 0.44

BMC indicates bone mineral content.

points during the study: immediately before and on the seventh day of the supplementation protocol for both the placebo and treatment conditions. No significant changes in body composition were observed during the course of the investigation (Table 1).

2.4. Dietetic analysis

To control for possible confounding effects of diet on cortisol [22,23], subjects were instructed to consume similar foods for 3 days before the exercise bout for each phase. A registered dietician and associated staff counseled and supervised the subjects to ensure no nutritional variation would confound the dependent variables. To assist participants in duplicating their diet during phase 1 and 2, subjects completed detailed 3-day diet diary records during both periods. Copies were made of phase 1 diet records and returned to subjects during phase 2 to enhance reproducibility. Food diaries were analyzed for energy and

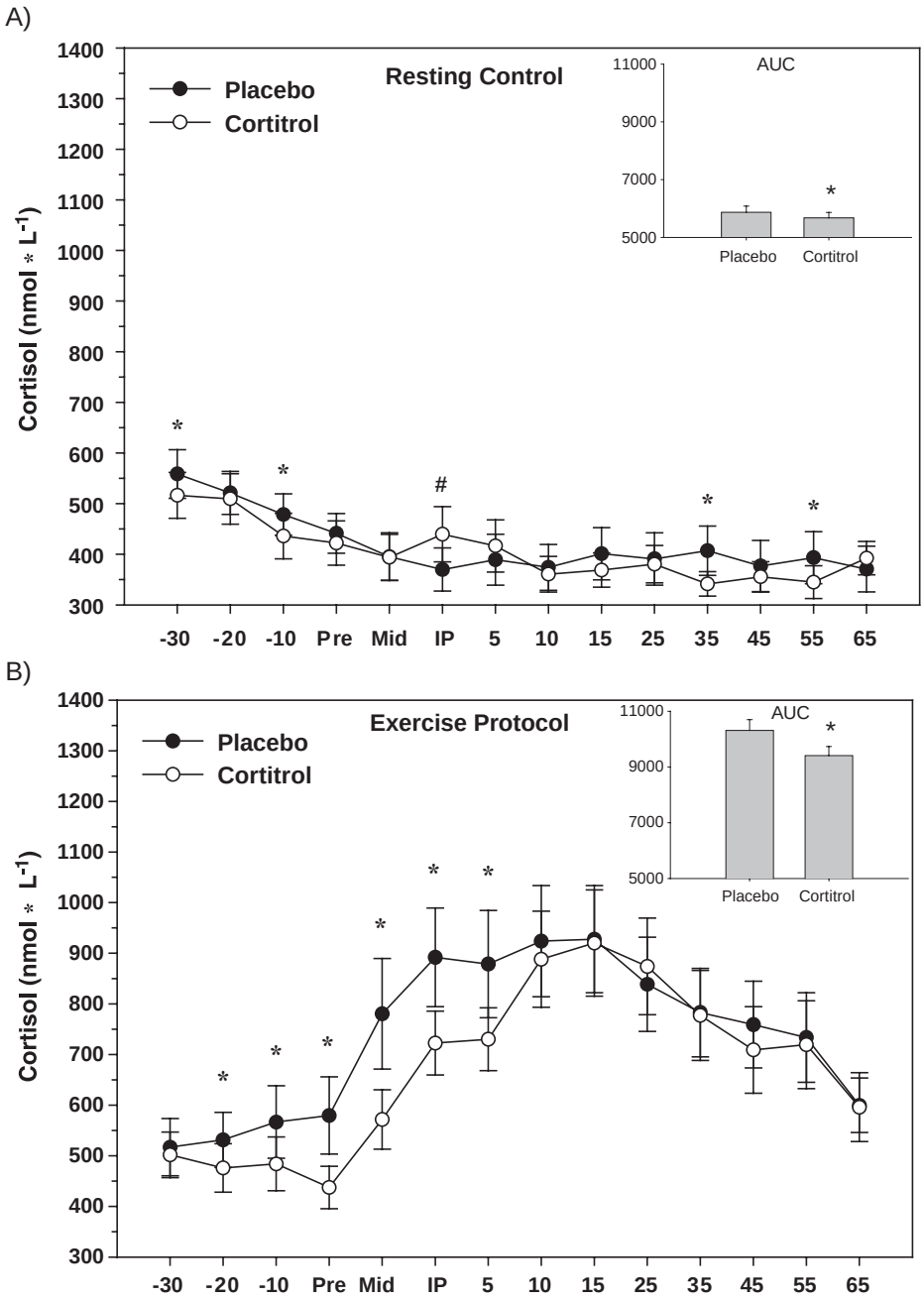

Fig. 2. A, Cortisol concentrations during the resting control day. *$P < .05$, significantly greater than corresponding Cortitrol value. #$P < .05$, significantly lower than corresponding Cortitrol value. Data are expressed as mean ± SE. Insert, Area under the curve comparison for resting day. *$P < .05$ from corresponding placebo condition. B, Cortisol concentrations during the exercise challenge day. *$P < .05$, significantly different than corresponding placebo value. Data are expressed as mean ± SE. Insert, Area under the curve comparison for exercise challenge day. *$P < .05$ from corresponding placebo condition.

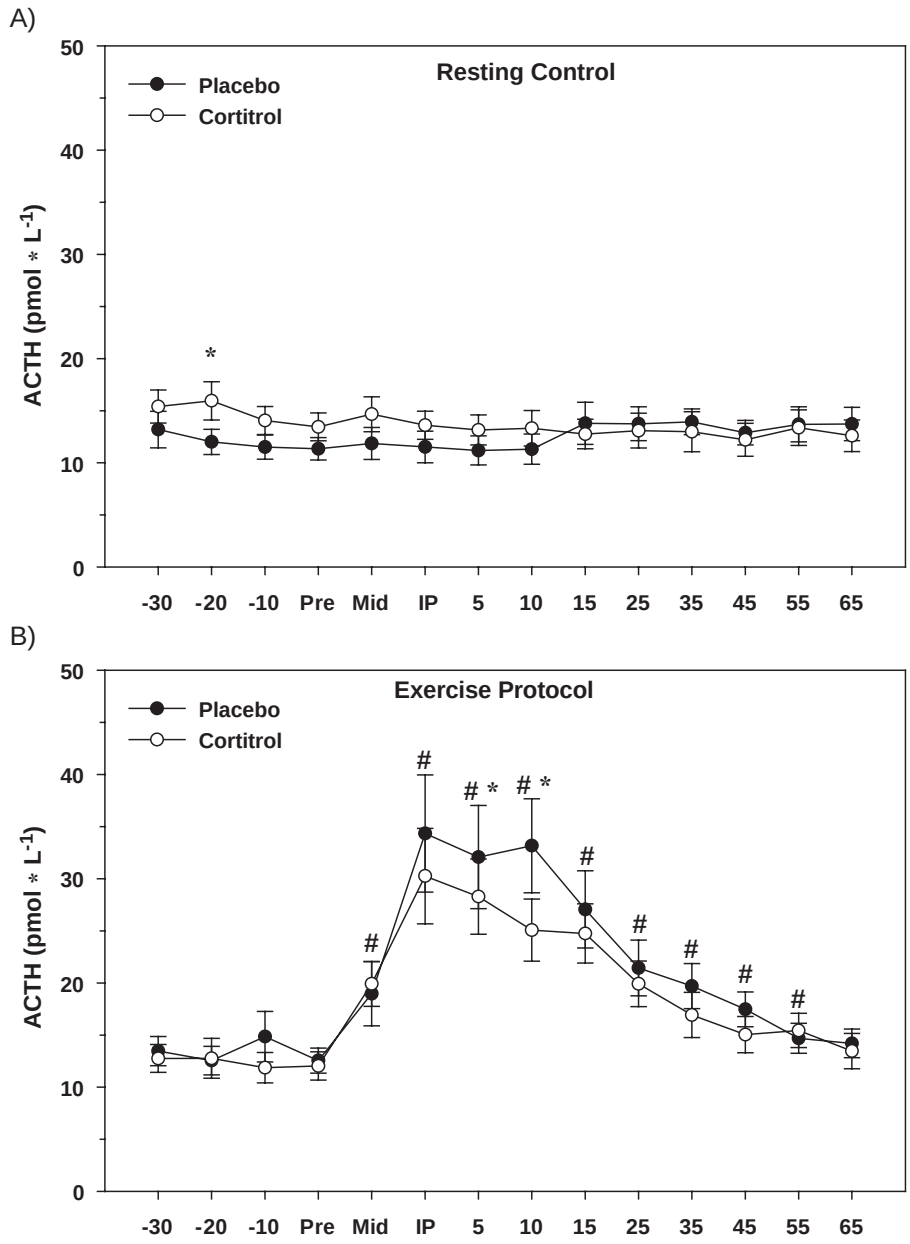

Fig. 3. A, Corticotropin concentrations during the resting control day. *$P < .05$ from corresponding placebo condition. B, Corticotropin concentrations during the exercise challenge day. *$P < .05$ from corresponding placebo condition. $^{\#}P < .05$ from corresponding pre-exercise time point. Data are expressed as mean ± SE.

macro/micronutrient content with NUTRITIONIST PRO (version 1.3, First Databank Inc, The Hearst Corporation, San Bruno, Calif).

2.5. Biochemical analyses

Serum concentrations of cortisol, total and free testosterone, and DHEA were determined in duplicate using commercially available enzyme immunoassay kits (Diagnostic Systems Laboratories, Webster, Tex) according to the manufacturer's procedures. Plasma corticotropin concentrations were determined using enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Webster, Tex). All samples were assayed in duplicate and were decoded only after analyses were completed (ie, blinded analysis procedure). For all procedures, samples were thawed only once before analysis. The minimum detection limits for cortisol, total and free testosterone, DHEA, and corticotropin were 2.76 nmol/L, 0.14 nmol/L, 0.66 nmol/L, 0.346 nmol/L and 0.264 pmol/L, respectively. In all cases, intra-assay and inter-assay variances were <10%.

Plasma growth hormone (GH) concentrations were determined in duplicate using an ^{125}I liquid-phase immunoradiometric assay (Nichols Institute Diagnostics, San Juan, Capistrano, Calif). This commercially available

immunoradiometric assay uses 2 monoclonal antibodies of high affinity and specificity for GH, each detecting a different epitope on the GH molecule. One of the antibodies was labeled for detection, whereas the other was coupled to biotin. The sensitivity for this assay using the B_o (2 SD) method was 0.04 ng/mL. Intra-assay variances for GH concentrations were less than 5%.

Plasma malondialdehyde (MDA) concentrations were determined using a thiobarbituric acid assay procedure as previously described [24,25] Briefly, 50 μL of unknown sample and MDA standard (0.61-19.44 μmol/L; 1,1,2,2-tetraethoxypropane standards) were pipetted into polypropylene test tubes. Then they were combined with 0.75 mL of 0.44 mol/L phosphoric acid stock solution and 0.25 mL of 42 mmol/L thiobarbituric acid (0.6 g of 4,6-dihydroxy-thiopyrimidine in 100 mL of dH_2O). Samples were further diluted using 0.45 mL dH_2O. All test tubes were then capped, sealed tight, and placed in a preheated water bath at

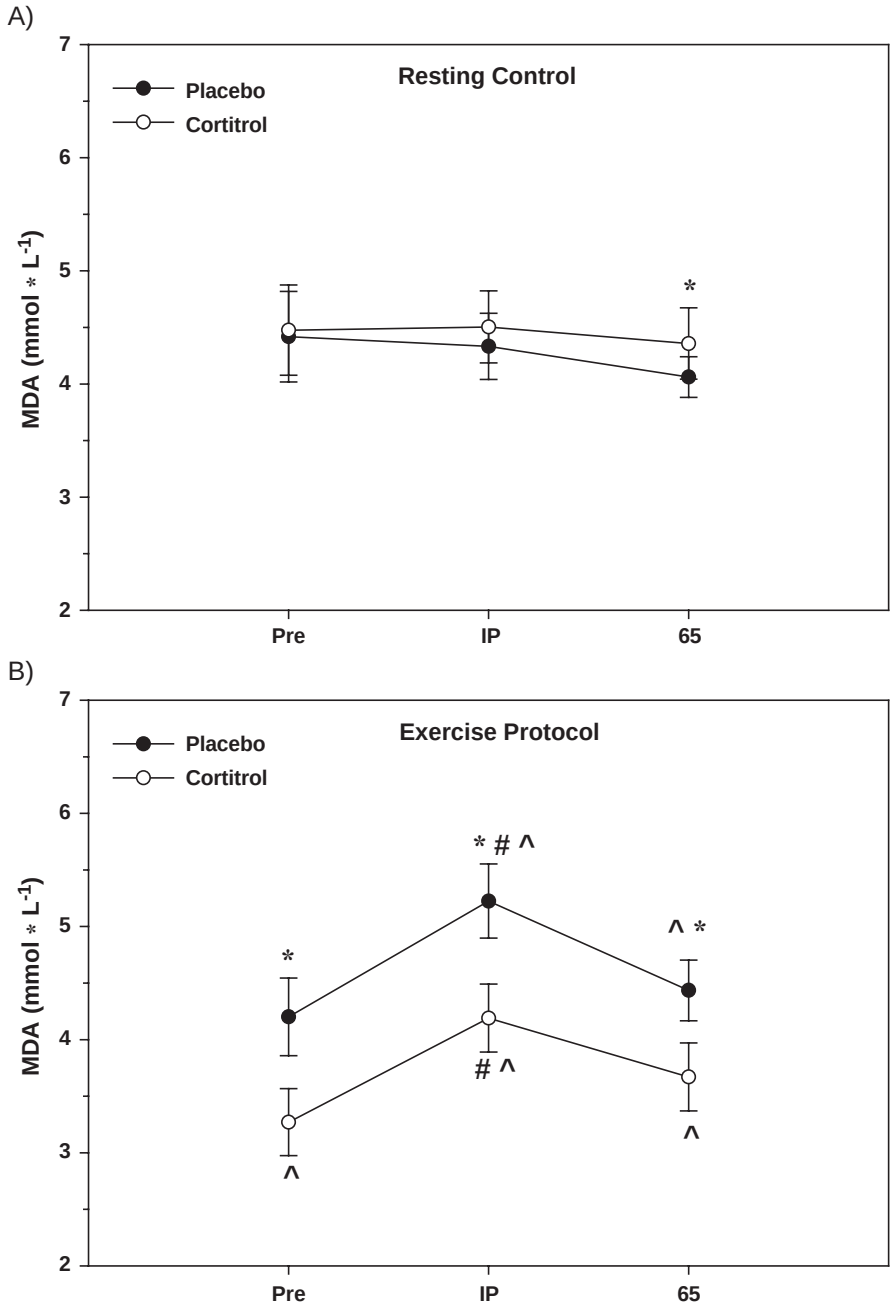

Fig. 4. A, Plasma MDA concentrations during the resting control day at the time points corresponding to pre-exercise (Pre), immediately post-exercise (IP), and 65 minutes post-exercise (65). *$P < .05$ from corresponding placebo condition. B, MDA responses during the exercise challenge day. *$P < .05$ from corresponding Cortitrol condition; $^{\#}P < .05$ from the corresponding treatment's pre-exercise value; ^$P < .05$ from plasma MDA concentrations resting day control values. Data are expressed as mean ± SE.

100°C for 60 minutes. After heating, samples were removed from the water bath and immediately placed into an ice water bath (0°C) until analysis. Samples remained on ice for no longer than 30 minutes before analysis; 1.5 mL of Me-NaOH stock solution was then added to all boiled samples and standards. All tubes were recapped, vortexed, and centrifuged at 3500 rpm for 5 minutes to sediment the precipitated plasma proteins. Plasma concentrations of MDA were determined in duplicate. One-millimeter extract of protein-free plasma was removed from each test tube without disturbing the sedimented precipitate. All samples and standards were transferred to 1.5-mL cuvettes and the absorbance was read at 532 nm using a spectrophotometer (Spectronic 401, Spectronic Instruments Inc, Rochester, NY). To eliminate inter-assay variance, all samples for this assay were analyzed in the same assay run. In all cases, intra-assay variances were less than 10%.

Plasma glucose and lactate concentrations were determined using an automated glucose/lactate analyzer (2300 Stat glucose/L-lactate analyzer, YSI, Inc, Yellow Springs, Ohio). Hemoglobin was analyzed in triplicate from whole blood using the cyanmethemoglobin method (Sigma Diagnostics, St Louis, Mo); hematocrit was analyzed in triplicate from whole blood via microcentrifugation and microcapillary technique. Plasma volume shifts after the workout were calculated using the formula of Dill and Costill [26].

2.6. Statistical analyses

Paired-sample t tests were used to examine differences between the 2 days of baseline resting values. Area under the curve (AUC) analyses were completed using standard trapezoidal statistical methods. Two-way analysis of variance with repeated measures was used to examine pairwise differences in hormonal concentrations and a 2-way analysis of variance (2 groups × 2 conditions) was used to evaluate the AUC data. Appropriate post hoc tests (ie, Fisher LSD or Tukey tests) were used when a significant F score resulted. All linear assumptions were tested and, when appropriate, $\log_{10}$ transformations were used and the data were reanalyzed. Using nQuery Advisor software (Statistical Solutions, Saugus, Mass), the statistical power for the n size used ranged from 0.80 to 0.90. Significance in this study was set at $P \leq 0.05$.

3. Results

There was no significant plasma volume shift pre- to post-exercise for either condition (placebo: +0.56% ± 5.51% placebo; Cortitrol: +4.12% ± 7.26%, $P > .05$). This was likely because of ad libitum water intake during the rest periods between sets and exercises.

There were no significant differences between Cortitrol and placebo supplementation, respectively, for dietary energy (9517 ± 3756 and 10752 ± 4246 kJ), and the percent energy from carbohydrate (48% ± 8% and 43% ± 13%), fat (31% ± 9% and 34% ± 12%), and protein (21% ± 2% and 23% ± 5%). This minimized the impact of dietary variation on endocrine responses between the 2 treatment conditions. The dietary records of all participants revealed that they had normal eating patterns; no fad diets and/or signs or eating disorders were revealed.

There were no pre-supplementation differences between conditions in serum cortisol concentrations (placebo: 479.1 ± 22.94 nmol/L; Cortitrol: 440.2 ± 142.3 nmol/L). Fig. 2A shows pairwise differences between placebo and Cortitrol at −30, −10, IP, +35, and +55 during REST. The insert of Fig. 2A demonstrates that cortisol AUC concentrations during REST were significantly lower for the Cortitrol supplementation condition.

Table 2
Values for plasma glucose and lactate during resting control and exercise days

	Glucose (mmol/L)		Lactate (mmol/L)	
	Placebo	Cortitrol	Placebo	Cortitrol
Resting control				
Baseline				
V-1	4.51 ± 0.52	4.38 ± 0.50	2.19 ± 0.76	2.60 ± 0.95
V-2	4.59 ± 0.46	4.43 ± 0.35	1.99 ± 0.58	2.26 ± 0.79
Pre-values				
−30	4.37 ± 0.59	4.55 ± 0.46	2.40 ± 1.00	2.22 ± 0.63
−20	4.47 ± 0.46	4.58 ± 0.44	2.23 ± 0.87	2.13 ± 0.58
−10	4.43 ± 0.44	4.54 ± 0.42	2.29 ± 0.90	2.10 ± 0.54
Pre	4.42 ± 0.49	4.50 ± 0.45	2.20 ± 0.91	2.10 ± 0.51
Mid-values				
Mid	4.38 ± 0.53	4.38 ± 0.42	2.05 ± 0.71	2.08 ± 0.51
Post-values				
IP	4.51 ± 0.39	4.43 ± 0.39	1.95 ± 0.72	1.96 ± 0.49
+5	4.49 ± 0.40	4.43 ± 0.45	1.94 ± 0.64	1.97 ± 0.46
+10	4.53 ± 0.39	4.47 ± 0.45	1.87 ± 0.65	1.88 ± 0.43
+15	4.45 ± 0.56	4.47 ± 0.43	1.91 ± 0.67	1.83 ± 0.46
+25	4.57 ± 0.34	4.48 ± 0.44	1.87 ± 0.64	1.83 ± 0.42
+35	4.61 ± 0.44	4.45 ± 0.47	1.84 ± 0.60	1.81 ± 0.44
+45	4.61 ± 0.39	4.43 ± 0.50	1.79 ± 0.51	1.84 ± 0.40
+55	4.65 ± 0.37	4.48 ± 0.56	1.75 ± 0.42	1.83 ± 0.40
+65	4.67 ± 0.41	4.47 ± 0.57	1.66 ± 0.44	1.74 ± 0.39
Exercise protocol				
Pre-exercise				
−30	4.36 ± 0.36	4.60 ± 0.37	2.22 ± 0.61	2.15 ± 0.62
−20	4.39 ± 0.37	4.60 ± 0.39	2.10 ± 0.59	2.08 ± 0.56
−10	4.40 ± 0.36	4.62 ± 0.37	2.22 ± 0.63	1.98 ± 0.51
Pre	4.40 ± 0.37	4.57 ± 0.34	2.81 ± 3.10	2.02 ± 0.55
Mid-exercise				
Mid	5.90 ± 1.13[a]	5.63 ± 0.83[a]	15.26 ± 3.26[b,a]	14.07 ± 2.91[b,a]
Post-exercise				
IP	6.42 ± 1.69[a]	6.09 ± 1.11[a]	16.94 ± 3.52[b,a]	16.59 ± 3.47[b,a]
+5	6.38 ± 1.67[a]	6.07 ± 1.15[a]	15.89 ± 3.78[b,a]	15.29 ± 3.70[b,a]
+10	6.22 ± 1.66[a]	5.93 ± 1.14[a]	14.38 ± 3.85[b,a]	14.19 ± 4.04[b,a]
+15	6.16 ± 1.56[a]	5.87 ± 1.14[a]	13.02 ± 3.87[b,a]	12.77 ± 3.86[b,a]
+25	5.89 ± 1.63[a]	5.67 ± 1.16[a]	10.46 ± 3.72[b,a]	10.26 ± 4.00[b,a]
+35	5.55 ± 1.60[a]	5.42 ± 1.19[a]	8.35 ± 3.40[b,a]	8.15 ± 3.46[b,a]
+45	5.17 ± 1.31[a]	5.13 ± 1.11[a]	6.85 ± 2.80[b,a]	6.67 ± 2.92[b,a]
+55	4.95 ± 1.08	4.88 ± 1.01	5.49 ± 2.29[b,a]	5.53 ± 2.49[b,a]
+65	4.72 ± 0.84	4.64 ± 0.97	4.71 ± 2.05[b,a]	4.64 ± 2.21[b,a]

Values are expressed as mean ± SD.
[a] $P < .05$ from corresponding resting control day time point.
[b] $P < .05$ from corresponding pre-exercise value.

Table 3
Values for total testosterone, free testosterone, and DHEA during resting control and exercise days

	Total testosterone (nmol/L)		Free testosterone (pmol/L)		DHEA (nmol/L)	
	Placebo	Cortitrol	Placebo	Cortitrol	Placebo	Cortitrol
Resting control day						
Baseline						
V-1	32.49 ± 12.69	30.18 ± 9.45	64.89 ± 37.19	63.10 ± 44.41	79.59 ± 24.32	84.92 ± 21.47
V-2	32.04 ± 13.42	30.30 ± 8.99	63.17 ± 31.27	58.84 ± 45.94	84.47 ± 20.84	86.75 ± 19.43
Pre-values						
−30	32.12 ± 11.55	30.68 ± 9.00	65.08 ± 26.76	61.80 ± 52.36	80.98 ± 21.61	86.63 ± 20.33
−20	31.47 ± 10.74	30.58 ± 8.88	76.70 ± 31.44	58.26 ± 48.39	75.62 ± 19.44	84.32 ± 21.10
−10	30.38 ± 10.90	29.51 ± 9.12	64.48 ± 31.33	61.58 ± 42.20	71.01 ± 19.04	79.59 ± 20.50
Pre	29.56 ± 10.42	29.48 ± 10.43	68.47 ± 28.52	59.80 ± 44.88	68.34 ± 17.66	75.97 ± 19.63
Mid-values						
Mid	29.06 ± 11.04	28.61 ± 9.53	71.50 ± 26.75	59.86 ± 44.27	65.23 ± 15.38[a]	75.42 ± 22.54
Post-values						
IP	29.66 ± 11.27	28.52 ± 9.33	65.40 ± 28.24	60.88 ± 43.07	66.71 ± 17.64	74.15 ± 20.10
+5	30.23 ± 11.01	28.81 ± 9.27	78.38 ± 26.14	56.71 ± 44.68	67.40 ± 18.05[a]	75.50 ± 20.19
+10	30.66 ± 11.52	30.05 ± 10.92	73.15 ± 27.68	60.87 ± 46.33	68.34 ± 20.46[a]	79.02 ± 18.56
+15	30.22 ± 11.23	29.84 ± 10.00	77.26 ± 33.23	66.76 ± 46.39	69.68 ± 20.26[a]	75.60 ± 20.33
+25	30.35 ± 11.26	29.83 ± 9.41	77.32 ± 34.94	66.73 ± 44.53	71.84 ± 20.15[a]	79.54 ± 18.46
+35	30.16 ± 10.77	30.21 ± 10.85	76.09 ± 35.49	68.59 ± 42.29	72.81 ± 18.37[a]	79.45 ± 17.45
+45	30.38 ± 10.76	29.81 ± 10.46	72.85 ± 28.83	70.78 ± 41.86	72.02 ± 17.01	79.21 ± 17.78
+55	30.15 ± 11.53	30.61 ± 9.38	73.76 ± 30.22	69.15 ± 40.04	71.50 ± 19.94	77.10 ± 15.01
+65	29.79 ± 11.57	29.38 ± 9.81	68.35 ± 29.58	63.18 ± 40.98	70.73 ± 18.89	75.26 ± 18.51
Exercise protocol						
Pre-exercise						
−30	29.52 ± 11.24	28.33 ± 8.10	64.64 ± 32.83	59.19 ± 39.32	83.26 ± 24.84	84.11 ± 20.87
−20	29.75 ± 11.18	28.24 ± 9.11	60.72 ± 31.37	66.51 ± 38.68	77.90 ± 24.03	85.06 ± 22.97
−10	28.47 ± 10.06	26.87 ± 8.32	59.82 ± 28.48	66.02 ± 42.49	74.24 ± 24.15	76.43 ± 18.78
Pre	28.83 ± 10.53	26.99 ± 8.49	59.80 ± 29.05[b]	67.62 ± 36.57[c,b]	73.58 ± 24.70	73.74 ± 17.36
Mid-exercise						
Mid	32.74 ± 11.98[c,b]	31.48 ± 10.57[c,b]	71.56 ± 38.97[c]	73.56 ± 45.51[c,b]	96.39 ± 24.29[c,b]	98.23 ± 18.62[c,b]
Post-exercise						
IP	35.20 ± 13.04[c,b]	34.48 ± 10.87[c,b]	82.74 ± 37.13[c,b]	80.06 ± 47.30[c,b]	109.44 ± 27.73[c,b]	111.39 ± 22.98[c,b]
+5	36.35 ± 12.85[c,b]	34.25 ± 10.46[c,b]	79.30 ± 36.54[c]	73.77 ± 45.67[c,b]	110.29 ± 27.65[c,b]	112.89 ± 28.42[c,b]
+10	36.01 ± 13.26[c,b]	34.59 ± 10.75[c,b]	74.42 ± 34.24[c]	77.09 ± 48.20[c,b]	109.67 ± 28.01[c,b]	112.53 ± 20.47[c,b]
+15	35.69 ± 13.15[c,b]	33.04 ± 10.22[c,b]	78.74 ± 33.88[c]	82.69 ± 63.63[c,b]	108.95 ± 26.32[c,b]	111.96 ± 21.64[c,b]
+25	32.54 ± 12.66[c]	31.34 ± 9.95[c]	67.87 ± 34.18[c]	75.26 ± 47.85[c,b]	104.24 ± 28.05[c,b]	107.14 ± 22.76[c,b]
+35	30.04 ± 11.59[c]	29.13 ± 9.69[c]	67.13 ± 39.64[c]	74.06 ± 45.56[c,b]	99.46 ± 30.91[b]	99.63 ± 24.33[c,b]
+45	28.78 ± 10.98	28.62 ± 10.26	56.57 ± 32.10[b]	66.10 ± 40.30	94.14 ± 31.43[b]	92.56 ± 25.42[c,b]
+55	27.82 ± 10.48	28.23 ± 10.47	55.09 ± 30.69[b]	67.64 ± 38.69	86.49 ± 30.42[b]	86.17 ± 28.10[c,b]
+65	27.26 ± 10.02	27.56 ± 10.16	53.77 ± 27.68[b]	56.95 ± 38.27	79.14 ± 28.97	84.50 ± 24.86

Values are expressed as mean ± SD.
[a] $P < .05$ from corresponding Cortitrol condition.
[b] $P < .05$ from corresponding resting control day time point.
[c] $P < .05$ from corresponding pre-exercise value.

During EX, subjects had significantly lower cortisol concentrations after Cortitrol supplementation than placebo at −20, −10, PRE, MID, IP, and +5 (Fig. 2B). Furthermore, total exposure to absolute molar concentrations of cortisol, as demonstrated by AUC analysis, was significantly lower during the Cortitrol phase of the trial than the placebo phase (Fig. 2B insert). There was a significant increase in cortisol during EX compared to REST.

Corticotropin responses are presented in Fig. 3. There were no significant differences between the resting baseline measures. In general, there were no significant differences between REST values, although a significant difference was found at −20 (see Fig. 4A). No differences were observed in AUC between the 2 conditions (placebo: 127.6 ± 69.7 pmol/L; Cortitrol: 131.7 ± 73.3 pmol/L). Corticotropin responses to EX are presented in Fig. 4B. As expected, plasma corticotropin significantly increased in response to EX and concentrations were elevated above PRE values through +55. Cortitrol treatment conditions were lower than placebo at +5 and +10. Corticotropin was significantly greater during EX than during REST. Corticotropin AUC was significantly lower for Cortitrol than placebo (placebo: 233.1.6 ± 142.9 pmol/L; Cortitrol: 209.0 ± 117.2 pmol/L).

Fig. 4A shows the MDA responses during the REST. Significant differences were noted at +65. Fig. 4B presents MDA data for EX. MDA values at PRE, IP, and +65 for

Cortitrol were significantly lower than placebo. Cortitrol values were significantly lower during EX than REST. There was an exercise-induced increase in MDA for both Cortitrol and placebo at IP.

Table 2 presents the metabolic profile of plasma glucose and lactate. No significant changes were noted in either variable during the baseline days (V-1 and V-2) or REST. As expected, plasma glucose and lactate were significantly elevated above PRE values post-exercise. Glucose values did not return to PRE values until +45; lactate values remained significantly elevated above PRE values through +65. Plasma glucose values for EX were significantly higher than corresponding REST values from MID to +45. Plasma lactate values for EX were significantly higher than corresponding REST values from MID to +65.

Table 4
Mean (±SD) values for GH during resting control and exercise days

	GH (μg/L)	
	Placebo	Cortitrol
Resting control day		
Baseline		
V-1	0.49 ± 0.61	0.17 ± 0.19[a]
V-2	0.20 ± 0.23	0.13 ± 0.13
Pre-values		
−30	0.63 ± 1.67	0.93 ± 2.45
−20	1.03 ± 2.81	1.80 ± 4.04[a]
−10	0.96 ± 1.95	2.36 ± 4.84[a]
Pre	1.49 ± 2.56	2.42 ± 4.42[a]
Mid-values		
Mid	2.77 ± 3.93	2.36 ± 3.73[b]
Post-values		
IP	2.49 ± 3.12[b]	1.76 ± 3.01[b]
+5	2.31 ± 2.61[b]	1.63 ± 2.96[b]
+10	2.23 ± 2.41[b]	1.53 ± 2.82[b]
+15	2.35 ± 2.29[b]	1.44 ± 2.72[b,a]
+25	2.33 ± 2.24[b]	1.36 ± 2.32[b,a]
+35	2.40 ± 2.69[b]	1.25 ± 1.82[b,a]
+45	2.10 ± 2.31[b]	1.13 ± 1.52[a]
+55	1.79 ± 1.98[b]	0.98 ± 1.35[a]
+65	1.47 ± 1.53[b]	0.85 ± 1.15[a]
Exercise protocol		
Pre-exercise		
−30	1.36 ± 2.77	1.72 ± 4.41
−20	1.52 ± 2.85	2.17 ± 4.23
−10	1.97 ± 3.75	2.61 ± 4.62
Pre	2.08 ± 3.66	2.61 ± 4.28
Mid-exercise		
Mid	16.70 ± 13.92[b]	13.96 ± 17.57[b]
Post-exercise		
IP	26.83 ± 17.19[b]	25.39 ± 19.58[b]
+5	25.82 ± 17.40[b]	25.19 ± 19.08[b]
+10	22.91 ± 16.31[b]	22.92 ± 18.44[b]
+15	20.27 ± 14.70[b]	22.00 ± 18.31[b]
+25	16.90 ± 13.89[b]	17.90 ± 15.21[b]
+35	14.04 ± 13.14[b]	14.79 ± 13.94[b]
+45	11.61 ± 12.01[b]	12.71 ± 13.36[b]
+55	8.55 ± 8.48[b]	10.69 ± 13.05[b]
+65	6.50 ± 6.50[b]	8.04 ± 10.08[b]

[a] $P < .05$ from corresponding resting control day time point.
[b] $P < .05$ from corresponding pre-exercise and baseline values.

Androgen concentration values are shown in Table 3. Total testosterone, free testosterone, and DHEA all followed a similar response pattern. No differences between the 2 baseline days (V-1 and V-2) or during REST were apparent. During EX, no significant differences between conditions were observed over the pre-exercise time frame. Significant exercise-induced increases were observed from MID to +35 in both conditions. All post-exercise values were significantly higher than corresponding REST values. Placebo DHEA values were significantly lower than Cortitrol values during REST.

Table 4 presents immunoreactive GH values. No significant differences were observed over the baseline days or during REST for either condition. There were significant exercise-induced increases in GH during EX compared to REST. During EX, there were significant pre- to post-exercise increases in GH concentration.

4. Discussion

It should be clear that the primary purpose of this study was to examine the ability of Cortitrol to modulate cortisol concentrations resulting from performance of a resistance exercise physical stress model. How any reductions in cortisol would impact physiological adaptations in various target tissue (eg, muscle and cell) cannot be determined with this experimental design and requires further experimentation. Thus, we wanted to know if Cortitrol could influence the stress response. We know that cortisol plays important regulatory roles in various physiological functions and complete elimination of the catabolic response would not be optimal in homeostatic regulation. It is also important to understand that elimination of the cortisol response to physical stress would not be optimal to physiological adaptations. However, positive changes in muscle size development have occurred with reductions of cortisol over the first 8 weeks of resistance training [27]. Nevertheless, when cortisol is produced in excess, receptors beyond the primary targets are exposed to higher than normal molar concentrations of cortisol producing effects that may in fact be counterproductive to optimal tissue repair and remodeling (eg, reductions in immune cell activation in the attempt to conserve glycogen breakdown in the muscle yet inhibit repair with limited B-T-cell function and macrophage abilities to clean up degraded proteins in muscle). It is the chronic elevations of cortisol beyond normal concentrations that are most concerning to optimal physiological function and can have a negative impact on many target tissues including muscle, bone, and immune cells. We wanted to create an acute stress response with high concentrations of cortisol using our “stress model” of high-intensity resistance exercise to determine the influence of Cortitrol supplementation on the cortisol response. This was done to determine the impact of the dietary supplement on a known physiological stress response of exercise to gain data on its physiological efficacy but its role in adaptive responses

cannot be determined from this experimental design. Data from this investigation show that Cortitrol plays an important modulatory role in dramatically reducing cortisol release before, during, and immediately after intense resistance exercise (Fig. 2). Furthermore, the accumulated reduction in cortisol over the 95-minute block of time on both the REST and EX days suggests that Cortitrol reduces the total tissue exposure to cortisol. A reduction in free radical formation, as measured by plasma MDA values, was also observed between the treatment conditions during EX. Thus, this study gives initial insight into the influence of Cortitrol as a general modulator of the adrenal stress response.

The findings of this study should be viewed as the effects of a composite supplementation formula consisting of various nutritional attributes, which together, work to reduce physiological stress. This formulation consists of a general vitamin/mineral complex and a proprietary blend of herbal compounds with known anticortisol effects [8,9,13,16]. Both components may be necessary to mediate the observed cortisol reductions.

A dearth of information exists as to the role of nutritional supplementation to impact stress responses of cortisol. Vitamin C has been implicated in reducing cortisol responses to physical exercise [28]. In prior work using resistance exercise, Marsit et al [4] showed a subtle reduction in cortisol after supplementation with 1000 mg vitamin C 24 hours before exercise. The treatment regimen used in the present study provided 250 mg of vitamin C, suggesting that ascorbic acid may have been a partial contributor to the positive effects observed in resting cortisol concentrations. In addition, magnolia bark extract, because of its antioxidant capacity [29], and phosphatidylserine, because of its effects on neuroendocrine responses to exercise [14,15], may have also contributed to the reduction in cortisol.

It is interesting to note that Cortitrol attenuated the cortisol response exclusively during the pre-exercise and early recovery period during EX, whereas no differences were apparent from +10 to +65. Subjects typically display an anticipatory rise in cortisol before exercise because of psychological fear of intense physical challenge [19]. Inherent to this model was the use of formal practice of the exercise challenge to specifically familiarize each subject with the dramatic physical demands of the exercise session [18,30]. Cortitrol supplementation diminished the magnitude of the anticipatory response and reduced cortisol concentrations into early recovery. These subtle effects implicate a physiological mechanism that does not disrupt cortisol's important hormonal feedback roles in response to, and recovery from, exercise (eg, glucose metabolism).

The tropic hormone corticotropin demonstrated no significant differences in the stimulatory signals to steroidogenesis during REST. Pulsatility of corticotropin may exert a major trophic effect but we did not measure corticotropin pulsatility in this study. Owing to this limitation we calculated AUC to act as a composite exposure measure. Reduced corticotropin AUC for Cortitrol indicated subtle alterations of the tropic hormone signal during EX. These data are consistent with a lower hypothalamic-pituitary signal for cortisol production, supporting a mechanism related to the second component of Cortitrol: the proprietary herbal combination containing magnolia bark, L-theanine, *Epimedium*, phosphatidylserine, and β-sitosterol. In particular, phosphatidylserine may have attenuated the corticotropin response to EX. Work by Monteleone et al [14,15] showed that, in addition to reduced cortisol concentrations, phosphatidylserine supplementation attenuated the corticotropin response to physical stress in healthy men. Our data indicate that part of the cortisol stress response, which was altered by the Cortitrol treatment, was mediated by subtle changes in the secretion of the regulatory hormone corticotropin.

Of particular interest was the differential responses of the Cortitrol and placebo treatments with regard to oxygen reactive species formation, as measured by MDA concentrations (see Fig. 4). This is a novel and unique finding that may have dramatic ramifications because free radical production after exercise is known to produce chemical damage to various target tissues. Although speculative, the significant reduction in MDA could be due to one or more of the antioxidant components of Cortitrol. One of the naturally occurring bioactive derivatives identified in magnolia officialis is the biphenolic compound magnolol, which has been shown to be a potent antioxidant [29,31]. β-Sitosterol has also been shown to have antioxidant properties that protect against lipid peroxidation [32]. Some studies also show vitamin C increases serum oxygen-radical absorbance capacity [33-35]. Thompson et al [36] specifically reported a reduction in plasma MDA during recovery from exercise after vitamin C supplementation. The precise role of cortisol in mediating the reduction in MDA remains unclear. It is plausible that these other antioxidant compounds worked alone or in concert to reduce MDA independent of any effect on circulating cortisol. Alternatively, there is some indication that elevated cortisol increases lipid peroxidation [37], and thus the decrease in cortisol would be associated with lower MDA levels. This finding implicates the role of Cortitrol in tissue repair and recovery, as well as in adrenal stress reduction.

The responses of plasma glucose and lactate to EX were consistent between conditions. Dramatic increases in plasma lactate (>15 mmol/L) demonstrated the extreme metabolic stress response to the exercise challenge [38]. Bush et al [39] has shown that high force exercise produces large lactate and epinephrine responses, which in part stimulate further release of cortisol. Lactate is correlated to epinephrine responses after exercise stress [39]; however, Cortitrol had no impact on metabolic responses (Table 2). Our data suggest the mechanism of Cortitrol is related to the adrenal gland and not to energy metabolism.

Resistance exercise has been classically shown to increase hypothalamic-pituitary gonadal hormones [18,21].

In general, no significant changes in androgen concentrations were observed between Cortitrol and placebo conditions (see Table 3). However, significantly lower cortisol values resulted in reduced cortisol/DHEA and increased testosterone/cortisol ratio, which would indicate a greater anabolic environment during these time points [40]. Reduced cortisol/DHEA ratio has been observed previously in marathon runners supplementing with β-sitosterol before the competition. This was associated with decreased inflammatory response and cortisol concentrations during the marathon recovery period [16]. In our study, the impact of such a response on inflammatory cytokines and immune cells remains speculative. However, future research may wish to investigate this potential role of Cortitrol. Nevertheless, the nutritional supplement did not impact gonadal function, again supporting an adrenal stress mechanism of action. Obviously, in such a multivariate environment, cortisol is but one factor in the mechanisms involved in the repair and remodeling physiological process.

The Cortitrol and placebo conditions showed similar GH responses after exercise (Table 4) despite a dramatic decrease in plasma cortisol responses to the exercise and early recovery. Although beyond the scope of this study, future novel investigations are needed to determine if another aggregate or molecular variant of GH beyond the one that is typically measured using various immunoassays (22 kDa) is affected by cortisol. Supporting such research, Hymer et al [41] demonstrated that different sizes of GH and GH aggregates exist in different GH-producing cells. Band 1 cells (pituitary cells with a density of <1.071 g/cm^3) produce and secrete more 22-kDa GH than band 2 cells (density >1.071 g/cm^3), which produce more GH aggregates. They [41] showed that hydrocortisone treatment of band 1 and band 2 pituitary cells was most detrimental to GH formation in band 2 cells, thus impacting higher molecular weight GH aggregates and binding proteins. Thus, future studies are needed to understand cortisol's influence on other forms or aggregates of GH and their effects on target tissues.

The results of this study imply that long-term supplementation with Cortitrol may have a favorable impact on chronic cortisol levels. Thus, the potential exists for improved muscular adaptation to resistance training, increased bone mineral density, and enhanced immune cell function. However, this study is limited in that it examined only acute responses to Cortitrol supplementation. Future studies will need to further examine some of the positive adaptations that may occur from chronic reduction in cortisol concentrations after Cortitrol supplementation.

In conclusion, we investigated the impact of Cortitrol supplementation on serum cortisol responses to intense physical stress via acute resistance exercise. We found that Cortitrol attenuated mean cortisol concentrations throughout a 95-minute period during a day of rest, and during the pre-resistance exercise and early exercise recovery periods. These results give validity to the formulation of Cortitrol as a nutritional supplement. This investigation also opens the door to many avenues of future research.

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