Vitamin E reduces peroxisomal fatty acid oxidation and indicators of oxidative stress in untrained, exercised rats treated with dehydroepiandrosterone (DHEA)

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The protective effect of vitamin E (Vit E) on oxidative stress induced by dehydroepiandrosterone (DHEA) treatment in untrained, exercised, male Sprague-Dawley rats was investigated. Thirty-two rats were treated with DHEA (0 or 100 mg/kg body weight/d i.p) and/or Vit E (0 or 1 g/kg diet) for 5 weeks. Untrained rats were exercised for 1 hr on a motorized rodent treadmill immediately prior to being killed. DHEA treatment decreased ($P < 0.05$) weight gain and the weight of fat pads, but increased relative liver weight compared with control rats. DHEA-treated rats supplemented with Vit E had greater final body weights and fat pad weights than rats treated with DHEA alone. DHEA-treated rats had significantly lower hepatic glucose-6-phosphate dehydrogenase activity but higher malic enzyme activity than control rats. Hepatic peroxisomes from DHEA-treated rats had a two-fold higher rate of fatty acid oxidation compared with control rats. Vitamin E supplementation of DHEA-treated rats maintained peroxisomal fatty acid oxidation at a level similar to controls. The specific activities of enzymes that are involved in protection against oxidative stress, viz, glutathione (GSH)-reductase, GSH-transferase, and catalase were higher in DHEA-treated rats compared with control rats. Alanine aminotransferase and aspartate aminotransferase, indicators of tissue damage, were also higher in DHEA-treated rats compared with control rats. In general, indicators of oxidative stress and damage in DHEA-treated rats supplemented with Vit E were similar to those in control rats, suggesting that Vit E has a protective effect against oxidative stress in untrained, exercised rats treated with DHEA.

Keywords: dehydroepiandrosterone; vitamin E; oxidative stress; rats; fatty acid oxidation

Introduction

Exercise of sufficient duration and intensity can increase the production of reactive oxygen species (ROS) H_2O_2, O_2^- or OH^-, especially in untrained subjects.[1-3] The interaction of ROS with unsaturated fatty acids can promote lipid peroxidation. Exercise has been shown to increase the amount of lipid peroxidation and elevate catalase activity in liver.[1] Antioxidant enzyme systems, such as superoxide dismutase (SOD), catalase, and several glutathione-related enzymes, provide a defense against exercise-induced oxidative stress. These enzymes reduce and/or conjugate ROS to less electrophilic and more soluble compounds. Non-enzymatic antioxidants such as glutathione (GSH) and vitamin E (Vit E) also protect against oxidative stress.[3] It has been shown that strenuous exercise can increase lipid and protein oxidation, thereby enhancing GSH and Vit E utilization.[2,4] In contrast, Vit E supplementation reduces lipid peroxidation at rest and following acute exer-

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cise[2,5] by quenching ROS and by maintaining normal levels of cellular thiols such as GSH.[2,6,7]

Hypolipidemic agents such as the adrenal steroid dehydroepiandrosterone (DHEA) represent another potential source of ROS.[8,9] Pharmacological doses of DHEA reduce metabolic efficiency by enhancing oxidation of energy substrates and decreasing lipid synthesis.[8–14] DHEA may reduce energy storage as fat by promoting microsomal and peroxisomal oxidation-reduction reactions, which are not directly coupled to oxidative phosphorylation.[15–19] As with high intensity exercise, increased oxidation-reduction reactions induced by DHEA treatment may cause oxidative damage. Some of the metabolic consequences of DHEA treatment are therefore similar to those found with exercise.

The objective of this study was to investigate the effects of DHEA treatment on indicators of hepatic oxidative stress and damage in untrained, exercised, male Sprague-Dawley rats, and to determine whether supplementation with Vit E protects against such potentially adverse effects of DHEA. Rats were exercised at a relatively high intensity to maximize conditions for oxidative stress. Hepatic antioxidant defense systems such as GSH peroxidase (GSH-P), transferase (GSH-T), and reductase (GSH-R); catalase; and SOD served as indicators of oxidative stress. Plasma levels of alanine aminotransferase (ALT/GTP) and aspartate aminotransferase (AST/GOT) provided information on hepatic and cardiac tissue damage.

Methods and materials

Animals

Four groups of eight male Sprague-Dawley rats (Holtzman Labs, Madison, WI USA) with a mean initial weight of 170 g were used. Rats were housed individually in hanging wire-mesh cages in a room regulated for temperature (21 ± 2° C), humidity (45–50%), and light (lights on 06:00–18:00). Animals were cared for in accordance with guidelines established by the Institute of Animal Resources of the National Research Council. Food and water were always available. Food intakes and body weight were determined weekly.

The treatment groups were as follows: control, DHEA, Vit E, and DHEA + Vit E. All rats were fed a semi-purified diet consisting of (g/kg): glucose, 650; casein, 100; lactalbumin, 100; corn oil, 50; cellulose (Alphacel), 50; AIN-76 mineral mix, 40; and AIN-76 vitamin mix, 10. Diets supplemented with Vit E (d,l-alpha-tocopheryl acetate, 250 IU/kg) were formulated to contain 1 g/kg additional Vit E, which would provide approximately five times the Vit E requirement of growing rats throughout the study. Diet ingredients were purchased from U.S. Biochemical Corp., Cleveland, OH USA. DHEA-treated rats were injected i.p. each morning for 5 weeks with 0.35 mol/kg body weight DHEA (DHEA-acetate, Sigma Chemical Co., St. Louis, MO USA) suspended in a 4:1 (vol/vol) saline:Emulphor EL 620 (Rhone-Poulenc, Cranbury, NJ USA) mixture. Non-DHEA–treated rats were injected with the vehicle alone at a dosage of 1 mL/kg body weight.

Rats were acclimated to the motorized rodent treadmill 2 days/wk, running 10 min/day at 10–15 m/min for 2 weeks. Just prior to being killed, rats were run for 1 hr at 21.5 m/min up a 12% grade. If a rat was unable to complete the 1 hr exercise run, time was recorded and the animal was immediately killed.

Sample collections

After an overnight fast and immediately after running for 1 hr, rats were killed by decapitation and truncal blood collected in chilled tubes containing EDTA. The plasma was harvested after centrifugation (4° C, 1640 × g, 25 min), dispensed into 1500-μL aliquots and frozen at −70° C. The liver was quickly excised then weighed in a tared ice-cold beaker containing the homogenization buffer (0.32 mol/L sucrose, 0.01 mol/L Tris-HCl, 1 mmol/L EDTA, pH 7.2). The epididymal and retroperitoneal fat pads were excised and weighed.

The liver was homogenized in four volumes of buffer with three downward strokes of a Potter-Elvehjem pestle (Kontes, Vineland, NJ) in a 30 mL ice-cold glass mortar powered by a 3/8 hp Craftsman drill (Sears Co, Greensboro, NC). Subcellular fractions were isolated by differential centrifugation in a Beckman (Beckman Instruments, Palo Alto, CA) high speed (J2-21) and ultra-centrifuge (L7-65) following an initial pelleting of nuclei and debris at 1000 × g for 10 min, 4° C, as follows: mitochondria and peroxisomes were obtained in the 10,000 × g, 20 min, 4° C pellet; microsomes (pellet) and cytosol (supernatant) were obtained by centrifuging the 10,000 × g supernatant at 100,000 × g for 60 min, 4° C.

Enzyme activities, plasma metabolites and body compositions

Plasma triglyceride and cholesterol levels and the activities (units/L plasma) of ALT/GTP and AST/GOT were determined spectrophotometrically using commercial kits (Sigma Diagnostic Procedures No. 336, 352, 59UV, and 58UV, respectively). The protein content of the liver fractions was determined according to the procedure of Bradford using a commercially available kit (Bio-Rad Protein Assay Kit, Bio-Rad Laboratories, Richmond, CA USA) and bovine serum albumin as standard. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and malic enzyme (EC 1.1.1.40) activities [units/(mg protein · min)] were determined spectrophotometrically in the cytosolic fraction as previously described.[20] Glutathione reductase (EC 1.6.4.2) activity [μmol/(mg protein · min)] was measured in the cytosolic fraction using oxidized glutathione as substrate and following the oxidation of NADPH spectrophotometrically at 340 nm.[21] Glutathione transferase (EC 2.5.1.18) activity [nmol/(mg protein · min)] was measured in the cytosolic fraction using 1 chloro-2,4-dinitrobenzene as substrate and monitoring the increase in absorbance at 340 nm.[22] Glutathione peroxidase (EC 1.11.1.9) activity [μmol/(mg protein · min)] was determined in an enzyme-coupled reaction in the 10,000 × g pellet and supernatant using H_2O_2 (selenium-dependent activity) and cumene hydroperoxide (total activity) as substrates by measuring the oxidation of NADPH at 340 nm.[23] Catalase activity [nmol/(mg protein · min)] was measured in the 10,000 × g pellet by measuring the disappearance of H_2O_2 spectrophotometrically at 240 nm.[24] Manganese-SOD (10,000 × g pellet) activity [μmol/(mg protein · min)] was determined spectrophotometrically by measuring the auto-oxidation of pyrogallol at 420 nm.[25] Peroxisomal palmitoyl-CoA oxidation [μmol/(mg protein · min)] was determined in the 10,000 × g pellet in the presence of KCN by measuring the reduction of NAD at 340 nm.[26] The liver lipid content was analyzed by gravimetric methods as previously described.[13]

Table 1 The effects of dehydroepiandosterone (DHEA) and vitamin E (Vit E) on body and tissue weights in exercised, male Sprague-Dawley rats*

Measurements	Control	DHEA	DHEA + Vit E	Vit E
Final body weight, g	430 ± 10^{a}	357 ± 11^{c}	396 ± 10^{b}	402 ± 10^{ab}
Relative liver weight, g/100g BW†	2.9 ± 0.2^{b}	3.5 ± 0.2^{a}	3.4 ± 0.2^{ab}	2.9 ± 0.2^{b}
Liver lipid content, g/100g	10.2 ± 2.0	14.1 ± 2.0	11.4 ± 2.0	8.7 ± 2.6
Epididymal fat pad weight, g	6.6 ± 0.5^{ab}	4.2 ± 0.5^{c}	5.8 ± 0.5^{b}	7.3 ± 0.5^{a}
Retroperitoneal fat pad weight, g	9.0 ± 0.5^{a}	4.4 ± 0.6^{c}	6.6 ± 0.5^{b}	8.5 ± 0.5^{a}

*Least-square means ± SEM within the same row not sharing a common superscript are significantly different ($P < 0.05$).
†Relative liver weight = g liver/100 g body weight.

Statistical analyses

Data were analyzed by least squares analysis of variance using the General Linear Models Procedure of The SAS System (SAS, Cary, NC USA). Differences between treatment means were considered significant at the $P < 0.05$ level.[27] Data are presented as arithmetic means ± the standard error of the mean.

Results

DHEA treatment significantly reduced final body weight and epididymal and retroperitoneal fat pad weights *(Table 1)* without affecting relative food intake (8.3 ± 0.1 g/100 g body weight). DHEA-treated rats supplemented with Vit E had greater final body weights and fat pad weights than rats treated with DHEA alone. DHEA treatment increased relative liver weight (g/100 g body weight) and hepatic malic enzyme activity while reducing glucose-6-phosphate dehydrogenase activity compared with control rats *(Table 2)*. Liver lipid content and plasma levels of triglycerides and cholesterol were not affected ($P > 0.05$) by treatment.

Specific activities of GSH-T, GSH-R, and catalase were 15, 23, and 100% higher, respectively, in rats treated with DHEA than in control rats *(Table 2)*. In contrast, DHEA-treated rats supplemented with Vit E had GSH-T, GSH-R, and catalase activities similar to controls. Total and selenium-dependent GSH-P and SOD activities were higher in rats supplemented with Vit E alone than in DHEA-treated rats supplemented with Vit E.

Because increased hepatic peroxisomal fatty acid oxidation is associated with oxidative damage and hepatocarcinogenesis due to generation of H_2O_2, the impact of DHEA and Vit E treatments on peroxisomal fatty acid oxidation were examined. Peroxisomal palmitoyl-CoA oxidation was approximately two-fold higher in DHEA-treated rats compared with control rats *(Figure 1)*. In contrast, supplementation of DHEA-treated rats with Vit E maintained peroxisomal fatty acid oxidation at a level similar to controls. To determine if DHEA-mediated increases in indicators of oxidative stress were associated with damage to tissues, ALT and AST activities in plasma were examined. Rats treated with DHEA had 21 and 63% higher activities of plasma AST and ALT, respectively, than control rats *(Figure 2)*. In contrast, supplementation of DHEA-treated rats with Vit E maintained AST and ALT activities at a level similar to controls. These results suggest that Vit E reduces oxidative damage induced by DHEA treatment in exercised rats by maintaining peroxisomal fatty acid oxidation at control levels, thereby limiting the production of ROS.

Discussion

The results of this study support the hypothesis that chronic treatment with pharmacological levels of DHEA in untrained, exercised rats increases oxidative stress, causing tissue damage and that Vit E supplementation alleviates this adverse response. Catalase activity, which converts H_2O_2 to H_2O, was increased two-fold by DHEA treatment, and Vit E supplementation maintained catalase activity at a level similar to controls. GSH-R activity, which reduces oxidized GSH, was increased by DHEA treatment and Vit E supplementation blocked this increase in GSH-R activity. GSH-T, which conjugates electrophiles and steroids, was increased ($P < 0.12$) by DHEA treatment and Vit E supplementation maintains GSH-T activity at a level similar to controls. Because the liver is the major site for steroid reduction and conjugation, hepatic GSH levels and antioxidant status may be compromised by treatment with DHEA at pharmacological levels. Vit E supplementation may alleviate this stress by quenching ROS and other metabolites of DHEA metabolism.

We attempted to increase the production of ROS in our animals in this study using a normal physiological process, such as exhaustive exercise, as demonstrated by Alessi and Goldfarb,[1] because we[9] had previously found that DHEA stimulated indicators of oxidative stress in sedentary animals, and that Vit E tended to prevent this response. Indeed, exercised control animals in this study had 4, 10, 10, 17, and 36% higher activities of SOD, GSH-T, catalase, AST, and ALT, respectively, than sedentary control animals in our previous study, which was conducted under identical experimental conditions.[9] Exercised DHEA-treated animals in this study had 15, 25, and 65% higher activities of SOD, peroxisomal fatty acid oxidation, and catalase, respectively, than sedentary DHEA-treated animals in our previous study.[9] In general, sedentary and exercised DHEA-treated rats supplemented with Vit E had lower activities of GSH-T, catalase, ALT, and AST than rats

Table 2 The effects of dehydroepiandrosterone (DHEA) and Vitamin E (Vit E) on hepatic lipogenic enzymes and enzymes that protect against oxidative stress in exercised,male Sprague-Dawley rats*

Measurements	Control	DHEA	DHEA + Vit E	Vit E
GSH-R†, μmol/(mg·min)	73 ± 3^{b}	90 ± 3^{a}	71 ± 3^{b}	80 ± 3^{b}
GSH-T‡, nmol/(mg·min)	808 ± 53^{bc}	931 ± 56^{ab}	739 ± 56^{c}	969 ±61^{a}
Total GSH-P§, μmol/(mg·min)	40 ± 3^{b}	32 ± 3^{bc}	28 ± 3^{c}	49 ± 3^{a}
Se-Dep. GSH-P‖, μmol/(mg·min)	27 ± 2^{ab}	22 ± 2^{bc}	21 ± 2^{c}	30 ± 2^{a}
Catalase¶, nmol/(mg·min)	897 ± 97^{b}	1780 ±104^{a}	976 ±91^{b}	1087 ± 104^{b}
SOD**, μmol/(mg·min)	1168 ± 48^{ab}	1057 ± 45^{b}	1041 ± 45^{b}	1212 ± 48^{a}
G6PD§§, units/(mg·min)	6.2 ± 0.5^{a}	3.6 ± 0.5^{b}	3.2 ± 0.5^{b}	6.8 ± 0.5^{a}
ME¶¶, units/(mg·min)	2.7 ± 0.3^{b}	3.6 ± 0.3^{a}	3.0 ± 0.3^{ab}	2.5 ± 0.3^{b}

*Least-square means ± SEM within the same row not sharing a common superscript are significantly different ($P < 0.05$).
†Glutathione reductase (GSH-R) activity per mg protein.
‡Glutathione transferase (GSH-T) activity per mg protein.
§Total glutathione peroxidase (GSH-P) activity per mg protein.
‖Se-dependent gluthathione peroxidase (GSH-P) activity per mg protein.
¶Catalase activity per mg protein.
**Manganese superoxide dismutase (SOD) activity per mg protein.
§§Glucose-6-phosphate dehydrogenase (G6PD) activity per mg protein.
¶¶Malic enzyme (ME) activity per mg protein.

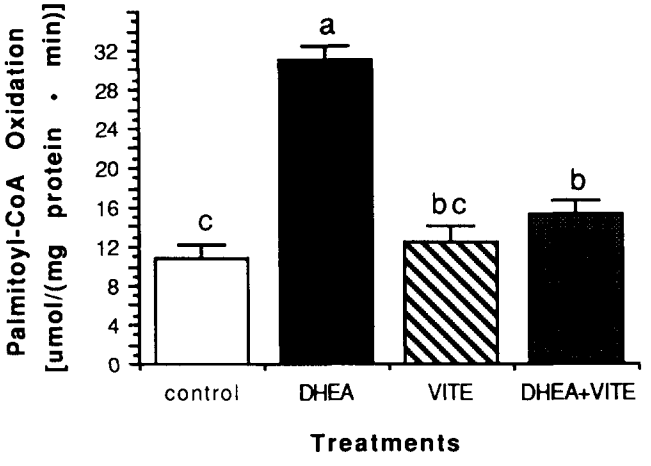

Figure 1 The effects of dehydroepiandrosterone (DHEA) and vitamin E (Vit E) on hepatic peroxisomal palmitoyl-CoA oxidation in exercised, male Sprague-Dawley rats. Bars represent means + SEM for each treatment group ($n = 8$). Bars not sharing a common superscript are significantly different ($P < 0.05$).

treated with DHEA alone. However, because there were no non-exercised control animals in the present study, the effect that exercise may have on indices of oxidative stress in animals treated with or without DHEA and Vit E can only be extrapolated and not confirmed. Our purpose for subjecting all four groups of animals to an acute bout of exhaustive exercise in this study was to maximize conditions in which oxidative stress occurs and test the effects of DHEA and Vit E under these conditions.

A protective effect of Vit E on GSH depletion and enzymes associated with GSH utilization during oxidative stress has been demonstrated.[3,6,7,28] Gohil et al.[4] demonstrated that submaximal physical exercise depleted GSH levels in blood, thereby lowering the GSH antioxidant system and resistance to oxidative damage. Sumida et al.[5] observed that Vit E supplementation prevented increases in serum indicators of oxidative damage following acute exhaustive exercise. Therefore, not only antioxidant enzymes, but non-enzymatic antioxidants such as GSH and Vit E may be utilized at an increased rate by exhaustive exercise or DHEA treatment, thereby jeopardizing normal pool levels of antioxidants.

The relationship between peroxisomal fatty acid oxidation and oxidative damage is thought to be due to the cytotoxic effects of H_2O_2. The activities of the aminotransferases ALT and AST, plasma indicators of oxidative damage from liver and heart, were increased by DHEA treatment. High fat diets and certain hypolipidemic agents, which have been linked to lipid peroxidation and hepatocarcinomas, increase hepatic peroxisomal beta-oxidation.[29] DHEA-treated rats supplemented with Vit E had ALT and AST activities similar to control rats, suggesting that Vit E protected against oxidative damage induced by DHEA by blocking the increase in peroxisomal fatty acid oxidation. This speculation is supported by Hennig et al.,[30]

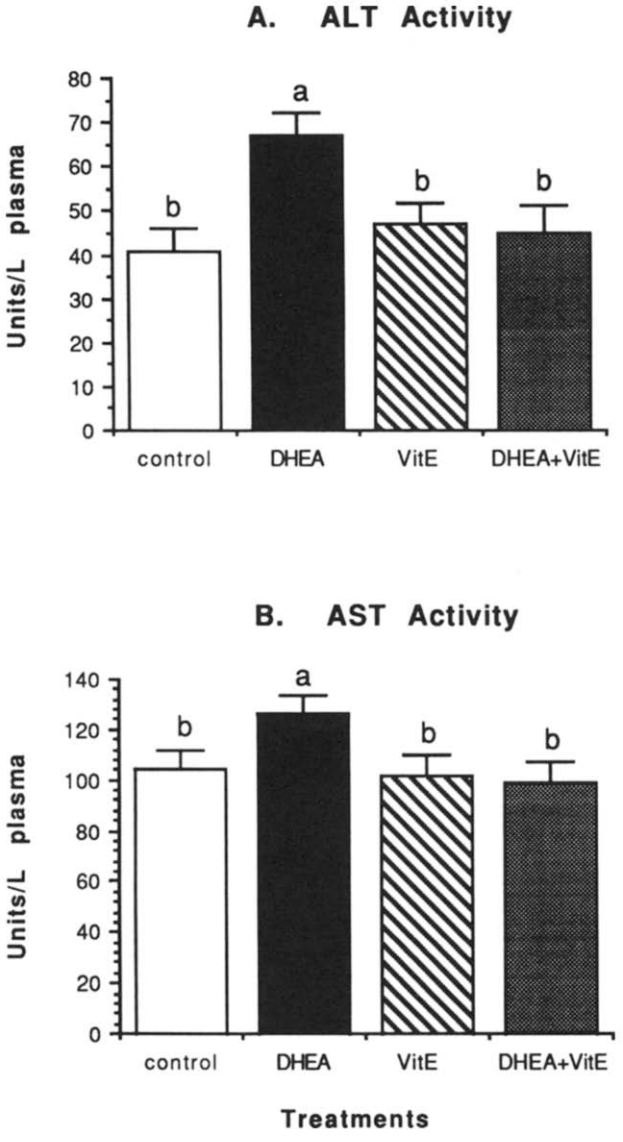

Figure 2 The effects of dehydroepiandrosterone (DHEA) and vitamin E (Vit E) on plasma alanine aminotransferase (ALT) (panel A) and aspartate aminotransferase (AST) (panel B) in exercised, male Sprague-Dawley rats. Bars represent means + SEM for each treatment group (n = 8). Bars not sharing a common superscript within each panel are significantly different ($P < 0.05$). One unit of ALT and AST activityis defined as the amount of enzyme that produces 1 mole of NAD per minute under the conditions of the assay procedure.

who demonstrated that the amount and type of fat that cells are exposed to affects both peroxisomal beta-oxidation and catalase activity. Peroxisomal beta-oxidation and catalase activity were greatest in endothelial cells exposed in vitro to the highest concentration of lipid and when the lipid source was linoleic acid compared with stearic, oleic, or linolenic acid. This stimulation of peroxisomal beta-oxidation and catalase activity by linoleic acid was prevented by supplementation with Vit E.[31] Similarly, we demonstrated that Vit E prevented the increase in peroxisomal beta-oxidation and catalase activity induced by DHEA treatment. We attempted to measure the amount of lipid peroxidation in liver homogenates using the thiobarbituric acid (TBA) procedure but were unsuccessful because our homogenization buffer contained sucrose, which interferes with the formation of the TBA-malondialdehyde complex.

The results of this study support the hypothesis that chronic administration of pharmacological levels of DHEA reduces weight gain and energy storage as fat, in part by enhancing hepatic peroxisomal fatty acid oxidation. Unlike mitochondrial fatty acid oxidation that reduces FAD^+, the first step of peroxisomal beta-oxidation couples the oxidation of fatty acids with the reduction of oxygen to form H_2O_2. This lowers metabolic efficiency due to a net loss of 2 ATP (1 FADH2) per cycle of beta-oxidation in peroxisomes. In addition, ROS generated by peroxisomal beta-oxidation cause oxidative damage and have been implicated in hepatocarcinogenesis.[3]

The actual mechanism by which DHEA increases peroxisomal fatty acid oxidation and indicators of oxidative stress and how Vit E supplementation prevents these events is not known. Tagliaferro et al. demonstrated that DHEA treatment in vivo stimulated whole body respiration[32] and adipose tissue lipolysis.[33,34] We have previously shown that DHEA treatment in vivo stimulated whole body respiration[35] and hepatocyte glucose oxidation,[8] and reduced fatty acid synthesis.[35] Together, these studies suggest that DHEA functions as an antiobesity agent by promoting peripheral lipid mobilization and hepatic uptake, thereby increasing substrate availability for beta-oxidation to meet the energy demands of steroid degradation. Such increased uptake of lipid in the liver would induce peroxisomal proliferation as previously reported[19,29–31,36] because peroxisomes chain-shorten long chain fatty acids for subsequent mitochondrial oxidation. Vit E may reduce this partitioning of lipids from periphery to liver by directly quenching ROS or reducing metabolites of DHEA degradation in the liver. In the present study, final body weights and fat pad weights were greater in DHEA-treated rats supplemented with Vit E compared with rats treated with DHEA alone, suggesting that Vit E may reduce overall energy utilization associated with DHEA degradation in the liver.

Together our data demonstrate that (1) high doses of DHEA stimulate hepatic peroxisomal fatty acid oxidation and indicators of oxidative stress and damage in untrained, exercised rats and (2) supplementation with Vit E alleviates these DHEA-mediated events. Studies to determine the direct versus indirect effects of DHEA on hepatocyte energy metabolism and how Vit E alters these effects are necessary to understand the mechanism of action of this antiobesity steroid.

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