The observation that the secondary injury at 3 days was almost completely blocked by prior treatment with a known antioxidant, polyethylene glycol-superoxide dismutase (PEG-SOD), supported a role of free radicals in the generation of the secondary injury (24). In contrast to the efficacy of PEG-SOD, the ability of vitamin E to protect muscles from contraction-induced injury has been much less predictable. In muscle fibers, vitamin E has the potential to act as a lipid-soluble antioxidant and to stabilize membranes (21). After vitamin E supplementation, whole body pliometric exercise by rats and humans has produced quite variable results in terms of its effect on the different indexes of muscle damage. For subjects who took vitamin E orally and subsequently ran downhill on a motor-driven treadmill, Meydani et al. (16) reported a protective effect based on a decreased activity of creatine kinase (CK) in the serum of the vitamin E-treated group at 5 days. In contrast, Jakeman and Maxwell (13) observed no effect of oral vitamin E treatment on either force deficit or the serum activity of CK during 12 days after box stepping. After downhill running performed by rats given a dietary supplement of vitamin E and by untreated rats, the vitamin E-treated rats showed no differences compared with untreated rats in the serum activity of CK, the force deficit, or the number of intact fibers per square millimeter in soleus muscles (23). In the latter study (23), a full interpretation is prevented by the lack of any knowledge of the recruitment or loading of the soleus muscle during downhill running, the small 20% force deficit, and the absence of any direct morphological data. Consequently, our purpose was to determine the role of vitamin E in both the initial and secondary aspects of contraction-induced injury.

The in situ extensor digitorum longus (EDL) muscle preparation of the mouse provides an accurate and reproducible model system for investigations of the full time course of the changes in force deficit and morphological damage with knowledge of the strain, average force, and number of contractions for a single muscle (4, 15). Despite the advantages of the preparation, the 8-mg mass of the mouse EDL muscle provides insufficient mass for the biochemical assays that we wished to undertake for investigations of free radical activity and the effects of vitamin E. Consequently, we developed an in situ EDL muscle preparation in the rat, which has a 15-fold greater muscle mass than does the mouse. We assumed that maximally activated EDL muscles in rats exposed to 225 stretches through a 20% strain would sustain a force deficit 3 days after the pliometric
The rats were anesthetized with pentobarbital sodium daily injections for 5 days. Control groups, with 5 rats in each treatment group, received (65 mg/100 g body mass ip). Additional anesthetic was administered periodically with caliper measurements of bundles of fibers dissected from the EDL muscle.

During each protocol of pliometric contractions, EDL muscles were set at L_o and then activated with a stimulation frequency of 100 Hz every 4 s. This produced an isometric contraction ~90% of P_o. The isometric contraction was held for 200 ms, and then the activated muscle was stretched through a strain of 20% relative to L_o at a velocity of 1.5 L/s. The stimulation ceased at the end of the stretch, and the relaxed muscle was returned to L_o. Each protocol consisted of three 5-min bouts of 75 contractions, with a recovery period of 5 min between bouts. After a rest period of 30 min, the P_o was measured again. The anesthetized rats were then removed from the apparatus, the incisions in the fascia and skin were closed separately, and the rats were placed in recovery cages.

For the untreated rats, the specific effects of pliometric contractions were compared with other types of contractions. Isometric contractions (n = 3) or stretches of passive muscles (n = 3) were substituted for the pliometric contractions but with the same level of activation, train rates, number of contractions, and rest periods used during the pliometric contraction protocol.

Evaluation of P_o. For the untreated rats, the P_o values of the experimental and the contralateral control EDL muscles were measured at 1 day (n = 6), 3 days (n = 8), 7 days (n = 7), 21 days (n = 7), or 42 days (n = 6) after the pliometric contraction protocol, whereas, after protocols of isometric contractions or stretches of passive muscles, the P_o values of the experimental and the contralateral control muscles were measured only at 3 days. For all vitamin E-treated and vehicle-treated rats, the P_o values of the experimental EDL muscles were determined at either 3 h or 3 days. For EDL muscles in each of the three groups, the P_o values measured after the contraction protocol are presented as a force deficit (Fig. 1). For P_o values measured during the first 7 days after a contraction protocol, the force deficit is the postprotocol P_o expressed as a percentage of the preprotocol P_o. For the untreated rats, an increase in body mass, muscle mass, and P_o occurred during the 6 wk of recovery. Consequently, after 7

**METHODS**

Animals. The experiments for the validation of the pliometric contraction protocol for EDL muscles were conducted on 43 Wistar rats that were 4 mo of age, and the comparisons of vitamin E treatment and vehicle treatment were performed on 33 Wistar rats that were 3 mo of age. The specific-pathogen-free rats were housed in a barrier facility in the Unit for Laboratory Animal Medicine at the University of Michigan. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

A preliminary study was designed to optimize the dosages and durations of the intravenous injections of α-tocopherol in ethanol. Five days of intravenous injections produced a stable muscle vitamin E content comparable to that achieved with 5 wk of dietary supplementation. The 5 days of vitamin E treatment produced increases in the vitamin E content of 2.0-fold in EDL, 2.8-fold in gastrocnemius, and 3.2-fold in soleus muscles. For the comparison of treatments, the rats (n = 33) were randomly assigned to a vitamin E-treated group (n = 18) injected intravenously with 200 µl of a 70% (wt/vol) solution of α-tocopherol in ethanol and a vehicle-treated group (n = 15) injected intravenously with an equivalent volume of ethanol. Thirteen vitamin E-treated and 10 vehicle-treated rats received daily injections into a tail vein for 5 days before the administration of the pliometric contraction protocol and were then tested at 3 h afterward (n = 6 and 5, respectively) on an additional day 5 days after the protocol, and then tested (n = 7 and 5, respectively). Nonexercised control groups, with 5 rats in each treatment group, received daily injections for 5 days.

Pliometric contraction protocol for situ EDL muscles of rats. The rats were anesthetized with pentobarbital sodium (65 mg/100 g body mass ip). Additional anesthetic was administered to maintain a depth of anesthesia that prevented responses to tactile stimuli. The distal tendon of the left EDL muscle was dissected free, and the peroneal nerve was exposed. The rat was placed on its side on a platform. The leg was attached securely to the platform by pinning the knee and tapping the foot. The body temperature was maintained at 37°C by a heating pad. The intact distal tendon of the EDL muscle was attached with a clamp to the arm of a servomotor-force transducer (model 6650, Cambridge Technology). An electrode was inserted under the peroneal nerve. Voltage and force transducer (model 6650, Cambridge Technology). An electrode was inserted under the peroneal nerve. Voltage and force transducer (model 6650, Cambridge Technology). An electrode was inserted under the peroneal nerve. Voltage and force transducer (model 6650, Cambridge Technology). An electrode was inserted under the peroneal nerve. Voltage and force transducer (model 6650, Cambridge Technology). An electrode was inserted under the peroneal nerve. Voltage and
days, the force deficit was calculated from the $P_o$ of the contralateral control muscle.

Muscle and blood sampling. After the final measurements of force development in situ, with the EDL muscle completely exposed, the $L_f$ was measured. Subsequently, both EDL muscles were removed from the rats. The estimated $L_f$ and the measured $L_f$ were within 3% of one another. The whole EDL muscles of untreated rats and the distal one-quarter of EDL muscles of vitamin E-treated and vehicle-treated rats were blotted, weighed, and frozen in isopentane cooled with dry ice for histological analysis. The remaining parts of the EDL muscles were frozen rapidly in liquid nitrogen for the additional biochemical assays. From each vitamin E-treated and vehicle-treated rat, part of the gastrocnemius muscle was removed and frozen rapidly in liquid nitrogen for vitamin E analysis. The gastrocnemius muscle was used for measurements of the muscle vitamin E content because the total masses of the exercised and control EDL muscles were required for biochemical analyses. Five milliliters of blood were removed from the abdominal aorta to determine serum activities of the muscle-derived enzymes, CK and PK. The blood was centrifuged at 500 $g$, and the serum was removed and stored at $-70^\circ$C. The anesthetized rat was euthanized with an overdose of anesthetic.

Muscle histology. Our direct assessments of muscle damage were made at 1 h in untreated rats ($n = 3$) and at 3 days in untreated, vehicle-treated, and vitamin E-treated rats. For the assessment, cross sections were cut from each muscle and stained with hematoxylin and eosin. In single sections of control and experimental muscles, the number of damaged fibers was determined with a Leitz image analyzer at a magnification of $\times 100$. Fibers were classified as intact or damaged. A damaged fiber was defined as a fiber that showed excessive swelling, degenerative changes in the cytosol, or no cytosolic elements with only a basement membrane remaining (15, 24). A fiber was classified as intact if no evidence of damage was observed. The number of damaged fibers was expressed as a percentage of the total number of fibers present in the cross-sectional area.

Biochemical analyses. The serum CK and PK activities were determined spectrophotometrically in the serum of nonexercised, vitamin E-treated, and vehicle-treated rats as described previously (14). The vitamin E content of samples of the gastrocnemius muscles was determined by using high-performance liquid chromatography techniques as described by Phoenix et al. (20).

Definition of damage to muscles. Following protocols of pliometric contractions, the severity of damage has been assessed by a variety of morphological, biochemical, and functional measures (8). We have assessed the magnitude of the damage with a direct measure of the number of damaged fibers observed with light microscopy of sections stained with hematoxylin and eosin and with an indirect measure of the force deficit. Neither variable provides an exact measure of the damage with a direct measure of the number of damaged fibers observed with light microscopy of sections stained with hematoxylin and eosin and with an indirect measure of the force deficit. Neither variable provides an exact measure of the force deficit. Neither variable provides an exact measure of the force deficit. Neither variable provides an exact measure of the force deficit.

<table>
<thead>
<tr>
<th>Table 1. Structural and functional measurements of EDL muscles of untreated, vehicle-treated, and vitamin E-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats</strong></td>
</tr>
<tr>
<td>Muscles</td>
</tr>
<tr>
<td>Body mass, g</td>
</tr>
<tr>
<td>Muscle mass, mg</td>
</tr>
<tr>
<td>Muscle length, cm</td>
</tr>
<tr>
<td>Fiber length, mm</td>
</tr>
<tr>
<td>CSAf, mm²</td>
</tr>
<tr>
<td>$P_o$, N</td>
</tr>
<tr>
<td>$sP_o$, KN/m²</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$; no. of rats. Measurements of untreated rats are performed on control contralateral muscles of rats from which recovery time of experimental muscles was 3 days or longer. EDL, extensor digitorum longus; CSAf, total fiber cross-sectional area; $P_o$, maximum isometric tetanic force; $sP_o$, normalized for CSAf; ND, not determined. CSA of muscle was calculated from the following equation: muscle mass (mg) × (mm) $\times 1.06$ (mg/mm²), where $L_f$ is fiber length and $1.06$ mg/mm² is density of skeletal muscle. *Significant difference compared with vehicle-treated and vitamin E-treated rats, $P < 0.05$. 

RESULTS

For EDL muscles measured in situ in untreated, vehicle-treated, and vitamin E-treated 3- to 4-mo old Wistar rats, the absolute $P_o$ values were 3.7, 3.2, and 3.3 N, respectively. The values for the $P_o$ were in good agreement with previously published data on the EDL muscles of young Wistar rats measured in vitro (6). The higher absolute body masses, muscle lengths, and absolute $P_o$ values of the 4-mo-old untreated rats compared with the 3-mo-old treated rats simply reflect the normal growth of the rats during the 1-mo period (Table 1). Compared with the vehicle-treated and the vitamin E-treated groups, the slightly higher values for the absolute forces of the untreated group were proportional to the increases in body masses. The specific $P_o$ of 257 kN/m² for the EDL muscles of young treated rats measured in situ (Table 1) is not different from the
values reported for EDL muscles in young mice measured in situ (4, 10) and in young rats measured in vitro (6). Specific P₀ values could not be calculated for the treated EDL muscles because of the need to immediately freeze the muscles for biochemical analyses.

The pooled value for the vitamin E content of the gastrocnemius muscles of the vehicle-treated rats (n = 15) was 5.2 ± 0.6 µg/g wet wt and that of the vitamin E-treated rats (n = 18) was 14.7 ± 3.3 µg/g wet wt. No difference was observed between the values for the vitamin E-treated rats at 3 h (11.9 ± 2.5 µg/g wet wt) and 3 days (8.7 ± 2.0 µg/g wet wt). Our values for the vitamin E content of muscles from vehicle-treated and vitamin E-treated rats were slightly lower than the values for rats treated with 5 wk of dietary supplementation (23). Despite the lower absolute values, our value for a threefold increase after vitamin E treatment was in excellent agreement with the relative increase reported after dietary supplementation (23). We conclude that we have collected valid data on force and achieved adequate vitamin E supplementation.

In untreated rats, during the pliometric contraction protocol, the force deficit during the last pliometric contraction was 94 ± 1% (Fig. 1). By 30 min after the pliometric contraction protocol, the force deficit was 63 ± 2%. The force deficits were 54 ± 3%, 64 ± 7%, and 55 ± 9% at 1, 3, and 7 days, respectively. No differences were observed among the force deficits between 30 min and 7 days. Thereafter, the force deficit diminished gradually, reaching control values by 42 days. Thirty minutes after the protocols of isometric contractions and stretches of passive muscles, the force deficits were 14 ± 5 and 4 ± 4%, respectively. In contrast to the substantial force deficit after the pliometric contraction protocol, 3 days after either isometric contractions or stretches of passive muscles, the force deficits were not different from zero, with the P₀ values 100 ± 4 and 103 ± 3% of their respective control values. These observations on EDL muscles of rats are in agreement with the reports of McCully and Faulkner (15) and Faulkner et al. (10), who found that protocols of isometric contractions and of stretches of passive EDL muscles of mice, respectively, result in an immediate small force deficit due to fatigue but do not cause injury.

For the vehicle-treated and vitamin E-treated rats, no differences between the groups were observed in the force deficits developed at any time during the 5-min bouts of pliometric contractions. The force deficits at the end of the protocol, at 30 min, and at 3 days were not different among the three groups. In addition, no differences were observed between the force deficits of the vehicle-treated and vitamin E-treated rats at 3 h (Fig. 1).

For the contralateral control EDL muscles in each of the three groups (Fig. 2A) and in the EDL muscles 1 h after the pliometric contraction protocol (Fig. 2B), cross sections stained with hematoxylin and eosin did not show abnormalities. In contrast, by 3 days after the pliometric contraction protocol was administered to untreated, vehicle-treated, and vitamin E-treated rats, 38 ± 5, 32 ± 5, and 29 ± 5%, respectively, of the fibers were classified as damaged (Table 2, Fig. 2, C–E). By 21 and 42 days, the number of intact fibers in the experimental muscles of the untreated rats was not different from the control value, but 5% of the fibers had central nuclei.

For the nonexercised rats, the serum CK activities of the vehicle-treated and vitamin E-treated rats were not different, but the serum PK activity of the vitamin E-treated rats was twofold greater than that of the vehicle-treated rats (Fig. 3). After the pliometric contraction protocol, the vehicle-treated rats showed a fourfold increase in the serum CK and PK activities at 3 h and a twofold increase at 3 days, whereas at both 3 h and 3 days after the protocol, the serum CK and PK activities of the vitamin E-treated rats were not different from those of the nonexercised rats (Fig. 3).

**DISCUSSION**

Pliometric contraction protocol for in situ muscles of rats. Despite a 15-fold difference in muscle mass, contraction-induced injury to in situ EDL muscles in rats was not dramatically different qualitatively or quantitatively from the more extensively investigated phenomenon in the in situ EDL muscles of mice (4, 5, 10, 15, 24). As with EDL muscles of mice, the recovery from fatigue was rapid and essentially complete by 3 h (10). Unlike the EDL muscles of mice, which display a substantial secondary increase in force deficit at 3 days (10), the plateau in the force deficit for EDL muscles of rats is similar to the pattern observed previously for muscle groups in humans (7, 17). The prolonged week-long plateau in the force deficit likely reflects different overlapping time courses for populations of fibers in terms of the onset of and recovery from injury.

For muscles of all species, between the initial injury and day 2, despite the substantial force deficits and electron-microscopic evidence of focal damage to single sarcomeres (5, 18), light-microscopic analysis of cross sections stained with hematoxylin and eosin showed no evidence of damaged fibers (10). In contrast, between 2 days and 5 days after contraction-induced injury, dramatic increases occur in the percentage of damaged fibers visible with light microscopy, the efflux of en-

<table>
<thead>
<tr>
<th>Time of</th>
<th>Total Number</th>
<th>Number of</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissection</td>
<td>of Fibers</td>
<td>Damaged Fibers</td>
<td>Damaged Fibers</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated rats</td>
<td>3</td>
<td>1 h</td>
<td>3,119 ± 70</td>
</tr>
<tr>
<td>7</td>
<td>3 days</td>
<td>2,611 ± 225</td>
<td>1,021 ± 168</td>
</tr>
<tr>
<td>Vehicle-treated rats</td>
<td>5</td>
<td>3 days</td>
<td>1,228 ± 221</td>
</tr>
<tr>
<td>Vitamin E-treated rats</td>
<td>5</td>
<td>3 days</td>
<td>1,419 ± 267</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. No damaged fibers were found in the contralateral muscles. Compared with untreated rats, lower number of fibers in vehicle-treated and vitamin E-treated rats is attributed to distal sectioning of muscles.

---

**Table 2. Number and percentage of intact and damaged fibers in EDL muscles of untreated, vehicle-treated, and vitamin E-treated rats dissected 1 h or 3 days after protocol of pliometric contractions**
zymes from injured muscles, and, for humans, reports of increased severity of muscle soreness (1, 8). These dramatic increases in a number of indexes of damage provide clear evidence of a more severe secondary injury (10, 15, 17), even when the force deficit does not change significantly (Ref. 17 and present study).

For damaged muscles of all species, after the secondary injury at 3 days, the force deficit and the number of damaged fibers show a gradual return to complete recovery. Full recovery is slightly more prolonged for the EDL muscles of rats than for those of mice (4, 10, 15). The prolongation of recovery is likely a function of the same relative damage with a 15-fold-greater muscle mass. After ischemic injury, the time course of regeneration is delayed for the 15-fold-larger EDL muscles of cats compared with that of rats (9). After contraction-induced injury to EDL muscles in mice, myotubes were observed in sections after 5 days (15), and the gradual increase in force development and muscle mass from this time onward was undoubtedly a function of the regeneration of portions of muscle fibers (6). The similarities, particularly regarding the onset and magnitude of the various aspects of contraction-induced injury, for EDL muscles of rats compared with the more
extensive amount of data on the EDL muscles of mice assured us of a viable rat model for subsequent investigations of biochemical events.

Vitamin E as an antioxidant. Potentially, vitamin E could protect against free radical damage by donating hydrogen atoms to free radicals and thus inhibiting a variety of degradative reactions associated with lipid peroxidation (3, 12). The lack of any difference in the force deficits or the percentage of fibers damaged in EDL muscles in the vitamin E-treated compared with vehicle-treated rats leads to the rejection of the hypothesis that vitamin E treatment protects muscle fibers from contraction-induced injury at the postcontraction times studied. The failure of vitamin E to protect muscle fibers from contraction-induced injury is consistent with the conclusions of Jakeman and Maxwell (13) and Warren et al. (23).

In studies of vitamin E treatment, a failure to demonstrate an increased content of vitamin E raises the possibility that the supplementation was inadequate (13). In the present study, as with the study of Warren et al. (23), direct evidence was provided of a threefold elevation in the content of vitamin E in the muscles of the vitamin E-treated rats, yet the force deficit was not decreased nor was the damage to fiber morphology diminished. Furthermore, the lack of protection afforded was not due a subsequent overwhelming of the antioxidant capacity of vitamin E. The almost threefold increase in the muscle content of vitamin E did not change between 3 h and 3 days when the oxidant stress in muscle fibers was likely at its greatest intensity. We conclude that vitamin E treatment before and after a protocol that induced severe (~50% force deficit) injury does not provide any additional protection beyond that provided by the endogenous vitamin E. The lack of any evidence that vitamin E treatment protects myofibers from contraction-induced injury is in sharp contrast to the almost complete absence of secondary injury after treatment of mice with PEG-SOD (24).

The marked discrepancy between the highly protective effects of PEG-SOD and the absence of any effect of vitamin E on the force deficit or the magnitude of the morphological damage may be related to the differential solubility of these compounds, the difference in the usual cellular location, or both factors. The lipophilic vitamin E is carried on plasma lipoproteins and incorporated into tissue membranes. Conversely, exogenously administered PEG-SOD is soluble in the aqueous phase of plasma and is not known to enter cells. The muscle damage 3 days after pliometric contraction protocol is associated with an inflammatory response (24). The free radical species generated by blood-borne neutrophils are both water soluble and initially located in extracellular sites. Consequently, these free radical species would be far more accessible to PEG-SOD than to vitamin E. We proposed previously that the secondary damage observed 3 days after the initial contraction-induced injury may be related to generation of free radicals by phagocytic cells that have infiltrated the damaged fibers (24) and that the PEG-SOD treatment reduced the damage by scavenging these cells. The observation that the membrane-associated vitamin E has no effect on this process is not surprising.

Vitamin E as membrane stabilization factor. At 3 h after the pliometric contraction protocol, the fourfold increase in the activities of CK and PK in the serum of the vehicle-treated rats indicated a significant efflux of enzymes from muscle fibers. The initial mechanical injury to the muscle fibers is induced by the combined effects of the strain, average force, and the number of contractions (5), and the efflux at 3 h suggests that the efflux was closely associated with the initial injury. The magnitude of the efflux of enzymes from muscle fibers after contraction-induced injury was attested to by the continued elevation of the serum activities 3 days later at values still twofold greater than resting activity levels. These observations of CK efflux from skeletal muscles of rats are consistent qualitatively with data on humans after pliometric exercise (17). After pliometric exercise, the activities of CK in the serum of humans can attain values 100-fold greater than resting values, with peaks 5 days after the initial injury (17). The
actual time of the peak of the CK efflux has not been determined in the single in situ muscle preparation in the rat, but it appears to occur within the first 3 days after the protocol (2). The earlier timing of the peak value for serum CK activity in rats compared with humans likely reflects the smaller muscle fiber cross-sectional areas and consequently the larger surface-to-volume ratios and shorter diffusion distances. The lower peak values may be a function of the smaller mass of the muscle injured, the shorter half-life of the CK in the rat, or both.

After contraction-induced injury, the absence of any change in the serum CK and PK of vitamin E-treated rats compared with the values for the nonexercised control rats as well as the 50% lower activities of CK and PK in the serum of vitamin E-treated compared with vehicle-treated rats suggest that during the first hours after a pliometric contraction protocol vitamin E prevents the loss of enzymes from muscle fibers (16, 19). After contraction-induced injury, vitamin E may stabilize muscle membranes by its interaction with membrane phospholipids (21). Whether the prevention of CK and PK efflux from muscle after contraction-induced injury is advantageous or disadvantageous to the muscle fibers will require further study of the recovery process after the injury.

The authors thank Richard Hinkle and Cheryl Hassett for assistance.

The Wellcome Trust and Physiological Society provided travel grants to A. McArdle. The research performed at the University of Michigan was supported by National Institute on Aging Grant AG-06157.

Address for reprint requests: J. A. Faulkner, Univ. of Michigan, Institute of Gerontology, Rm. 1056, 300 N. Ingalls, Ann Arbor, MI 48109-2007.

Received 4 March 1996; accepted in final form 6 May 1997.

REFERENCES


