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Burn and Smoke Inhalation Injury in Sheep Depletes Vitamin E: Kinetic studies using deuterated tocopherols

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Abstract

To test the hypothesis that burn and smoke injury will deplete tissue α -tocopherol and cause its faster plasma disappearance, deuterium-labeled vitamin E was administered to sheep exposed to both surface skin burn and smoke insufflation that causes injury similar to human victims of fire accidents. Two different protocols were used: 1) deuterated vitamin E was administered orally with food at 0 time (just prior to injury), or 2) the labeled vitamin E was administered orally with food the day prior to injury. The animals that had been operatively prepared seven days before, were anaesthetized, then received both 40% body surface area 3rd° burn and 48 breaths of cotton smoke, or sham injuries. All were resuscitated with Ringer's lactate solution (4 ml/kg/% BSA burn/24h) and mechanically ventilated. Blood samples were collected at various times after vitamin E dosing. In both studies the depletion of plasma α -tocopherol was faster in the injured sheep. The sheep given deuterated vitamin E 24 h prior to injury had similar maximum α -tocopherol concentrations at similar times. The exponential rates of α -tocopherol disappearance were 1.5 times greater and half-lives were 12 h shorter ($p < 0.05$) in the injured sheep. In separate studies, various tissues were obtained from sheep that were sacrificed from 4 h to 48 h after injury. The liver α -tocopherol concentrations in sheep killed at various times after injury appear to show a linear decrease at a rate of 0.1 nmol α -tocopherol/g liver per hour, suggesting that the liver is supplying α -tocopherol to maintain the plasma and lung α -tocopherol concentrations, but that this injury is so severe that the liver is unable to maintain lung α -tocopherol concentrations. These findings suggest that α -tocopherol should be administered to burn patients to prevent vitamin E depletion and to protect against oxidative stress from burn injury.

INTRODUCTION

In the United States, about 100,000 people require hospitalization and 5,000 deaths occur each year because of burn injury (1). Additionally, 70% of fire victims who die within 12 h of insult have inhalation injury (2,3). Combined burn and smoke inhalation injury is typically associated with a systemic inflammatory response and increased reactive oxygen species (ROS) (4,5). ROS can result in cell membrane destabilization, enzyme inactivation, increases in capillary permeability and vascular reactivity (6,7) All these modifications strongly resemble many of the prominent characteristics of circulatory burn shock and distant organ injury (8).

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We have previously described the pathophysiology of acute lung injury in sheep subjected to the combined burn and smoke inhalation injury (9,10). The tissue injury included an increase in pulmonary vascular permeability to both fluid and protein (11). Although the exact mechanism of the acute lung injury in thermal damage is not completely understood, we have shown that ROS play a critical role in this pathological process (12). Additionally, stimulated neutrophils release ROS that affect the microcirculation of the lung (13).

Both enzymatic and non-enzymatic antioxidants are important ROS defense mechanisms (14). But given that vitamin E is a lipid-soluble antioxidant that prevents propagation of lipid peroxidation (15), it potentially plays a critical role in protection from burn and smoke injury. In burned patients, plasma α -tocopherol concentrations typically decline rapidly and remain depressed for more than 7 days after injury (16).

We hypothesized that burn and smoke injury will deplete tissue vitamin E and thus cause its faster disappearance from the plasma. To test this hypothesis we have used stable isotope-labeled vitamin E in a well-established model of the human acute respiratory distress syndrome that is sheep exposed to both injury burn and smoke insufflation.

METHODS

Deuterated vitamin E

RRR- α -5-(C²H₃) tocopheryl acetate and *all rac*- α -5,7-(C²H₃)₂ tocopheryl acetate (*d*₃-*RRR*- α -tocopheryl acetates and *d*₆-*all rac*- α -tocopheryl acetates, respectively) were a gift from the Natural Source Vitamin E Association (NSVEA). The compounds were determined to be 96% *RRR*- α -tocopheryl acetate and 93% *all rac*- α -tocopheryl acetate by weight. Their isotopic purities at their nominal level of deuteration were 84% (*d*₀:4.0%; *d*₁: 2.0%; *d*₂: 9.7%) and 86% (*d*₀,*d*₁: <0.1%; *d*₂:0.1% *d*₃:0.8%, *d*₄: 1.3%; *d*₅: 11.2%), respectively. The *d*₃-*RRR*- and *d*₆-*all rac*- α -tocopheryl acetates were encapsulated in gelatin capsules as 1:1 mixtures of 75 mg each, diluted in α -tocopherol-stripped corn oil. The *RRR/all rac* ratio in the capsules was determined by GC/MS to be 0.98. The two forms of vitamin E paralleled each other in the plasma with the *d*₆-*RRR*- α -tocopherol concentrations at roughly half those of *d*₃-*RRR*- α -tocopherol (17); therefore, only *d*₃-*RRR*- α -tocopherol data are reported.

Animal Care

Animals were cared for in the Investigative Intensive Care Unit at the University of Texas Medical Branch (UTMB Galveston, TX), which is approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. The UTMB Animal Care and Use Committee approved the experimental procedures. National Institutes of Health and American Physiological Society guidelines for animal care and use were strictly followed. The animals were fed a chow diet containing vitamin E 30 IU per pound diet (66 mg dl- α -tocopheryl acetate per kg) along with hay for at least 2 weeks prior to study. The vitamin E requirements of a 50 kg sheep is 15 IU (15 mg dl- α -tocopheryl acetate) (18).

Animals were studied while awake. The sheep had access to food and ate in a regular manner. They showed no outward signs of liver damage, nor was there histologic evidence of liver damage.

Surgical Preparation and Injury

Sheep were surgically prepared for chronic study, as described previously (19-21). Briefly, a Swan-Ganz thermal dilution catheter (model 93A-1317-F, Edwards Critical Care Division; Irvine, CA) was inserted through the right external jugular vein for the measurement of cardiac output and the core body temperature and measure the central venous pressure to evaluate fluid

resuscitation. An arterial catheter (16 Gauge, 24 inch. Intracath, Becton Dickinson; Sandy, UT) was inserted into the right femoral artery for the measurement of arterial blood gas. The caudal mediastinal lymph node was cannulated (Silastic medial grade tubing, 0.025 in. ID, 0.047 in, OD, Dow Corning; Midland, MI) according to a modification of the technique described by Staub and colleagues (22). The contribution to the node was removed by ligation of the tail of the caudal mediastinal lymph node and cauterization of the systemic diaphragmatic lymph vessels (20). After the operation, the sheep were allowed 5-7 days to recover from the operative procedure and given food and water ad libitum.

The smoke and burn injury protocol was described previously (19-21). Briefly, under induction of anesthesia with 10 mg/kg ketamine (Ketalar; Parke-Davis; Morris Plains, NJ), a tracheotomy was performed, and a cuffed tracheostomy tube (10-mm diameter, Sheiley; Irvine, CA) was inserted. Anesthesia was maintained with halothane. Using the Bunsen burner, a third-degree flame burn of 20% of the total body surface area was made on one flank. Thereafter, inhalation injury was induced while the sheep was in the prone position. A modified bee smoker was filled with 50 g burning cotton toweling and was connected to the tracheostomy tube via a modified endotracheal tube containing an indwelling thermistor from a Swan-GanzTM catheter. During the insufflations procedure, the temperature of the smoke did not exceed 40° C. The sheep were insufflated with a total of 48 breaths of cotton smoke. After smoke insufflations, another 20% total body surface area, third-degree burn was made on the contralateral flank.

The resuscitation protocol was described previously (19-21). Immediately after the injury, anesthesia was discontinued and the animals were allowed to awaken and were mechanically ventilated with a Servo ventilator (model 900C, Simens-Elena; Solna, Sweden) throughout the next 48 h experimental period. Ventilation was performed with a positive endexpiratory pressure (PEEP) of 5 cm H₂O and a tidal volume of 15 ml/kg. The respiratory rate was set to maintain normo-capnea. For the first 3 h post injury, all animals received an inspired oxygen concentration (FiO₂) of 100% to expedite the removal of CO; thereafter, FiO₂ was adjusted to maintain the arterial oxygen saturation >90%. These respiratory settings allowed rapid carboxyhemoglobin clearance after smoke inhalation.

During the experiment, fluid resuscitation was performed with Ringer's lactate solution following the formula (4 ml/%burn surface area / kg body wt for the first 24h and 2 ml / % burned surface area /kg body wt per day for the next 48 h) (20). During this experimental period, the animals were allowed free access to food, but not to water, to allow accurate determination of fluid balance. Fluid balance was constantly monitored.

Measured physiological variables were not considered valid until the animals were fully awake and standing, which usually occurs within 1 h post injury. Arterial blood was measured with a blood gas analyzer (model IL1600; Instrumentation Laboratory; Lexington, MA). The data were corrected for core body temperature. Lung lymph flow was measured with a graduated test tube and stopwatch. Lymph and blood samples were collected in EDTA tubes, and the total protein concentration in plasma (Cp) and lymph (CL) were measured with a refractometer (National Instrument; Baltimore, MD). Thereafter pulmonary microvascular permeability index (PI) was calculated by the following equation: $PI = QL * (CL/CP)$, where QL is lung lymph flow (ml/h) (20).

When all measurements were completed, the animals were anesthetized with ketamine and humanely euthanized by administration of 60 ml saturated potassium chloride solution. Immediately after this procedure, the right lower lobe of the lung was harvested for pathological examination and the measurement of wet/dry weight ratio, corrected for the content of blood,

as described by Pearce et al. (23). Airway obstruction score was measured by method described by Cox et al. (24)

Sheep received both 40% body surface area 3rd° burn and 48 breaths of cotton smoke, or sham injuries. All were resuscitated with Ringer's lactate solution (4 ml/kg/% BSA burn/24h) and mechanically ventilated. In addition to the deuterated vitamin E study, additional sheep were analyzed using the same protocol at various intervals up to 75 h after injury to evaluate tissue α -tocopherol depletion.

Study design—48 h protocol

Deuterated vitamin E was administered orally to each animal with food at 0 time, then the animals were injured, while under deep surgical planes of anesthesia as described above. The sheep received both 40% body surface area 3rd° burn and 48 breaths of cotton smoke (n=4), or sham injuries (n=4). All were resuscitated with Ringer's lactate solution (4 ml/kg/% BSA burn/24h) and mechanically ventilated. Blood samples were collected in EDTA at approximately 0, 3, 6, 9, 12, 15, 18, 24, 30, 36, 42, 48 h after vitamin E dosing. At 48 h after injury, the animals were deeply anesthetized with ketamine and sacrificed by injection of 10 ml of saturated KCl.

Study design—75 h protocol

To allow optimal absorption of the deuterated vitamin E, the dose was administered orally with food to animals the day prior to injury (24 h). Blood samples were collected in EDTA at intervals up to 75 h. At 24 h after administration of the labeled vitamin E, a blood sample was obtained, then the sheep were anaesthetized and at 26 h, received both 40% body surface area 3rd° burn and 48 breaths of cotton smoke (n=4), or sham injuries (n=3). All were resuscitated with Ringer's lactate solution (4 ml/kg/% BSA burn/24h) and mechanically ventilated. At 75 h, the animals were anesthetized with ketamine and sacrificed by administrations of saturated KCl.

Study design for non-labeled experiments

Sheep were instrumented in the same fashion as above. They were injured and resuscitated in the same fashion as the previous groups. The sheep were anesthetized and euthanized and the organs harvested frozen in liquid nitrogen and sent for analysis by overnight freight service to the Linus Pauling Institute, OSU.

Analyses

Briefly, plasma was isolated from blood drawn into tubes containing EDTA by centrifugation at 3000 rpm for 10 min at 5°C and transferred into plastic tubes. Aliquots were kept at -80°C until shipped on dry ice by overnight courier to the Linus Pauling Institute, OSU where they were kept frozen at -80°C until analysis. Labeled and unlabeled α -tocopherols from sheep given deuterated tocopherols were extracted and analyzed by liquid chromatography-mass spectrometry as described previously (25). Tissues from additional animals were analyzed for α - and γ -tocopherol concentrations, as described (26).

Plasma total cholesterol and triglycerides were measured by enzymatic assays (Sigma, St. Louis). Plasma lipids are the sum of the molar concentrations of cholesterol and triglycerides.

Lung conjugated dienes (CDs) were measured according to Till et al. (27). Lung tissue (1.0 g) was homogenized with 0.8 mL of distilled water, then extracted twice using a 2:1 (vol/vol) mixture of chloroform and methanol. The chloroform extract was dried with N₂, the residue reconstituted with 1.5 mL of heptane and read spectrophotometrically at 233 nm (Spectronic 1001, Milton Roy, Houston, TX). Lung wet/dry ratios were estimated by drying the tissue and

a concomitant blood sample, then weighing until a constant weight was achieved for each sample, as described previously (28).

Mathematical and Statistical Analyses

Areas under the plasma d₃-α-tocopherol/lipids concentration curves (AUC) were calculated using the trapezoidal rule for each sheep for each trial. Time to maximal concentration (T_{max}) and maximal concentrations (C_{max}) were identified by visual inspection of the data. Apparent first order kinetic rates of α-tocopherol disappearance (FDR) were determined by linear least-squares regression of the semilogarithmic concentration (mmol α-tocopherol/mol lipids) versus time data using the linest function (Excel, Microsoft, Redmond, WA) and half-lives were calculated as $t_{1/2} = \ln 2 / \text{disappearance rate constant}$, as previously described (29).

The statistical significance of the data was determined using ANOVA (JMP, SAS Institute Inc., Cary, NC 27513). Results (reported as means ± SE) were considered to be significant at the 95% confidence level (P<0.05). Tissue concentrations were evaluated by ANOVA following log transformation of data; Tukey's Multiple Comparison Test was used for paired comparisons.

RESULTS

Plasma Lipid Concentrations

Plasma lipids (cholesterol and triglyceride concentrations) remained relatively constant throughout both protocols and were not statistically different between sham and injured animals in either experiment (Figure 1 and Table 1). Plasma total α-tocopherol (the sum of the labeled and unlabeled α-tocopherols, Figure 1) at each time point would be expected to remain constant if the output of α-tocopherol from the liver was sufficient maintain the normal plasma concentrations because the labeled α-tocopherol disappears from the plasma as it is replaced (30). As can be seen there were fluctuations in both lipids and α-tocopherol concentrations. These fluctuations, likely caused by infusions of electrolyte solutions during burn management, necessitated correcting plasma α-tocopherol concentrations by total lipids. This correction is necessary because plasma vitamin E is transported entirely within lipoproteins (31).

Outcomes—48 h protocol

In this protocol, the labeled vitamin E (75 mg each d₃-RRR-α-tocopheryl acetates and d₆-all rac-α-tocopheryl acetates) was given immediately prior to the injury protocol (only the d₃-RRR-α-tocopherol is reported). Plasma d₃-α-tocopherol/lipid ratios followed different patterns in the sham and injured animals (Figure 2). In the burn and smoke injured animals, the exponential rates of increase (R_I) of the d₃-α-tocopherol/lipid concentrations were slower (p<0.01) (Table 2), suggesting that tocopherol absorption may have been slowed by the injury and/or oxidation of the vitamin. Importantly, the exponential rates of decrease (R_D) were greater (p<0.05) in the injured animals, suggesting that the injury caused faster α-tocopherol depletion, likely as a result of oxidative stress. The faster vitamin E depletion in the injured sheep resulted in α-tocopherol half-lives that were 8 h shorter than those of the sham animals (p<0.05). Neither the peak concentrations (C_{max}), nor the times of maximum concentration (T_{max}) were different between the two groups.

The areas under the d₃-α-tocopherol/lipid concentration curves (AUCs) over the 48 h study period were not statistically different between the two groups despite the slower appearance and the faster disappearance of d₃-α-tocopherol/lipid in the burn and smoke group. This lack of statistically significant difference is most likely attributable to the variability in responses observed in the injured animals.

Outcomes—75 h protocol

In the preceding study, the labeled vitamin E was given immediately prior to the injury, which might have limited the dose absorbed and caused an apparent faster vitamin E disappearance. To eliminate this possible explanation, in the 75 h study the sheep were given the same dose of deuterated vitamin E (75 mg each d₃-RRR- α -tocopheryl acetates and d₆-all rac- α -tocopheryl acetates) the day before the injury to allow the plasma d₃- α -tocopherol/lipid concentrations to reach a maximum prior to the injury. The effectiveness of this strategy was confirmed in that both injured and sham treated animals had similar d₃- α -tocopherol/lipid T_{max}s and C_{max}s (Table 3).

To calculate the exponential rates of disappearance, the data from 24 to 75 h was evaluated. These times were chosen as 24 h was approximately the peak in plasma d₃- α -tocopherol/lipid concentrations (Table 3), and the concentrations between 24 and 75 h were approximately linear on a natural log plot, as has been previously observed in humans (32).

Plasma d₃- α -tocopherol/lipid concentration ratios from two representative sheep illustrate that the burn and smoke injured sheep had faster apparent first order rates of vitamin E disappearance, despite both sheep having similar d₃- α -tocopherol/lipid ratios prior to the injury (Figure 3). Importantly, the exponential rate constants of α -tocopherol disappearance were 1.5 times greater ($p < 0.01$) in the injured compared to the shams (Table 3) and as a result the α -tocopherol half-lives were 12 h shorter, emphasizing that the burn and smoke injury depleted plasma vitamin E.

Plasma Unlabeled Vitamin E Concentrations

Plasma unlabeled vitamin E might also be expected to be decreased by the burn and smoke injury. During the 48 h trial, the average plasma unlabeled α -tocopherol/lipid concentrations in the plasma were not statistically different ($p < 0.07$) in the sham compared with the burn and smoke injured group (1.4 ± 0.4 vs 1.0 ± 0.3 vs. mmol/mol, respectively). During the 75 h trial, prior to the burn and smoke injury both groups had similar plasma α -tocopherol/lipid concentrations (1.8 ± 0.2 vs 1.7 ± 0.1 mmol/mol, respectively), however, the injury caused decreased α -tocopherol/lipid concentrations in the burn and smoke group (1.3 ± 0.1 mmol/mol) compared with the sham group (1.9 ± 0.1 mmol/mol, respectively, $p < 0.001$). These data confirm our expectations that the burn and smoke injury depletes plasma vitamin E.

Tissue Unlabeled Vitamin E Concentrations

In separate studies, tissues were obtained from various sheep that were sacrificed from 4 h to 48 h after injury. The α -tocopherol concentrations were measured in heart, kidney, liver and lung (Table 4). Only the liver and lung show a significant α -tocopherol depletion comparing sham and injured animals at 48 h. These data suggest that the liver supplies the lung with α -tocopherol and the injury depletes not only lung α -tocopherol, but liver α -tocopherol as well.

To evaluate more closely the α -tocopherol status in response to injury, only samples of liver and lung that were obtained simultaneously from each animal are shown (Table 5). Additionally, the lung wet/dry ratios and the lung α -tocopherol per dry weight are shown. Although the wet/dry ratios of the lungs show that the lungs were edematous, this finding does not explain the lower lung α -tocopherol concentrations because irrespective of the denominator (g dry weight or wet weight) the injury resulted in significantly lower α -tocopherol concentrations. These data again emphasize the simultaneous α -tocopherol depletion from lung and liver.

Markers of oxidative stress

α -Tocopherol depletion is likely a result of lipid peroxidation, suggesting that animals receiving a combination burn and inhalation injury have undergone marked oxidative stress. Conjugated diene concentrations were measured in lungs from similarly treated sheep. These data demonstrate that by 18 h there is a substantive increase in lipid peroxidation (Table 6).

DISCUSSION

Burn and smoke injury in sheep causes a marked depletion of lung and liver α -tocopherol and a faster exponential rate of plasma α -tocopherol disappearance that resulted in a shortening of its half-life by about 10 h (Tables 2 and 3). These data, along with increased concentrations of conjugated dienes (Table 6), support the hypothesis that burn and smoke injury causes lipid peroxidation that depletes vitamin E. Moreover, the data suggest that vitamin E supplementation may limit the injury caused by burn and smoke. Indeed, as a proof of principle study, we found oral administration of vitamin E supplements prior to injury limited the extent of the injury (33). Furthermore, nebulization of an emulsion containing α -tocopherol into sheep lungs post-injury minimized the increased pulmonary transvascular fluid flux and hypoxia seen with combined burn and smoke inhalation in our ovine model (34).

An alternative explanation for the more rapid exponential disappearance rate of α -tocopherol is that its loss could be a result of its escape from the plasma compartment and that the measured loss would not be a result of oxidation but rather due to "third spacing". First, α -tocopherol circulates entirely within plasma lipoproteins; molecules that are very large relative to most proteins in the circulation. We believe the loss of lipoproteins from the circulation is unlikely based on the data shown in Table 1 and in Figure 1, which demonstrate that the plasma lipids remained relatively constant during our studies. Secondly, we continuously monitor the fluid balance of the animals, and closely regulate fluid balance because we are well aware of this potential confounder (35). Additionally, we have measured the lung lymph α -tocopherol and even after severe injury and application of additional vitamin E by nebulization to the lung, we do not find appreciable concentrations of tocopherols in the lymph (unpublished observations, Traber et al.). Therefore, we do not believe that "third spacing" effects are the cause of the increased vitamin E loss in the injured sheep. Although the lung wet/dry ratios indicated that the lungs were edematous in the injured sheep, the lung α -tocopherol concentrations were lower irrespective of whether the concentrations were expressed per dry or per wet weight (Table 5).

The tissue data presented herein demonstrates the critical role of the liver in maintaining body vitamin E concentrations. It is generally accepted that the hepatic α -tocopherol transfer protein is responsible for facilitating the efflux of α -tocopherol from the liver into the plasma for delivery to the tissues via lipoproteins (36). The liver α -tocopherol concentrations in sheep sacrificed at various times after injury indicate that there is a linear decrease at a rate of 0.1 nmol α -tocopherol/g liver per hour ($p < 0.001$). This depletion accounts for a nearly 2000 nmol α -tocopherol loss from the liver over 48 h; by contrast, the lung only sustained a 1000 nmol α -tocopherol loss (assuming both lungs and liver are approximately 500 g (37)). The plasma deuterated α -tocopherol kinetic data shows an approximate 6000 nmol α -tocopherol loss from the plasma α -tocopherol pool over 48 h. Taken together these data suggest that the liver is supplying α -tocopherol to maintain the plasma and lung α -tocopherol concentrations, but that this injury is so severe that the liver is unable to maintain plasma α -tocopherol concentrations. That is, the plasma kinetic data indicates a loss of 6000 nmols over 48 h, 1/3 from the liver and 1/6 from the lungs, with half from other tissues or from diet. It is remarkable that the injury is to the skin and lungs, yet the liver suffers the greater loss of vitamin E. Although it is likely that the liver is exporting vitamin E, alternative mechanisms are also possible. It is well recognized that lipoproteins carry lipid hydroperoxides (38-41), and likely deliver them to

liver, therefore, oxidation of vitamin E may also take place in the liver. Following combined burn and smoke inhalation injury in sheep, markers of liver damage progressively increased, reaching statistical significance by 12 h for serum aspartate aminotransferase (AST, $p < 0.05$) and by 18 h for serum alanine aminotransferase (ALT) (Traber, et al. unpublished observations). It should be noted that many burn patients have fatty liver (42), a metabolic consequence that has been suggested to be a result of oxidative stress in the liver (43,44), but this is phenomenon is usually observed after a longer period of time subsequent to the injury. At least up to 48 h, the liver appears to be able to maintain export of α -tocopherol to the plasma.

Although sheep have markedly lower plasma α -tocopherol concentrations than do humans, this is likely due to markedly lower plasma lipid concentrations in sheep (Table 1, Figure 1). Sheep have both low and high density lipoproteins (LDL and HDL) as their major circulating lipoproteins. We recently demonstrated that mice that were genetically unable to secrete very low density lipoproteins (VLDL) from their livers, that they were able, however, to maintain normal tissue vitamin E concentrations, likely as a result of HDL α -tocopherol transport (45). Thus, we believe that although the lipid levels are lower in sheep than in humans, it is likely they have similar mechanisms to humans for secretion and maintenance of plasma α -tocopherol by the liver. We believe these data are applicable to humans because we have also seen that human cigarette smokers have increased vitamin E disappearance rates (46). Moreover when the smokers' ascorbic acid status was normalized by two weeks supplementation with vitamin C, then vitamin E disappearance rates were also normalized (47). It should be noted that sheep can synthesize their own vitamin C, so lack of vitamin C should not be the cause of the more rapid vitamin E disappearance in the studies reported herein. Previous studies have also shown that patients with severe burn injury and additional trauma had virtually no vitamin E in their circulation (16). These patients did not survive if their vitamin E levels were not restored. The data from the sheep suggest that low liver vitamin E concentrations will result in impaired maintenance of tissue and plasma vitamin E and thus inadequate antioxidant protection. The data in tables 4 and 5 indicate that the sheep in the sham group had lung α -tocopherol concentrations of 5-7 nmol/g tissue; these are much lower than in humans who have been reported to have vitamin E concentrations in lung of approximately 20-25 nmol/g and in liver from 16 to 95 nmol/g (17). However, the α -tocopherol concentrations we observed in the sheep are similar to those previously reported by others. For example, sheep plasma concentrations (from control animals prior to any treatments) have been reported from 1.5 to 5.5 μ mol/L when consuming diets from 15 to 50 mg vitamin E/kg diet (48-52). Tissue α -tocopherol concentrations from sheep fed a diet containing lower levels of vitamin E (8 mg/kg diet) were liver, 2.25 nmol/g; lung, 4.85 nmol/g; heart, 6.73 nmol/g; and kidney, 2.23 nmol/g with plasma α -tocopherol concentrations of 1.30 μ mol/L. Thus, the sham animals reported herein have an α -tocopherol status within that expected for sheep consuming adequate vitamin E diets.

The findings presented here further suggest that a downward spiral in protection against lipid peroxidation will allow continued tissue injury and additional oxidative damage and suggest the importance of maintaining adequate α -tocopherol status in burn and smoke injured patients.

In summary, the combination of smoke inhalation and burn injury in the chronic ovine cardiopulmonary model results in marked vitamin E depletion.

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Figure 1. Plasma lipids and total α -tocopherol concentrations

Plasma lipids (cholesterol (C) and triglycerides (TG), left panels) and total α -tocopherol (sum of labeled and unlabeled α -tocopherol) are shown for each time point (mean $\pm$ SE) for sham (n=4) compared with burn and smoke injured (n=4) animals in the 48 h experiment (upper panels) and for sham (n=3) compared with burn and smoke injured (n=4) animals in the 75 h experiment (lower panels). To convert cholesterol to mg/dl divide by 0.0259, to convert triglycerides to mg/dl divide by 0.0113

Figure 2. Plasma d₃-α-Tocopherol Concentration Curves

Plasma divide₃-α-tocopherol/lipids (means + SE) are shown for each time point for sham (n=4) compared with burn and smoke injured (n=4) animals in the 48 h expt. Sheep were given the deuterated vitamin E (d₃-RRR-α-tocopheryl acetate and d₆-all rac-α-tocopheryl acetate, 75 mg each) at 0 time, then the injury was administered and blood samples taken at various times, as indicated.

Figure 3. Plasma d_3 - α -Tocopherol Disappearance Kinetics in Animals Given the Dose 26 h Prior to Injury

Plasma d_3 - α -tocopherol/lipids are shown for representative animals from each group. Sheep were given the deuterated vitamin E ((d_3 -RRR- α -tocopheryl acetate and d_6 -all rac- α -tocopheryl acetate, 75 mg each) at 0 time, then at 26 h the injury protocol was administered and blood samples taken at various times, as indicated.

Table 1
Plasma Lipids Averaged Over the Time Course of the Experiments

Experiment	Injury	Cholesterol mmol/L	Triglycerides mmol/L	Lipidsmmol/L ¹
48 h	Sham (n=4)	1.02 ± 0.14 ²	0.10 ± 0.01	1.11 ± 0.14
	Burn & Smoke (n=4)	1.04 ± 0.06	0.14 ± 0.01	1.18 ± 0.07
75 h	Sham (n=3)	0.95 ± 0.03	0.13 ± 0.01	1.08 ± 0.03
	Burn & Smoke (n=4)	1.23 ± 0.13	0.13 ± 0.04	1.36 ± 0.17

¹To convert cholesterol to mg/dl divide by 0.02586, to convert triglycerides to mg/dl divide by 0.01129, lipids = sum cholesterol plus triglyceride concentrations.

²mean±SE

Table 2
Plasma d₃- α -Tocopherol/Lipids Kinetic Parameters in 48 h Experiment

	Tmax (h)	Cmax (mmol/mol)	AUC (mmol/mol.h)	Rate_I(h⁻¹)	Rate_D(h⁻¹)	Half-Life (h)
sham (n=4)	20 ± 2	0.18 ± 0.01	5.6 ± 0.5	0.35 ± 0.03	0.022 ± 0.002	32 ± 3
burn & smoke (n=4)	18 ± 2	0.15 ± 0.05	3.8 ± 1.2	0.15 ± 0.06	0.030 ± 0.003	24 ± 3
p-value	NS	NS	NS	P<0.01	P<0.05	P<0.05

Shown are means ± SE. <at>Tmax = Time to maximum concentration, Cmax=peak concentration, AUC=area under the curve; apparent first order kinetic rates, Rate_I = exponential rate of increase, Rate_D = exponential rate of decrease.

Table 3
Plasma d₃- α -Tocopherol/Lipids Kinetic Parameters in 75 h Experiment

	Tmax (h)	Cmax (mmol/mol)	AUC (mmol/mol.h)	Rate_D (h⁻¹)	Half-Life (h)
sham (n=3)	22 ± 4	0.23 ± 0.06	5.2 ± 1.0	0.022 ± 0.002	32 ± 4
burn & smoke (n=4)	20 ± 1	0.25 ± 0.04	4.0 ± 0.4	0.035 ± 0.002	20 ± 1
p-value	NS	NS	NS	P<0.01	P<0.01

Shown are the mean ± SE, Tmax = Time to maximum concentration, Cmax=peak concentration, AUC=area under the curve (from 24 to 75 h), Rate_D = exponential rate of decrease.

Table 4
Vitamin E Concentrations from Various Tissues from Animals Sacrificed at the Indicated Interval After Injury

		α -tocopherol (nmol/g wet weight)			
		Heart	Kidney	Liver	Lung
Sham	n=	22.1 $\pm$ 1.5 11	3.1 $\pm$ 0.6 12	10.0 $\pm$ 1.0 ^a 20	5.8 $\pm$ 0.4 ^c 22
Burn & Smoke Injury					
4 Hours	n=	22.1 $\pm$ 1.8 6	3.6 $\pm$ 0.3 6	12.0 $\pm$ 2.2 ^b 6	6.3 $\pm$ 1.1 6
8 Hours	n=	21.1 $\pm$ 2.0 6	3.8 $\pm$ 0.5 6	10.1 $\pm$ 1.5 5	5.6 $\pm$ 0.6 7
12 Hours	n=	19.7 $\pm$ 1.3 4	3.9 $\pm$ 0.6 4	7.4 $\pm$ 1.3 4	5.7 $\pm$ 0.7 4
24 Hours	n=	24.1 $\pm$ 2.8 9	4.2 $\pm$ 0.6 8	9.4 $\pm$ 1.5 7	6.6 $\pm$ 0.6 ^d 8
48 Hours	n=	22.5 $\pm$ 2.0 8	5.2 $\pm$ 0.5 9	5.8 $\pm$ 1.0 ^{a,b} 19	4.2 $\pm$ 0.4 ^{cd} 27

Values (means $\pm$ SE) with the same superscript are significantly different, $p < 0.05$ by Tukey's Multiple Comparison Test

Table 5
Vitamin E Concentrations of Lung and Liver from the Same Animals 48 h After Injury

Tissue	Injury	n/group	α -Tocopherol (nmol/g wet wt)	Wet/Dry Ratio	α -Tocopherol (nmol/g dry wt)
Lung	Sham	15	6.76 $\pm$ 0.58	5.29 $\pm$ 0.27	34.5 $\pm$ 3.0
	Burn & Smoke	10	4.24 $\pm$ 0.68	6.04 $\pm$ 0.16	26.2 $\pm$ 4.4
	p-value =		0.005	0.010	0.044
Liver	Sham	15	9.30 $\pm$ 0.90		
	Burn & Smoke	10	4.88 $\pm$ 0.83		
	p-value =		0.001		

Table 6% Baseline Lung Conjugated Dienes (mean $\pm$ SE)

Time (h)	B&S (n=10)	sham (n=4)	P =
0	100%	100%	
3	116 % $\pm$ 8 %	99 % $\pm$ 2 %	0.214
6	111 % $\pm$ 7 %	94 % $\pm$ 4 %	0.178
12	118 % $\pm$ 5 %	97 % $\pm$ 5 %	0.034
18	124 % $\pm$ 4%	94 % $\pm$ 12 %	0.009
24	137 % $\pm$ 5 %	112 % $\pm$ 9 %	0.021
36	141 % $\pm$ 6 %	84 % $\pm$ 10 %	0.001
48	136 % $\pm$ 6 %	109 % $\pm$ 6 %	0.017

The baseline values were set at 100% with Burn and Smoke group equal 0.143 ± 0.006 and sham equal 0.136 ± 0.016 .