Anti-peroxidation effects of vitamin E on low density lipoprotein and milk fat globule membrane of lactating goats: in vivo versus metal ion challenge in vitro

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Abstract

Ruminants are animals with mild oxidation risk considering characteristics of their plasma. The purpose of the present study was to determine if surplus vitamin E supplementation further improved their peroxidation status. Four lactating goats (\textit{Capra ibex ibex}) receiving a single intramuscular injection of 3000 IU \textit{d}-\textit{\alpha}-tocopheryl acetate were monitored daily for a week. Plasma and milk levels of thiobarbituric acid reactive substances (TBARS) were measured to estimate the peroxidation status of overall body and mammary gland, respectively. Lipid hydroperoxide content of low density lipoprotein (LDL) and milk fat globule membrane (MFGM) were determined both immediately after isolation and after metal ion challenge to evaluate their spontaneous peroxidation in vivo and peroxidation susceptibility in vitro, respectively. The results showed that while plasma and milk levels of \textit{\alpha}-tocopherol peaked at day 2, the corresponding TBARS dropped to their lowest weekly levels. Content of preformed lipid hydroperoxides in LDL and MFGM remained unchanged \((P<0.05)\) during the study period, while peroxidation in LDL and MFGM on extended exposure to Cu\textsuperscript{2+} was prevented by vitamin E enrichment. Therefore, surplus vitamin E was beneficial to the peroxidation-resistant mechanisms of the overall body and those specifically within mammary gland of lactating goats. Furthermore, peroxidation resistance of LDL and MFGM to copper challenge in vitro also benefited from vitamin E enrichment despite the fact their endogenous peroxidation seemed unresponsive. The hypothesis that peroxidation of LDL and MFGM of lactating goats in situ, which proceeds with metal ion-independent mechanisms, is minor was discussed.

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1. Introduction

Cattle, sheep, goats and deer are representatives of the suborder Ruminantia within the order Artiodactyla. Ruminants are unique in some physiological parameters compared to non-ruminants and humans. Levels of glucose and lipids in their plasma (Chapman, 1980) are generally low. Furthermore, high density lipoprotein (HDL) constitutes as much as 80\% of the total plasma lipoproteins in sheep, goats and deer, whereas low density lipoprotein (LDL) is present in lower amounts (Chapman, 1980). In the plasma of cattle, sheep and deer, very low density lipoprotein (VLDL) and chylomicrons are present at very low levels (Christie, 1981). Nevertheless, the impression that ruminants might have a more favorable peroxidative status than species with different characteristics is yet theoretical.

Vitamin E (in the form of \textit{\alpha}-tocopherol) is the major lipid-soluble antioxidant of lipoproteins and biomembranes (Packer and Fuchs, 1993). Lipid peroxidation, as a result of free radical attack, leads to the loss of membrane integrity and compromised cellular function. Peroxidation-modified LDL is aberrant in recognition interaction with their...
disposal cells (Esterbauer et al., 1989). Studies indicated that cholesterol content, vitamin E to cholesterol ratio and antioxidant content of LDL affected its susceptibility to metal ion-induced oxidation (Frei and Gaziano, 1993; Tribble et al., 1994). On the other hand, the extent of peroxidation of erythrocytes, but not plasma, in dairy cows was sensitive to exposure to hot environments (Bernabucci et al., 2002). Under more stressful conditions such as mastitis, although recruitment of bovine neutrophils to mammary gland is a prerequisite of immune defense, hydroxy radicals released by infiltrated neutrophils caused mammary cell injury (Ledbetter et al., 2001). This peroxidation-associated mechanism was demonstrated in an in vitro model where antioxidants were proved useful to prevent mammary tissue damage during bovine mastitis (Boulanger et al., 2002).

Amide biomembrane protection, increasing the concentration of α-tocopherol in milk can improve the oxidative stability of milk (Focant et al., 1998). Supplementing vitamin E in diet, enriched α-tocopherol content of the plasma lipid fraction in a saturable way in dairy cows, and the increase of α-tocopherol content of milk is a function of α-tocopherol content of plasma (Weiss and Wyatt, 2003). Amount of vitamin E associated with the milk fat globule membrane (MFGM) is considered the main determinant of the oxidative stability of milk. MFGM derives from the apical portion of mammary epithelial membrane surrounding and enveloping the fat globules as they are extruded from the cells. It has been demonstrated that freshly prepared MFGM simulates mammary epithelial membrane in fatty acid and antioxidant compositions (Palmquist and Schanbacher, 1991; Jensen and Nielsen, 1996). Consequently, peroxidative extent and susceptibility of MFGM would appropriately reflect in real time those properties of mammary epithelial membrane.

The beneficial effect of supplemental vitamin E in lactation and stress relief is inconsistent (Paula-Lopes et al., 2003; LeBlanc et al., 2002). Cows with marginal pretreatment vitamin E status that received an injection of vitamin E tended to have reduced risk of peripartum disease (LeBlanc et al., 2002). It is not known, however, whether a more favorable physiological peroxidative status prior to supplementation will attenuate the effect of vitamin E.

As a phenolic compound, the antioxidant activity of vitamin E relies on its ability to donate hydrogen from the hydroxyl group attached to the chromanol ring to reactive chain-propagating radicals (Kagan et al., 2003). So far, metal ion- and alkylperoxyl radical-catalyzed reactions are most commonly used as in vitro systems to predict peroxidative susceptibility of lipid milieus. However, it is predictable that in vivo peroxidation reactions are far more complicated than those in vitro models. The purpose of the present study was to evaluate the effectiveness of excessive vitamin E supplementation on further improvement of peroxidation status of lactating goats. Plasma and milk levels of thiobarbituric acid-reactive substances (TBARS) were measured to estimate the peroxidation status of overall body and mammary gland, respectively. Milk traits were used to monitor the general function of mammary gland. Lipid hydroperoxide contents of LDL and MFGM were determined both immediately at the time of preparation and after metal ion challenge to evaluate the effects of vitamin E on their spontaneous peroxidation in vivo and peroxidation susceptibility in vitro, respectively.

2. Materials and methods

2.1. Experimental goats

This study was conducted in the university goat farm, National Chung Hsing University, Taichung, Taiwan. Four lactating, non-pregnant Alpine goats (Capra ibex ibex) were used in this study. They were in weeks 2–4 of the second lactation cycle with milk yield of 2.5–3.5 kg/day. The experimental goats were apparently healthy without clinical signs of mastitis. Bermuda hay and alfalfa pellet were given ad libitum. A corn and soybean meal-based commercial concentrate (Fu-Shao Feed Industry, Taichung, Taiwan) formulated to meet NRC requirements (NRC, 1984) for dairy cow (17% crude protein, 72% total digestible nutrient, as-fed basis) was fed before milking (0600 and 1700 h) in quantities proportional to milk production. The total vitamin E content (sums of α-, β-, γ- and δ-tocopherol) determined with HPLC for the concentrate, Bermuda hay, and alfalfa pellet was 208.7, 5.5 and 9.0 mg/kg as-fed, respectively.

2.2. Vitamin E injection and sampling

This study covered a 2-week period. Experimental goats received placebo injection in the beginning of the first week and received vitamin E injection in the beginning of the second week. Injections were applied intramuscularly above the thigh bone immediately before evening milking. D-α-Tocopheryl acetate (3000 IU, Sigma, St. Louis, MO, USA) was diluted 1:3 with 20% ethyl alcohol in Tween-80 (Sigma). This dosage was the same used parenterally in cows prior to calving for evaluation of neutrophil function (Hogan et al., 1992) and was similar to that used orally in sheep per week for bioavailability study (Hidiroglou and Singh, 1991).

Samplings were conducted every day during the study period after evening milking. Blood samples were taken by venipuncture from the jugular vein into tubes containing 1 μM butylated hydroxytoluene (BHT) in 3 mM EDTA. They were placed on ice until taken to the laboratory. Plasma was harvested immediately after centrifugation at 1200×g for 10 min. Aliquots (1 ml) of plasma were stored at −20 °C for the analysis of total TG, total cholesterol, α-tocopherol and
TBARS. Plasma samples for lipoprotein electrophoresis and LDL isolation were stored at 4 °C and used within 2 days. Samples (700 ml) of composite milk were collected from each goat at each milking during the 2-week study period. Immediately after collection, about 600 ml was used to prepare MFGM and 1 ml aliquots for TBARS analysis. At least 40 ml of each sample was preserved with 0.2% potassium dichromate and kept at 4 °C until analysis within 2 days for milk traits (Milko Scan 255, Foss Electric, Hillerød, Denmark) and somatic cell count (SCC) (Fossmatic 250, Foss Electric). Other samples of 1.5 ml milk were stored at −20 °C for triplicate analysis of α-tocopherol. Samples of each milking were analyzed separately and results of two milkings were pooled for each day.

2.3. Plasma TG and cholesterol analysis

Contents of TG and cholesterol in plasma were determined using commercial kits (Wako, Osaka, Japan) on an automatic analyzer (type 7050, Hitachi, Tokyo, Japan).

2.4. Analysis of α-tocopherol content in plasma and milk

A reversed-phase HPLC procedure modified from that of Hidiroglou and Atwal (1989) was applied to measure α-tocopherol content in plasma and milk samples. Briefly, 0.3 ml plasma or 0.5 ml milk were combined with 0.1 ml 20 μg/ml δ-tocopherol (internal standard) in ethanol/BHT solution and mixed well. Ethanol (2 ml) and hexane (5 ml) were added, vortex-mixed for 3 min, and centrifuged for 3 min at 100×g. The organic layers were pipetted into polypropylene vials and evaporated under a stream of nitrogen. The residue was immediately redissolved in 200 μl methanol, vortex-mixed thoroughly and stored at −20 °C. We subsequently injected 20 μl of this solution into the HPLC apparatus. Solutions containing 1–20 μg/20 μl of α-tocopherol and δ-tocopherol (Sigma) were prepared as calibration curve for samples.

HPLC was performed with a Shimadzu LC-10 AD pump (Shimadzu, Tokyo, Japan) equipped with a Kanto 4.6×250 mm RP-C18 column (5 μm, Kanto Chemical, Tokyo, Japan) at 40 °C in Shimadzu CTO-6A column oven. The Hitachi F-4500 fluorescence spectrophotometer (excitation=285 nm, emission=330 nm) was coupled with a software Data module. The mobile phase was pure methanol at a flow rate of 1 ml/min.

2.5. Plasma lipoprotein electrophoresis

The Helena Titan Gel Lipoprotein Electrophoresis System (Helena Laboratories, Beaumont, TX, USA) was used for the separation of plasma lipoprotein on agarose gel. The distribution of the four major lipoprotein fractions was computed automatically by the Helena EDC densitometer at 525 nm (Helena Laboratories) and expressed as percentage of total lipoproteins.

2.6. Isolation of LDL from plasma

LDL was isolated from plasma by sequential ultracentrifugation in a KBr solution within a density gradient of 1.006–1.063 g/ml following the method described by Chapman et al. (1981). Sodium azide (0.02%), EDTA (0.04%), BHT (10 μM) and phenylmethylsulfonyl fluoride (0.35 mg/ml) were added to plasma samples before centrifugation. Immediately before the oxidation challenge, the isolated LDL stock solution was dialyzed against phosphate-buffered saline (PBS) for 24 h. The buffer was changed four times. After dialysis, this EDTA- and BHT-free LDL solution was stored at 4 °C and used for oxidation studies within 24 h.

2.7. Preparation of MFGM from fresh milk

MFGM was isolated from fresh milk according to the method described by Palmquist and Schanbacher (1991) and modified by Jensen and Nielsen (1996). Sucrose (50 g/kg) was added to a 560 ml fresh milk sample and loaded into 16×50 ml centrifugation tubes (35 ml/tube). These tubes were overlaid with 15 ml PBS and centrifuged at 1500×g for 20 min at room temperature, and the tubes were held at 4 °C overnight. The cream harvested from the top of the tubes was frozen in a nitrogen atmosphere at −20 °C until churning. The MFGM was prepared by churning the thawed cream followed by centrifuging at 48,000×g for 25 min at 4 °C to collect the red-brown mass at the bottom of the tubes. The MFGM was suspended in 2 ml PBS and kept at −20 °C for purity examination with electrophoresis and oxidation studies.

2.8. Quantification of protein content

The concentration of protein in LDL and MFGM stock solutions was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

2.9. Oxidation of LDL and MFGM with copper ion

Oxidation experiments on LDL and MFGM were modified from those of Kuzuya et al. (1991) and Lamb and Leake (1992). Briefly, LDL and MFGM stock solutions were diluted with PBS to a final protein concentration of 100 and 500 μg/ml, respectively. Oxidation was initiated by the addition of copper sulfate (final concentration, 5 μM) and 37 °C incubation. A series of LDL solution samples (100 μl) were removed from 37 °C at 10 min, 3 and 6 h post oxidation while the MFGM solution was removed at 3 h. Oxidation was stopped by adding EDTA (final concentration 0.3 mM) and BHT (final concentration 10 μM) and chilling in an ice bath. LDL and MFGM solutions exposed to various times of oxidative challenge were stored at −20 °C before determination of peroxidation product.
2.10. Calibration of MFGM purity by SDS-PAGE

Isolated MFGM was examined for purity by SDS-PAGE on 10% polyacrylamide gel combined with densitometer scanning (Laemmli, 1970; Cavaletto et al., 1999). Percentage of contaminating casein was determined by densitometer to adjust for the content of true MFGM protein.

2.11. TBARS content in plasma, milk and MFGM

TBARS content was measured to estimate the amount of lipid peroxidation products (Esterbauer and Cheeseman, 1990) in plasma, milk and MFGM preparation. 1,1,3,3-Tetraethoxypropane, one of the end products of lipid peroxidation, served as a standard in the concentration range 0.5–10 nmol/ml to represent TBARS content.

2.12. Lipid peroxide levels in LDL determined by ferrous oxidation–xylenol orange assay

Hydroperoxides content of LDL was determined using a commercial kit (PeroXOquant™ quantitative peroxide assay: lipid compatible formulation, Pierce, Rockford, IL, USA). This kit is developed based upon the oxidation of ferrous to ferric ions under acidic conditions by lipid hydroperoxides. The ferric ion indicator dye xylenol orange [1-cresolsulfonphthalein-3,3′-bis(methyliminodiacetic acid) sodium salt] was used to bind ferric ion to produce a colored (blue-purple) complex with an extinction coefficient of $1.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 560 nm, with an absorbance maximum between 550 and 600 nm (Jiang et al., 1992; Nourooz-Zadeh et al., 1994).

Fig. 1. Concentration of α-tocopherol in plasma and milk of lactating goats (C. ibex ibex) before and different days after vitamin E injection. Single dose of 3000 IU D-α-tocopheryl acetate was applied intramuscularly, and α-tocopherol was determined by HPLC as described in Materials and methods. Values are means±S.E.M. of four animals. *Significantly different from the before value, $P<0.05$. 
2.13. Statistical analysis

Results were expressed as mean±S.E.M. of the four experimental goats. Results of milk analysis were pooled from the two milkings each day. Student’s *t*-test (SAS, 1996) was used to compare baseline values obtained as means over the week following placebo injection (before injection) and values at various days after vitamin E injection. Differences were considered significant at *P*<0.05.

3. Results

3.1. α-Tocopherol content in plasma and milk after vitamin E injection

Concentration of α-tocopherol in plasma of lactating goats before vitamin E supplementation (Fig. 1) was 6.46±3.36 μg/ml. After vitamin E injection, plasma concentration of α-tocopherol remained unchanged (*P*>0.05) at the first day but rose abruptly at the second day to over fourfold (28.71±2.91 μg/ml). From day 3 to day 6, plasma concentration of α-tocopherol declined gradually while remaining higher (*P*<0.05) than the pre-injection level. At day 7, α-tocopherol concentration in plasma was higher (13.54±2.91 μg/ml) but was not statistically different than that before vitamin E injection.

Concentration of α-tocopherol in milk before vitamin E supplementation (Fig. 1) was 1.01±0.11 μg/ml. Concentration of milk α-tocopherol was elevated (*P*<0.05) by vitamin E injection. Peak concentration of milk α-tocopherol was observed at day 2 (8.06±0.60 μg/ml) post-injection. The pattern of change in α-tocopherol content of milk and plasma during the week following vitamin E injection appeared similar. Overall, the concentration of α-tocopherol in milk was about one eighth of that in plasma.

3.2. Characteristics of plasma lipids and milk traits after vitamin E injection

Single parenteral injection of high dose of vitamin E to lactating goats exerted no apparent effect (*P*>0.05) on the characteristics of plasma lipids and the major milk traits (Table 1). Plasma TG was less in quantity in contrast to plasma total cholesterol. The ratio of TG to total cholesterol was about 1:4.5 to 1:5.

Distribution of the major lipoprotein fractions in plasma was estimated using a scanner to compute the density of electrophoresis bands as described in Materials and methods. The alpha-lipoprotein band corresponded to HDL and the beta-lipoprotein band corresponded to LDL. VLDL migrated between HDL and LDL as the pre-beta lipoprotein band. Chylomicrons, when present, stayed at the point of application. The results (Table 1) indicate that HDL was the most abundant lipoprotein fraction (representing about 60% of the total lipoprotein) in plasma of lactating goats, followed by LDL (27%) and VLDL (12%). Chylomicrons represented about 1%. After vitamin E injection, significant (*P*<0.05) elevation of LDL and depression of HDL were observed at the second day. The distributions of chylomicrons at the first day and VLDL at the seventh day were decreased after vitamin E injection. Since chylomicrons were present in much lower amounts than the rest of lipoproteins and as the variation occurred at day 7, it was not likely directly related to vitamin E injection; these changes were not included in the discussion of this report.

Milk yield and content of the major milk components of experimental goats were not changed (*P*>0.05) during the

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<td>Effect of vitamin E injection* on plasma lipids and milk traits of lactating goats (C. ibex ibex)</td>
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Values are expressed as mean±S.E.M. of four animals and two milkings (milk traits) each day.

* Single dose (3000 IU) of D-α-tocopheryl acetate was injected intramuscularly.

* Significantly different from the value before vitamin E injection, *P*<0.05.
post-injection week (Table 1). As the levels of plasma TG, plasma total cholesterol and milk fat did not apparently vary during the study period, no correction based on lipid content was attempted when referring to the concentration of α-tocopherol in plasma and milk.

3.3. Levels of TBARS in plasma and milk after vitamin E injection

In the present study, TBARS in plasma and milk were determined to estimate the peroxidation status of lactating goats in situ. The levels of TBARS in plasma before vitamin E injection (Fig. 2) (1.62±0.07 nmol/ml) and the first day after injection (1.56±0.08 nmol/ml) were not appreciably different (P>0.05). However, TBARS in plasma dropped drastically (P<0.05) to 0.29±0.08 nmol/ml at the second day after injection, which was less than 20% of the basal level. Whereas the levels of plasma TBARS rebounded prominently to 1.19±0.08 nmol/ml at the third day, it was still lower (P<0.05) than that before vitamin E supplementation. Slight elevation of plasma TBARS continued after day 3. By day 7, plasma TBARS (1.54±0.08 nmol/ml) returned to the pretreatment level (P>0.05).

The profile of levels of TBARS in milk following vitamin E injection (Fig. 2) paralleled that of plasma. Lower levels of milk TBARS (P<0.05) remained throughout the post-injection week.

3.4. Preformed and after copper ion induction of lipid peroxides in LDL

The amount of lipid hydroperoxides in LDL was estimated using a ferrous oxidation–xylenol orange assay system. Preliminary studies indicated that the TBA test failed to detect differences in the lipid hydroperoxide content of LDL from goats caused by vitamin E supplementation and by copper ion incubation (data not shown). The level of preformed lipid hydroperoxides in freshly isolated LDL (10 min) was not apparently different (P>0.05) before and after vitamin E supplementation (Fig. 3). Incubation with copper

![Fig. 2. Concentration of thiobarbituric acid reactive substances (TBARS) in plasma and milk of lactating goats (C. ibex ibex) before and different days after vitamin E injection. Single dose of 3000 IU d-α-tocopheryl acetate was applied intramuscularly. Values are means±S.E.M. of four animals. *Significantly different from the before value, P<0.05.](image)
ion for 3 and 6 h accelerated the formation of lipid hydroperoxides in LDL before vitamin E supplementation. Therefore, more \( P<0.05 \) lipid hydroperoxides were accumulated in LDL before vitamin E supplementation as compared to those accumulated in LDL at 2 and 4 days post vitamin E injection.

3.5. Preformed and after copper ion induction of lipid peroxides in MFGM

The amount of lipid hydroperoxides in MFGM was expressed as TBARS content. As displayed in the upper panel of Fig. 4, the amount of preformed lipid hydroperoxides in newly prepared MFGM was not \( P>0.05 \) different before and after vitamin E supplementation. On the other hand, as shown in the lower panel of Fig. 4, incubation with copper ion for 3 h increased the levels of TBARS in all MFGM preparations, regardless of times from vitamin E injection. Furthermore, the accumulation of lipid hydroperoxides was slower \( P<0.05 \) in MFGM during the period from day 2 to day 5 post vitamin E injection.

4. Discussion

In the present study, we assessed the effects of vitamin E on peroxidation status of ruminants that have long been assumed to be physiologically at a low oxidative risk due to their plasma lipid characteristics. We studied the overall peroxidation status of lactating goats and their peroxidation status in mammary gland—an organ with high metabolic rates during lactation. Effects of antioxidants on mammary gland function may be more pronounced during lactation than otherwise because of the increased release of oxidative radicals associated with higher metabolic activity. Overall peroxidation status was indicated in this study using the content of peroxidation products (TBARS) in plasma. Content of peroxidation products in plasma represents the compromised outcome of whole blood resistance to free radical aggression, taking into account all the body reserves since blood circulates through every kind of molecular and enzymatic antioxidant equipment. It is the result of the overall antioxidant defense plus the overall oxidative stress being experienced in the animal. Based on similar concepts, peroxidation status in mammary gland was indicated in our study using the content of peroxidation products in milk.

The results we obtained for plasma lipids of lactating goats were not outside of predicted values for ruminants, if not totally similar to other related studies. The levels of plasma TG and total cholesterol of lactating goats obtained in the present study were within the range reported previously for mature goats not indicating sex and physiological stage (Christie, 1981). A comparative study on male ruminants in Saudi Arabia (Al Senaidy, 1996) reported values of plasma cholesterol for sheep and plasma TG for bulls of about half of those we obtained for lactating goats. The greater amount of plasma lipids represents not only characteristics of goats, but might also indicate the demand of lipid substrates by secreting mammary glands. As for lipoprotein profiling, the percentage of HDL in plasma lipoprotein of lactating goats obtained was within the range of 60% (sheep) to 90% (cattle) reported elsewhere (Christie, 1981). That for LDL in lactating goats was found closer to the upper edge in a relatively wider variation from 6% (cattle) to 34% (sheep) for ruminants (Christie, 1981). Taking into account the cholesterol plus cholesterol ester composition of HDL and LDL of ruminants, which amounts to 60–80% and 57–61%, respectively (Christie, 1981), the...
The majority of plasma cholesterol of lactating goats would be carried in the form of HDL and much less in LDL. This is in extreme contrast to humans where LDL cholesterol comprises about 6/7 of the total plasma cholesterol and HDL cholesterol comprises the remaining 1/7 (Qureshi et al., 1991). Based on the above observations, it is very likely that, as a member of ruminants, goats ought to experience much less internal oxidative stress than humans.

Pretreatment \( \alpha \)-tocopherol concentrations in plasma were similar between lactating goats in our study and lactating cows (Jukola et al., 1996; Trout et al., 1998), but were much lower in camel, bulls and sheep (Al Senaidy, 1996). Jensen and Nielsen (1996) reported that the basal level of \( \alpha \)-tocopherol in plasma of cows fed a vitamin E-poor diet was in the range 1.36–1.79 mg/mL, whereas cows fed a diet adequate in vitamin E had a basal plasma level of 3.82 mg/mL. The pretreatment \( \alpha \)-tocopherol concentration in plasma of lactating goats of the present study is well above the adequate level. Accordingly, \( \alpha \)-tocopherol concentration in milk of lactating goats of the present study was relatively high among studies. A significant relationship between contents of \( \alpha \)-tocopherol in milk and plasma of dairy cows has recently been recorded following the enrichment of \( \alpha \)-tocopherol in the plasma lipid fraction using vitamin E in diets of various degrees of polyunsaturated fatty acid composition (Weiss and Wyatt, 2003). Although the \( \alpha \)-tocopherol contents of isolated LDL and MFGM in our study were not determined, the results of Gurusinghe et al. (1988) and Jensen and Nielsen (1996) indicated that their contents of \( \alpha \)-tocopherol would have increased accordingly with the elevation of \( \alpha \)-tocopherol in plasma and milk after vitamin E injection.

Fig. 4. Concentration of thiobarbituric acid reactive substances (TBARS) in milk fat globule membrane (MFGM) of lactating goats (C. ibex ibex) before and different days after vitamin E injection. Single dose of 3000 IU \( d \)-\( \alpha \)-tocopheryl acetate was applied intramuscularly. TBARS preformed in situ (upper panel) and TBARS accumulated after incubation with \( \text{Cu}^{2+} \) for 3 h (lower panel) were determined. Values are means \( \pm \) S.E.M. of four animals. *Significantly different from before value, \( P<0.05 \).
Observing the changes in levels of peroxides in both plasma and milk of lactating goats following vitamin E injection (Fig. 2), it is suggested that the effect of surplus vitamin E, at the present administration regime and dosage, in further improving the overall peroxidation-resistant mechanisms of lactating goats and those specifically in mammary gland is best at day 2 post-injection and is effective for about a week. On the contrary, surplus vitamin E was apparently not effective in decreasing the extent of peroxidation of LDL (Fig. 3, 10 min) and MFGM (Fig. 4, upper panel) that occurred endogenously prior to isolation. The lack of responsiveness to vitamin E enrichment of in situ LDL and MFGM peroxidation can be approached in two ways. First, it is likely that antioxidant concentrations in LDL and MFGM are already more than sufficient to neutralize the endogenous oxidative attack. Study found out that marginal pretreatment vitamin E status has been an important factor for vitamin E to be effective in reducing the risk of retained placenta. (LeBlanc et al., 2002). It is not practical, though, to accurately measure in vivo peroxidation of LDL and MFGM as much of the lipid hydroperoxides were reported formed mostly ex vivo (Frei and Gaziano, 1993). Secondly, vitamin E might not necessarily add to the overall antioxidant activity under the biological environments of LDL and MFGM. Considering the differences in sensitivity to anti-peroxidation protection by surplus vitamin E between biological fluids (plasma and milk) and individual lipid milieus (LDL and MFGM), apparently peroxidation reactions proceeding within the more restrained biological environments were somehow different from those of wider scopes and more versatile mechanisms. Frei and Gaziano (1993) indicated that LDL subjects to metal ion-dependent and -independent oxidative stress with different susceptibilities. They stated that vitamin E is capable of exerting either prooxidant activity against aqueous peroxy radical-induced LDL peroxidation or antioxidant activity against metal ion-induced LDL oxidation. In primates and rabbits, the protective effects of vitamin E supplementation against experimental atherosclerosis (Wojcicki et al., 1991; Verlangieri and Bush, 1992) and against coronary heart disease observed in epidemiologic studies (Rimm et al., 1993; Stampfer et al., 1993) suggest that LDL oxidation occurs inside these animals by a metal ion-dependent mechanism. We postulate that the actions of vitamin E in lactating goats depend on the type(s) of peroxidation reaction that prevails in LDL and MFGM before isolation. The hypothesis that the spontaneous peroxidation occurring in LDL and MFGM inside lactating goats by a metal ion-independent mechanism is worth further investigation.

The acceleration of LDL and MFGM peroxidation by metal ion challenge was also measured in the present study to assess the effect of vitamin E enrichment. This methodology is commonly used to reflect peroxidation susceptibility of LDL (Ramireq and Aguilera, 1998), but was applied for the first time to assess MFGM peroxidation. Our results indicated that vitamin E supplementation attenuated the extent of in vitro peroxidation of both LDL (Fig. 3, 3 and 6 h) and MFGM (Fig. 4, lower panel) following copper ion challenge. Both lipid milieus, coincident with previous observations on TBARS levels in plasma and milk, were seen most responsive during day 2 to day 4 post-injection when the enrichment of vitamin E was most prominent. This result could again be viewed from two directions. First, vitamin E better benefits conditions of extreme oxidative attack when the requirement of antioxidant exceeds the endogenous store. It has been noticed that effects of antioxidants on reproductive function were more pronounced during heat stress because of the increased metabolic rates associated with cellular hyperthermia (Paula-Lopes et al., 2003), as elevated temperature increases liver peroxidation (Ando et al., 1997) and activity of enzymes involved in free radical production such as xanthine oxidase (Skibba et al., 1989). Also, vitamin E is effective as an antioxidant only under the circumstances of metal ion-induced peroxidation. This result indirectly confirms our previous assumptions that in vivo peroxidation of LDL and MFGM in lactating goats is relatively minor and proceeds with metal ion-independent mechanisms.

In conclusion, single injection of a high dosage of vitamin E to lactating goats effectively improved the peroxidation-resistant mechanisms of the overall body and those specifically within mammary gland. Although endogenous peroxidation of the two important lipid milieus, LDL and MFGM, seemed not positively responsive, surplus vitamin E did increase their resistance to copper ion challenge in vitro. This study provides indirect evidences that peroxidation of LDL and MFGM of lactating goats in situ is minor and proceeds with metal ion-independent mechanisms.

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References


