Vitamin E Supplementation of Human Macrophages Prevents Neither Foam Cell Formation Nor Increased Susceptibility of Foam Cells to Lysis by Oxidized LDL

Reto Asmis, Jennifer Jelk

Abstract—Several studies in macrophage cell lines, rodent macrophages, and animal models of atherosclerosis suggest that vitamin E may prevent the formation of foam cells. We tested this hypothesis in a recently developed, fully autologous in vitro model of human foam cell formation. During maturation, macrophages continuously increased their α-tocopherol/total cholesterol ratio, demonstrating that these cells accumulate α-tocopherol at an even higher rate than cholesterol. In the presence of unsupplemented serum, we observed no correlation between serum vitamin E levels and the increase in the cellular α-tocopherol/total cholesterol ratio. In contrast, under supplemented conditions, a 3.1-fold increase in the mean serum α-tocopherol/total cholesterol ratio resulted in a corresponding mean 3.5-fold increase in the cellular α-tocopherol/total cholesterol ratio. Vitamin E loading had no effect on the lipid composition of macrophages and did not affect their growth. Foam cell formation was stimulated in mature unsupplemented and vitamin E–loaded macrophages for 1 week with 50 μg autologous aggregated low density lipoprotein (LDL) in the presence of unsupplemented and vitamin E–loaded serum, respectively. We observed no effect of vitamin E supplementation on the formation of foam cells. However, foam cell formation resulted in a 36% and 44% reduction in the cellular α-tocopherol/total cholesterol ratio in unsupplemented and vitamin E–supplemented foam cells, respectively. The loss of vitamin E was accelerated with increasing concentrations of aggregated LDL and was accompanied by an increase in the susceptibility of these foam cells to succumb to the cell lytic effects of oxidized LDL (OxLDL). However, vitamin E supplementation did not protect macrophages or foam cells from OxLDL-mediated cell lysis, suggesting that vitamin E loss in foam cells is not the cause of their increased susceptibility to cell lysis. Our results suggest that the beneficial effects of vitamin E on cardiovascular disease observed in humans are due neither to a reduction in the propensity of macrophages to form foam cells nor to an increased resistance of these cells to cytolytic OxLDL. (Arterioscler Thromb Vasc Biol. 2000;20:2078-2086.)

Key Words: vitamin E ■ oxidized LDL ■ macrophages ■ foam cells ■ cell death

Data from a large number of studies strongly suggest that a suboptimal status of any major dietary antioxidant, including vitamin E, increases the risk of cardiovascular disease (for review, see Reference 1). In particular, the Nurses’ Health Study2 and the Health Professionals Follow-Up Study3 support the concept that the use of vitamin E supplements is associated with a decreased risk of coronary heart disease. Probably the most intriguing link between plasma antioxidants and atherogenesis is the oxidative modification of LDL as put forward in the “LDL oxidation hypothesis”4,5 and the “antioxidant hypothesis of atherosclerosis.”6–8 LDL oxidation appears to play an important role in atherosclerosis.9 In vitro, oxidized LDL (OxLDL) possesses a wide array of potentially atherosclerotic properties.10 It is implicated in the recruitment of monocytes into the subendothelial space11 and in the inhibition of tissue macrophage motility.12 In particular, the cytotoxic effects of OxLDL on endothelial and smooth muscle cells13 as well as on macrophages14 are likely to contribute to the progression of atherosclerotic lesions. In humans, massive vitamin E supplementation improves the oxidation resistance of isolated LDL,15 which might explain the antiatherosclerotic properties of vitamin E. However, vitamin E determines only ≈20% of the oxidation resistance of LDL.16,17 Furthermore, evidence suggests that OxLDL may not exist in plasma and that only oxidation of LDL in the subendothelial space is of importance in the initiation and progression of atherosclerotic lesions.10 Hence, the beneficial effects of vitamin E could extend beyond the prevention of LDL oxidation. Data obtained from experiments in macrophage cell lines18–20 and mouse peritoneal macrophages21 as well as from ex vivo22 and in vivo studies in rodents23–27 suggest that vitamin E prevents foam cell formation, the hallmark of the earliest detectable atherosclerotic lesions. We tested this hypothesis in human monocyte–derived...
The methods for isolation and culture of human monocyte-derived macrophages, lipid extraction, and lipoprotein analysis are described in detail. The isolation of LDL was performed using a discontinuous gradient density and flotation ultracentrifugation in a TL-100 ultracentrifuge. The remaining cell fractions were washed with PBS to remove platelets. Human mononuclear cells were isolated by density gradient centrifugation on low endotoxin Ficoll-separating solution, washed, and cultured in polytetrafluoroethylene (Teflon) bags for 2 weeks as described elsewhere. Autologous serum was obtained from the remaining 50 mL of blood. Macrophage yields and their rates of adherence were essentially quantitative, as shown previously.

**Lipoproteins**

Autologous human LDL was isolated by discontinuous gradient density and flotation ultracentrifugation in a TL-100 ultracentrifuge (Beckman Instruments) equipped with a TLA-100.4 fixed-angle rotor, as described previously. LDL was concentrated by ultracentrifulation in Centricon-100 concentrators (Amicon) and was further purified by gel filtration chromatography on Excellulose GF-5 columns (Pierce). To prepare aggregated lipoproteins, 2 mg of -acetyl-L -alanyl- L -glutamine (2 mmol/L, Biochrom), nonessential amino acids (1% [vol/vol], Biochrom), penicillin G/streptomycin (100 U/mL and 100 µg/mL, respectively, Biochrom), and HEPES (10 mmol/L, Fluka Chemie AG) were added. All solutions were routinely tested for endotoxin (LAL-Pyrogen test, Skan AG). Endotoxin levels of the plates were analyzed with a Personal Laser Densitometer (Molecular Dynamics). Spot integration was performed with ImageQuant (Molecular Dynamics). Standard curves were designed to fit with the SigmaPlot graphics program (Jandel Scientific), as described previously. Cholesterol, cholesteryl oleate, and trioleate were used as lipid mass standards. The molecular weight of cholesteryl oleate (651) was used to calculate total cholesterol levels in macrophages as nanomoles per microgram DNA.

**α-Tocopherol Measurements**

α-Tocopherol determinations were performed after lipid extraction of cells or serum (see above). Lipid extracts were separated by reverse-phase high-performance liquid chromatography with a 5-mm C-18 Brownlee column (220×4.6 mm, Applied Biosystems) and methanol used as an eluant (1.5 mL/min). α-Tocopherol was detected fluorometrically with a Jasco Spectrofluorometer (model 821-FP, Japan Spectroscopic Co) set at an excitation wavelength of 295 nm and an emission wavelength of 325 nm.

**Measurement of Cellular DNA**

To determine the cell number in each well, the amount of DNA was determined fluorometrically with use of the fluorescent dye 4',6-diamidino-2-phenylindole as described by Brunk et al. Fluorescence was measured in a fluorescence spectrophotometer (model 204-A, Perkin-Elmer) set to an excitation wavelength of 360 nm and an emission wavelength of 450 nm, with both slits set to 10 nm. Cellular DNA content is 9.1 µg DNA per 10^6 cells, as determined previously.

**Assays for Serum Lipids**

Serum triglyceride and cholesterol levels were measured with commercial kits (Unimate 5, Hoffmann LaRoche AG).

**Membrane Integrity Assay**

Membrane integrity was assessed by the [3H]adenine release method described by Reid and Mitchinson. Briefly, macrophages were loaded with [8-3H]adenine (1 µCi/mL, Amersham) in culture medium with 5% human autologous serum for 2 hours and washed twice with PBS for 10 minutes at 37°C. Cells were then incubated for 48 hours with culture medium, either alone or supplemented with Cu2+ -oxidized LDL; the radioactivity released into the supernatant was measured. LDL oxidation and purification were performed, as described previously. Intracellular radioactivity was measured after cell lysis with 1% (by volume) Triton X-100. Radioactivity was determined by scintillation counting.

**Results**

Macrophages Accumulate Vitamin E During Their Differentiation

Differentiation of monocytes into 2-week-old mature macrophages is characterized by cell growth and a concurrent increase in vitamin E content. This increase is due to vitamin E uptake and the synthesis of vitamin E-containing lipids.
Massive increase in cellular free cholesterol and triglycerides while cholesteryl ester levels decrease. In adherent cultures, 2-week-old mature macrophages continue to grow in size and in the process accumulate more cholesterol and triglycerides. In the present study, we show that vitamin E is also accumulated during this process. For these experiments, 2-week-old mature macrophages from 8 male volunteers, aged 32 to 53 years, were incubated with autologous serum and also after 1 week (day 7, Figure 1). On both days, supplemented macrophages had a 3.5-fold higher level of vitamin E than did unsupplemented cells, which corresponds with the mean increase in serum vitamin E after supplementation.

Increasing Cellular Vitamin E Does Not Affect Neutral Lipid Levels in Human Macrophages

To assess the effect of vitamin E supplementation on the cellular neutral lipid composition, we extracted macrophages from 8 donors on day 0 and day 7 and analyzed their neutral lipid content. Vitamin E loading affected neither mean cellular cholesterol (Figure 2A) nor mean cholesteryl ester (Figure 2B) levels. Mean triglyceride levels in α-tocopherol–loaded macrophages were increased by 18% and 15% on day 0 and day 7, respectively, but these increases were not statistically significant (Figure 2C; by Student t test, P=0.25 and P=0.31, respectively).

Increasing Cellular Vitamin E Does Not Affect Foam Cell Formation

We evaluated the effect of vitamin E loading of human macrophages on their ability to transform into foam cells. Unsupplemented and vitamin E–loaded macrophages from 8 individuals were incubated with 50 μg aggregated autologous LDL to stimulate cholesteryl ester accumulation. Foam cell formation was measured as the aggregated LDL-induced increase in cholesteryl esters compared with macrophages incubated in the absence of aggregated LDL. Changes in cholesteryl ester levels due to cell growth were measured separately in each experiment, and values for foam cell formation were corrected accordingly. Vitamin E supplemen-
Foam Cell Formation Dramatically Decreases Cellular Vitamin E Levels

The transformation of human macrophages into foam cells induced by 50 μg aggregated LDL is characterized by a sharp increase in cholesteryl esters, whereas cholesterol and triglyceride levels essentially remain unchanged. In the present study, we show that lipid-standardized α-tocopherol levels decrease dramatically during foam cell formation (Figure 4). We observed an average reduction of the α-tocopherol/total cholesterol ratio in unsupplemented foam cells of 36 ± 15% and 44 ± 12%, respectively. However, the cellular concentration of vitamin E (expressed as picomoles α-tocopherol per microgram DNA) essentially remained unchanged in unsupplemented cells (+1 ± 9%) and declined only marginally in vitamin E-supplemented foam cells (−18 ± 13%).

Foam Cell Formation Increases Susceptibility to OxLDL-Induced Cell Lysis

As an integral part of the cellular antioxidant defense, vitamin E protects cell membranes from oxidative damage. To test whether the observed reduction in cellular vitamin E results in an increased susceptibility to oxidative damage, we incubated human macrophages with 100 and 200 μg aggregated LDL to induce massive foam cell formation. For both concentrations of aggregated LDL, we observed a 30-fold...
growth of massive foam cell formation, we also observed a net loss of vitamin E from 179 to 93 and 74 pmol/mg cholesterol ratio decreased from 32.7 to 9.0 and 7.7 pmol/mmol, respectively. Interestingly, under these conditions of massive foam cell formation, we also observed a net loss of cellular vitamin E from 179 to 93 and 74 pmol/mg DNA, respectively. However, this loss of vitamin E only marginally affected the rate of survival of foam cells cultured under serum-free conditions (Figure 5, control). In contrast, foam cells exposed for 48 hours to 100 μg OxLDL in the absence of serum showed a marked increase in the loss of membrane integrity compared with control macrophages. In the presence of serum, no cytotoxicity of OxLDL was observed in macrophages or foam cells (not shown). Foam cells with a vitamin E level of 9.0 μmol/mmol total cholesterol were as resistant to 50 μg OxLDL as were control macrophages. However, foam cells with a vitamin E level of 7.7 μmol/mmol showed a marked increase in membrane damage. In the presence of 100 μg OxLDL, cell death increased to 42% in macrophages and 56% and 86% in foam cells with a vitamin E/total cholesterol ratio of 9.0 and 7.7 μmol/mmol, respectively.

Loss of Cellular Vitamin E Does Not Correlate With Increased Cell Lysis by OxLDL

Macrophages from 4 individuals were transformed into foam cells as described above (see Foam Cell Formation Increases Susceptibility to OxLDL-Induced Cell Lysis) and exposed to 100 μg OxLDL to induce cell lysis. For all 4 individuals, we obtained results similar to the ones shown in Figure 5. Cellular vitamin E levels of foam cells decreased with increasing levels of aggregated LDL. Concurrently, increasing the amount of aggregated LDL to induce foam cell formation resulted in an increased susceptibility to cell lysis. However, when cell lysis was plotted against either cellular vitamin E levels (expressed as picomoles α-tocopherol per microgram DNA, Figure 6) or against the cellular α-tocopherol/total cholesterol ratio (not shown), we observed no statistically significant correlation between the parameters.
E-loaded foam cells were 28.1, 134.6, 18.7, and 59.8
E-loaded macrophages and unsupplemented and vitamin
Cellular vitamin E levels of unsupplemented and vitamin
unsupplemented or vitamin E–supplemented autologous serum.

cells were incubated for 48 hours either in the absence (control)
macrophages to transform into foam cells varies
We have shown in a previous study that the propensity of human
marginal.
were unsupplemented cells, although the increase was only
induced cell lysis in either cell type. Vitamin E–supplemented
protection by vitamin E supplementation from OxLDL-
and foam cells, respectively, we observed no significant
increased susceptibility to OxLDL-induced cell lysis.

Increasing Cellular Vitamin E Does Not Protect
Macrophages and Foam Cells From Cell Lysis
by OxLDL
To verify that cellular vitamin E does not protect cells from
the cell lytic properties of OxLDL, macrophages were loaded
with vitamin E. Unsupplemented and vitamin E–supple-
mented macrophages as well as unsupplemented and vitamin
E–supplemented foam cells were exposed to cytolytic
OxLDL for 48 hours (Figure 7). Even though cellular vitamin
E levels were increased 4.8-fold and 3.2-fold in macrophages
E–loaded macrophages and unsupplemented and vitamin
E–loaded foam cells were 28.1, 134.6, 18.7, and 59.8
µmol/mmol total cholesterol, respectively. Half of the cells from
each group were harvested, and neutral lipids and α-tocopherol
levels were measured; the other half of the cells were loaded
with [3H]adenine, as described in Methods. [3H]Adenine–loaded
cells were incubated for 48 hours either in the absence (control)
or presence of 100 µg Cu2+-oxidized LDL (OxLDL).

(r = 0.47 and r = 0.46, respectively). These results suggest that
the loss of vitamin E observed during the transformation of
macrophages into foam cells is not the cause of their
increased susceptibility to OxLDL-induced cell lysis.

Discussion
We have shown in a previous study that the propensity of human
macrophages to transform into foam cells varies >7-fold among
male volunteers, whereas foam cell formation in an individual
varies <25% over a 10-month period.28 Earlier results from
vitamin E supplementation studies in rodents,22–27 rodent mac-
rophages,21 and macrophage cell lines18–20 have suggested that
variations in the vitamin E levels of human macrophages may
account for the large variations in foam cell formation that we
had observed between different individuals. Therefore, we in-
vestigated whether human foam cell formation is attenuated by
vitamin E.

To prevent artifacts and minimize the variability of our
results, we obtained loaded and untreated macrophages from
the same blood sample of a given donor to eliminate any
intraindividual differences. The serum concentrations of α-toc-
opherol that we have reported were higher than those
reported in other studies for healthy donors from Austrian,38
Scottish,39 or Finnish cohorts40 but were within the range
determined for other Swiss cohorts.38,41,42 Finally, to prevent
artifacts due to solvents such as ethanol or dimethyl sulfox-
ide, we used a technique to supplement serum that does not
require organic solvents and, instead of forming mainly
liposomes, incorporates vitamin E into lipoproteins.31

In good agreement with results from an earlier study by
Garner et al,41 we have shown in our previous study that
human monocytes cultured in autologous serum from fasted
subjects increase their neutral lipid mass dramatically during
their differentiation into mature macrophages.28 In the present
study, we demonstrate that this also holds true for vitamin E.
Not only do mature macrophages continue to accumulate
vitamin E, but they accumulate vitamin E at a higher rate than
cholesterol, as evidenced by the increase in the α-tocopher-
ol/total cholesterol ratios. We measured α-tocopherol/total
cholesterol ratios of 1 to 2 µmol/mmol in 1-day-old mono-
cytes (R.A., unpublished data, 2000). After 2 weeks of
nonadherent culture, this ratio increased to 10.1 to 26.4
µmol/mmol (day 0, Table). During the course of the exper-
iments, eg, from day 0 to day 7, the α-tocopherol/total
cholesterol ratio increased further for all donors (on average,
2.2-fold). The triglyceride content of human monocytes
increases 270-fold during the same 3-week maturation peri-
od.28 Therefore, it is likely that the maturing monocyte-
derived macrophage increases its α-tocopherol content in an
attempt to protect the large numbers of newly acquired fatty
acids from oxidation.

Interestingly, the serum α-tocopherol level in the present
study was no predictor of the degree of vitamin E accumu-
lation in macrophages from a given donor (Table). Even
though the transfer of α-tocopherol from lipoproteins to
macrophages appears to occur mainly by diffusion,31 not all
lipoproteins may contribute equally. Ziouzenkova et al38
reported in a study of 59 healthy human subjects that no
correlation was found between the α-tocopherol content of
plasma and LDL. Hence, the distribution of lipoprotein
species, their vitamin E levels, and their respective numbers
in serum are likely to determine the rate of vitamin E
transport into macrophages. By increasing the overall vitamin
E content of a given serum in vitro, ie, loading by diffusion,
we increased the ability of all lipoproteins that act as vitamin
E donors to transfer α-tocopherol to macrophages. The
degree of vitamin E supplementation most likely depends on
the total lipid content of the lipoproteins, not their initial
vitamin E level. This would explain why a 3.1-fold increase
in the serum α-tocopherol/cholesterol ratio resulted in a
3.5-fold increase in the cellular α-tocopherol/cholesterol ratio
despite the apparent lack of correlation between serum and
cellular vitamin E levels under unsupplemented conditions.

We had shown in our previous study that no “oxidative
burden,” eg, LDL oxidation, is required to induce foam cell
formation.28 In fact, OxLDL must be aggregated to induce

Figure 7. Vitamin E supplementation of macrophages and foam
cells does not increase resistance to cell lysis by OxLDL.
Unsupplemented macrophages (open bars) and vitamin
E–loaded macrophages (shaded bars) from the same blood
sample were plated and cultured for 3 days either in the absence (macrophages) or presence of 200 µg aggregated LDL
per milliliter (foam cells), in the presence of the corresponding
unsupplemented or vitamin E–supplemented autologous serum.
Cellular vitamin E levels of unsupplemented and vitamin
E–loaded macrophages and unsupplemented and vitamin
E–loaded foam cells were 28.1, 134.6, 18.7, and 59.8
µmol/mmol (day 0, Table). During the course of the exper-
iments, eg, from day 0 to day 7, the α-tocopherol/total
cholesterol ratio increased further for all donors (on average,
2.2-fold). The triglyceride content of human monocytes
increases 270-fold during the same 3-week maturation peri-
od.28 Therefore, it is likely that the maturing monocyte-
derived macrophage increases its α-tocopherol content in an
attempt to protect the large numbers of newly acquired fatty
acids from oxidation.

Interestingly, the serum α-tocopherol level in the present
study was no predictor of the degree of vitamin E accumu-
lation in macrophages from a given donor (Table). Even
though the transfer of α-tocopherol from lipoproteins to
macrophages appears to occur mainly by diffusion,31 not all
lipoproteins may contribute equally. Ziouzenkova et al38
reported in a study of 59 healthy human subjects that no
correlation was found between the α-tocopherol content of
plasma and LDL. Hence, the distribution of lipoprotein
species, their vitamin E levels, and their respective numbers
in serum are likely to determine the rate of vitamin E
transport into macrophages. By increasing the overall vitamin
E content of a given serum in vitro, ie, loading by diffusion,
we increased the ability of all lipoproteins that act as vitamin
E donors to transfer α-tocopherol to macrophages. The
degree of vitamin E supplementation most likely depends on
the total lipid content of the lipoproteins, not their initial
vitamin E level. This would explain why a 3.1-fold increase
in the serum α-tocopherol/cholesterol ratio resulted in a
3.5-fold increase in the cellular α-tocopherol/cholesterol ratio
despite the apparent lack of correlation between serum and
cellular vitamin E levels under unsupplemented conditions.

We had shown in our previous study that no “oxidative
burden,” eg, LDL oxidation, is required to induce foam cell
formation.28 In fact, OxLDL must be aggregated to induce
cholesteryl ester accumulation in human macrophages. According to the “response-to-retention” hypothesis, aggregated and fused LDL particles are likely to be the first modifications of LDL that occur in the vessel wall. Therefore, in the present study, we used in vitro aggregated LDL as a model of early atherogenic LDL to induce foam cell formation. However, we observed no effect of vitamin E supplementation on cholesteryl ester accumulation or any other lipid parameter we measured in our fully autologous human foam cell model. This suggests that in humans, the effect of vitamin E supplementation on early lesion formation is not due to an altered propensity of macrophages to transform into foam cells. However, increased vascular vitamin E levels may reduce the formation of foam cell–inducing aggregates, which in turn would result in fewer foam cells. In apoE-deficient mice, vitamin E supplementation not only reduces LDL oxidation but also its state of aggregation. The reduction in both parameters was accompanied by a decrease in the aortic lesion area. At least in this mouse model of atherogenesis, vitamin E supplementation appears to reduce foam cell formation indirectly by preventing the oxidative modification and subsequent aggregation of LDL.

Contrary to our results in human macrophages, several reports on experiments in cell lines suggest that vitamin E supplementation directly reduces foam cell formation. Particularly, the evidence from experiments in cell lines should be treated with caution. Macrophage cell lines, in contrast to human macrophages, are characterized by high proliferation rates and very low vitamin E levels, which makes these cell lines poor models of human foam cell formation. Exposure to high levels of vitamin E might significantly affect their “normal” lipid metabolism.

Data from several animal studies appear to support an inhibitory role of vitamin E on foam cell formation and the appearance of fatty streaks. Vitamin E levels in the diet of laboratory animals vary dramatically from country to country. This may explain why at least one study in C57BL/6 mice found no reduction in fatty streak formation after vitamin E supplementation. In some studies, animals were fed α-tocopherol doses that were 10-fold to 30-fold higher than those in the animals’ regular diet. Hence, extrapolation of data from animal models to the human may not be warranted, at least not with regard to vitamin E supplementation and foam cell formation. In particular, macrophages from animals kept on a low vitamin E diet (30 mg/kg), as in France or the United States, are likely to respond differently to atherogenic stimuli, especially after massive vitamin E supplementation, than are cells from rodents that continuously received a high vitamin E diet (200 mg/kg), as in Germany.

In human cardiovascular disease, epidemiological data support a protective role of antioxidants. However, intervention trials have yielded rather mixed results. Our results would predict that vitamin E supplementation in humans would not significantly reduce lesion formation because the development of fatty streaks, the hallmark of early atherosclerotic lesions, would not be prevented. Indeed, in humans, vitamin E supplementation showed a modest effect at best on the development and progression of atherosclerosis. The observed modest benefits of vitamin E may be due to protective effects on the endothelium or the inhibition of smooth muscle cell proliferation rather than a reduction in foam cells.

Although vitamin E has no effect on human foam cell formation, we found a dramatic effect of aggregated LDL on macrophage α-tocopherol levels. At low concentrations of aggregated LDL (50 μg/mL), only the α-tocopherol/total cholesterol ratio was reduced, but the total cellular vitamin E level was not significantly affected. The average α-tocopherol/total cholesterol ratio in native LDL isolated from 21 Swiss male volunteers is 3.80 ± 0.59 μmol/mmol (R.A., unpublished data, 2000), which is 2.7-fold to 11.8-fold lower than the cellular α-tocopherol/total cholesterol ratio in mature macrophages (see Table). Therefore, the massive uptake and accumulation of aggregated LDL would contribute far more cholesterol than vitamin E to the cells and thereby reduce the cellular α-tocopherol/total cholesterol ratio. In the presence of higher concentrations of aggregated LDL (100 and 200 μg/mL), macrophages showed a marked reduction not only in their cellular α-tocopherol/total cholesterol ratio but also in their α-tocopherol/DNA ratio; i.e., they actually lost vitamin E per cell. On the basis of our studies in P388D1 cells, we believe that concurrently with the uptake of aggregated LDL–derived cholesterol, some cellular cholesterol may already be exported or released by the cells in an attempt to reduce the rapidly increasing cellular cholesterol levels. Because vitamin E tends to migrate along with cholesterol, it is very likely that under these conditions of massive foam cell formation, significant amounts of cellular vitamin E leave the macrophages along with cholesterol.

Surprisingly, the net loss of vitamin E was not correlated with the increased susceptibility of foam cells to OxLDL-induced cell lysis. Furthermore, supplementation of macrophages and foam cells with vitamin E showed no protection from the cell lytic effect of OxLDL. Therefore, we conclude that the loss of vitamin E during the formation of foam cells is not the cause of their increased susceptibility to cell lysis. Why foam cells become more susceptible to cell lysis is unclear at this time. Because foam cell death may play a crucial role in the destabilization of atherosclerotic plaques, our results would indicate that vitamin E supplementation should not show any substantial benefits with regard to the clinical outcomes of plaque rupture. However, the Cambridge Heart Antioxidant Study (CHAOS), a randomized, placebo-controlled trial, demonstrated a significant reduction of cardiovascular events by vitamin E. Patients in the CHAOS study received high doses (400 to 800 IU/d) of vitamin E. The “LDL oxidation hypothesis” predicts that vitamin E should prevent the oxidative modifications of LDL. However, according to more recent studies, it is unlikely that vitamin E supplementation would have reduced LDL oxidation in lesions from these patients. Whereas the α-tocopherol/cholesterol ratio is decreased in lesions, vitamin E levels are elevated in atherosclerotic plaque compared with healthy arterial tissue. Nevertheless, oxidized lipids and cytotoxic OxLDL are abundant in these advanced lesions. If vitamin E supplementation does not prevent or even reduce LDL oxidation in advanced lesions, the observed beneficial effects of high doses of vitamin E must lie elsewhere. A cytoprotective effect of extracellular vitamin E, similar to the one seen with serum in our macrophage lysis assay, may account for some of the benefits. The prevention of necrotic foam cell...
death most certainly would slow down the process of plaque destabilization, which otherwise would lead to plaque rupture and its clinical manifestations. However, at the high doses of vitamin E administered in the CHAOS study, the anti-thrombotic properties of vitamin E may have also contributed significantly to the observed reduction in cardiovascular events.

In conclusion, we suggest that the beneficial effects observed after the treatment of patients with vitamin E are not due to a reduced propensity of macrophages to transform into foam cells. Nor does vitamin E supplementation reverse the increased susceptibility of foam cells to OxLDL-induced cell lysis. Instead, the protection by increased extracellular α-tocopherol of macrophages and, in particular, foam cells from the cell lytic activity of OxLDL may explain at least some of the beneficial effects of vitamin E.

Acknowledgments

This work was supported by grants from the Swiss National Science Foundation and from Henkel Corp, La Grange, Ill. We would like to thank Kirsten Gallagher for critical discussions regarding this manuscript and for the assistance in its preparation.

References


Vitamin E Supplementation of Human Macrophages Prevents Neither Foam Cell Formation Nor Increased Susceptibility of Foam Cells to Lysis by Oxidized LDL
Reto Asmis and Jennifer Jelk

doi: 10.1161/01.ATV.20.9.2078
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/9/2078

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/