

## 5. Vitamin E

### 5.1 Role of vitamin E in human metabolic processes

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers (1). Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defence system normally protects cells from the injurious effects of endogenously produced free radicals as well as from species of exogenous origin such as cigarette smoke and pollutants. Should our exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (2), then damage to biological molecules may occur. There is considerable evidence that disease causes an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralize excess radicals, may play an important role in modifying the development of disease.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The term “vitamin E” refers to a family of eight naturally-occurring homologues that are synthesized by plants from homogentisic acid. All are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologues (*d*- $\alpha$ -, *d*- $\beta$ -, *d*- $\gamma$ -, and *d*- $\delta$ -) have a saturated 16-carbon phytyl side chain, whereas the four tocotrienols (*d*- $\alpha$ -, *d*- $\beta$ -, *d*- $\gamma$ -, and *d*- $\delta$ -) have three double bonds on the side chain. There is also a widely available synthetic form, *dl*- $\alpha$ -tocopherol, prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts; these isomers are differentiated by rotations of the phytyl chain in various directions that do not occur naturally.

For dietary purposes, vitamin E activity is expressed as  $\alpha$ -tocopherol equivalents ( $\alpha$ -TEs). One  $\alpha$ -TE is the activity of 1 mg *RRR*- $\alpha$ -tocopherol (*d*- $\alpha$ -tocopherol). To estimate the  $\alpha$ -TE of a mixed diet containing natural forms of vitamin E, the number of milligrams of  $\beta$ -tocopherol should be multiplied by 0.5,  $\gamma$ -tocopherol by 0.1, and  $\alpha$ -tocotrienol by 0.3. Any of the synthetic all-*rac*-

$\alpha$ -tocopherols (*dl*- $\alpha$ -tocopherol) should be multiplied by 0.74. One milligram of the latter compound in the acetate form is equivalent to 1 IU of vitamin E.

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, making them unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability (3).

The major biological role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation—a series of chemical reactions involving the oxidative deterioration of PUFAs (see Chapter 8 on antioxidants). Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (4). Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (5).

Absorption of vitamin E from the intestine depends on adequate pancreatic function, biliary secretion, and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilization within mixed bile salt micelles, uptake by enterocytes, and secretion into the circulation via the lymphatic system (6). Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates the vitamin E molecules, solubilizes the vitamin E, and then transports it to the brush border membrane of the enterocyte, probably by passive diffusion. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolysed in the small intestine before absorption.

Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein, lipase. During this process tocopherol can be transferred to high-density lipoproteins (HDLs). The tocopherol in HDLs can transfer to other circulating lipoproteins, such as LDLs

and very low-density lipoproteins (VLDLs) (7). During the conversion of VLDL to LDL in the circulation, some  $\alpha$ -tocopherol remains within the core lipids and is thus incorporated in LDL. Most  $\alpha$ -tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognizing apolipoprotein A-I and A-II present on HDL (8).

Although the process of absorption of all the tocopherol homologues in the diet is similar, the  $\alpha$  form predominates in blood and tissue. This is due to the action of binding proteins that preferentially select the  $\alpha$  form over other forms. In the first instance, a 30-kDa binding protein unique to the liver cytoplasm preferentially incorporates  $\alpha$ -tocopherol in the nascent VLDL (9). This form also accumulates in non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the membranes of mitochondria and endoplasmic reticulum in the heart and lungs (10).

Hepatic intracellular transport may be expedited by a 14.2-kDa binding protein that binds  $\alpha$ -tocopherol in preference to the other homologues (11). Other proteinaceous sites with apparent tocopherol-binding abilities have been found on erythrocytes, adrenal membranes, and smooth muscle cells (12). These may serve as vitamin E receptors which orient the molecule within the membrane for optimum antioxidant function.

These selective mechanisms explain why vitamin E homologues have markedly differing antioxidant abilities in biological systems and they illustrate the important distinction between the *in vitro* antioxidant effectiveness of a substance in the stabilization of, for example, a food product and its *in vivo* potency as an antioxidant. From a nutritional perspective, the most important form of vitamin E is  $\alpha$ -tocopherol; this is corroborated in animal model tests of biopotency which assess the ability of the various homologues to prevent fetal absorption and muscular dystrophies (Table 5.1).

Plasma vitamin E concentrations vary little over a wide range of dietary intakes. Even daily supplements of the order of 1600 IU/day for 3 weeks only increased plasma levels by 2–3 times and on cessation of treatment, plasma levels returned to pretreatment levels in 5 days (13). Similarly, tissue concentrations only increased by 2–3 times when patients undergoing heart surgery were given 300 mg/day of the natural stereoisomer for 2 weeks preoperatively (14). Kinetic studies with deuterated tocopherol (15) suggest that there is rapid equilibration of new tocopherol in erythrocytes, liver, and spleen but that turnover in other tissues such as heart, muscle, and adipose tissue is much slower. The brain is markedly resistant to depletion of, and repletion with, vitamin E (16). This presumably reflects an adaptive mechanism to avoid detrimental oxidative reactions in this key organ.

TABLE 5.1

**Approximate biological activity of naturally-occurring tocopherols and tocotrienols compared with *d*- $\alpha$ -tocopherol**

Common name	Biological activity compared with <i>d</i> - $\alpha$ -tocopherol (%)
<i>d</i> - $\alpha$ -tocopherol	100
<i>d</i> - $\beta$ -tocopherol	50
<i>d</i> - $\gamma$ -tocopherol	10
<i>d</i> - $\delta$ -tocopherol	3
<i>d</i> - $\alpha$ -tocotrienol	30
<i>d</i> - $\beta$ -tocotrienol	5
<i>d</i> - $\gamma$ -tocotrienol	Unknown
<i>d</i> - $\delta$ -tocotrienol	Unknown

The primary oxidation product of  $\alpha$ -tocopherol is  $\alpha$ -tocopheryl quinone that can be conjugated to yield the glucuronate after prior reduction to the hydroquinone. This glucuronide is excreted in the bile as such or further degraded in the kidneys to  $\alpha$ -tocopheronic acid glucuronide and hence excreted in the bile. Those vitamin E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile (17). Some vitamin E may also be excreted via skin sebaceous glands (18).

## 5.2 Populations at risk for vitamin E deficiency

There are many signs of vitamin E deficiency in animals, most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked by traces of peroxidized PUFAs in the diets of animals with low vitamin E status include cardiac or skeletal myopathies, neuropathies, and liver necrosis (19) (Table 5.2). Muscle and neurological problems are also a consequence of human vitamin E deficiency (20). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis.

The assessment of the vitamin E requirement for humans is confounded by the very rare occurrence of clinical signs of deficiency because these usually only develop in infants and adults with fat-malabsorption syndromes or liver disease, in individuals with genetic anomalies in transport or binding proteins, and possibly in premature infants (19, 21). This suggests that diets contain sufficient vitamin E to satisfy nutritional needs.

Work with several animal models (22) suggests that increasing intakes of vitamin E inhibits the progression of vascular disease by preventing the oxi-

TABLE 5.2

**Diseases and syndromes in animals associated with vitamin E deficiency and excess intakes of polyunsaturated fatty acids**

Syndrome	Affected organ or tissue	Species
Encephalomalacia	Cerebellum	Chick
Exudative diathesis	Vascular	Turkey
Microcytic anaemia	Blood, bone marrow	Chick
Macrocytic anaemia	Blood, bone marrow	Monkey
Pancreatic fibrosis	Pancreas	Chick, mouse
Liver necrosis	Liver	Pig, rat
Muscular degeneration	Skeletal muscle	Pig, rat, mouse
Microangiopathy	Heart muscle	Pig, lamb, calf
Kidney degeneration	Kidney tubules	Monkey, rat
Steatitis	Adipose tissue	Pig, chick
Testicular degeneration	Testes	Pig, calf, chick
Malignant hyperthermia	Skeletal muscle	Pig

Source: provided by GG Duthie, Rowett Research Institute, Aberdeen, United Kingdom.

dation of LDL. It is thought that oxidized lipoprotein is a key event in the development of the atheromatous plaque, which may ultimately occlude the blood vessel (23).

Human studies, however, have been less consistent in providing evidence for a role of vitamin E in preventing heart disease. Vitamin E supplements reduce *ex vivo* oxidizability of plasma LDLs but there is no correlation between *ex vivo* lipoprotein oxidizability and endogenous vitamin E levels in an unsupplemented population (24). Similarly, the few randomized double blind placebo-controlled intervention trials conducted to date with human volunteers, which focused on the relationship between vitamin E and cardiovascular disease, have yielded inconsistent results. There was a marked reduction in non-fatal myocardial infarction in patients with coronary artery disease (as defined by angiogram) who were randomly assigned to take pharmacologic doses of vitamin E (400 and 800 mg/day) or a placebo in the Cambridge Heart Antioxidant Study involving 2000 men and women (25). However, the incidence of major coronary events in male smokers who received 20 mg/day of vitamin E for approximately 6 years was not reduced in a study using  $\alpha$ -tocopherol and  $\beta$ -carotene supplementation (26). Furthermore, in the Medical Research Council/British Heart Foundation trial involving 20536 patients with heart disease who received vitamin E (600 mg), vitamin C (250 mg) and  $\beta$ -carotene (20 mg) or a placebo daily for 5 years, there were no significant reductions in all-cause mortality, or in deaths due to vascular or non-vascular causes (27). It was concluded that these antioxidant supplements provided no measurable health benefits for these patients.

Epidemiological studies suggest that dietary vitamin E influences the risk of cardiovascular disease. Gey et al. (28) reported that lipid-standardized plasma vitamin E concentrations in middle-aged men across 16 European countries predicted 62% of the variance in the mortality from ischaemic heart disease. In the United States both the Nurses Health Study (29), which involved 87 000 females in an 8-year follow-up, and the Health Professionals Follow-up Study of 40 000 men (30) concluded that persons taking supplements of 100 mg/day or more of vitamin E for at least 2 years had approximately a 40% lower incidence of myocardial infarction and cardiovascular mortality than those who did not. However, there was no influence of dietary vitamin E alone on incidence of cardiovascular disease when those taking supplements were removed from the analyses. A possible explanation for the significant relationship between dietary vitamin E and cardiovascular disease in European countries but not in the United States may be found in the fact that across Europe populations consume foods with widely differing amounts of vitamin E. Sunflower seed oil, which is rich in  $\alpha$ -tocopherol, tends to be consumed more widely in the southern European countries where a lower incidence of cardiovascular disease is reported, than in northern European countries where soybean oil, which contains more of the  $\gamma$  form, is preferred (31) (Table 5.3). A study carried out which compared plasma  $\alpha$ -tocopherol and  $\gamma$ -tocopherol concentrations in middle-aged men and women in Toulouse (southern France) with Belfast (Northern Ireland) found that the concentrations of  $\gamma$ -tocopherol in Belfast were twice as high as those in Toulouse;  $\alpha$ -tocopherol concentrations were identical in men in both countries but higher in women in Belfast than in Toulouse ( $P < 0.001$ ) (32).

It has also been suggested that vitamin E supplementation (200–400 mg/day) may be appropriate therapeutically to moderate some aspects of degenerative diseases such as Parkinson disease, reduce the severity of neurologic disorders such as tardive dyskinesia, prevent periventricular haemorrhage in pre-term babies, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers (33). However, very high doses may also induce adverse pro-oxidant effects (34), and the long-term advantages of such treatments have not been proven. In fact, a double blind study to determine the influence of vitamin E (200 mg/day) for 15 months on respiratory tract infections in non-institutionalized persons over 60 years found no difference in incidence between groups, but that the number of symptoms and duration of fever and restricted activity were greater in those receiving the vitamin (35).

TABLE 5.3

**Cross-country correlations between coronary heart disease mortality in men and the supply of vitamin E homologues across 24 European countries**

Homologue	Correlation coefficient, <i>r</i>
Total vitamin E	-0.386
<i>d</i> - $\alpha$ -tocopherol	-0.753 <sup>a</sup>
<i>d</i> - $\beta$ -tocopherol	-0.345
<i>d</i> - $\gamma$ -tocopherol	-0.001
<i>d</i> - $\delta$ -tocopherol	0.098
<i>d</i> - $\alpha$ -tocotrienol	-0.072
<i>d</i> - $\beta$ -tocotrienol	-0.329
<i>d</i> - $\gamma$ -tocotrienol	-0.210

<sup>a</sup> The correlation with *d*- $\alpha$ -tocopherol is highly significant ( $P < 0.001$ ) whereas all other correlations do not achieve statistical significance.

Source: based on reference (31).

### 5.3 Dietary sources and possible limitations to vitamin E supply

Because vitamin E is naturally present in plant-based diets and animal products and is often added by manufacturers to vegetable oils and processed foods, intakes are probably adequate to avoid overt deficiency in most situations. Exceptions may be during ecologic disasters and cultural conflicts resulting in food deprivation and famine.

Analysis of the FAO country food balance sheets indicates that about half the  $\alpha$ -tocopherol in a typical northern European diet, such as in the United Kingdom, is derived from vegetable oils (31). Animal fats, vegetables, and meats each contribute about 10% to the total per capita supply and fruit, nuts, cereals, and dairy products each contribute about 4%. Eggs, fish, and pulses contribute less than 2% each.

There are marked differences in per capita  $\alpha$ -tocopherol supply among different countries ranging from approximately 8–10 mg/person/day (e.g. Finland, Iceland, Japan, and New Zealand) to 20–25 mg/person/day (e.g. France, Greece, and Spain) (31). This variation can be ascribed mainly to the type and quantity of dietary oils used in different countries and the proportion of the different homologues in the oils (Table 5.4). For example, sunflower seed oil contains approximately 55 mg  $\alpha$ -tocopherol/100 g in contrast to soybean oil that contains only 8 mg/100 ml (36).

TABLE 5.4

**Vitamin E content of vegetable oils (mg tocopherol/100 g)**

Oil	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	$\alpha$ -tocotrienol
Coconut	0.5	0	0.6	0.5
Maize (corn)	11.2	60.2	1.8	0
Palm	25.6	31.6	7.0	14.3
Olive	5.1	Trace	0	0
Peanut	13.0	21.4	2.1	0
Soybean	10.1	59.3	26.4	0
Wheatgerm	133.0	26.0	27.1	2.6
Sunflower	48.7	5.1	0.8	0

Source: reference (36).

#### 5.4 Evidence used for estimating recommended intakes

In the case of the antioxidants (see Chapter 8), it was decided that there was insufficient evidence to enable a recommended nutrient intake (RNI) to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. Despite its important biological antioxidant properties, there is no consistent evidence that supplementing the diet with vitamin E protects against chronic disease. The main function of vitamin E, which appears to be that of preventing oxidation of PUFAs, has nevertheless been used by the present Consultation as the basis for proposing RNIs for vitamin E because of the considerable evidence in different animal species that low levels of vitamin E combined with an excess of PUFAs give rise to a wide variety of clinical signs.

There is very little clinical evidence of deficiency disease in humans except in certain inherited conditions where the metabolism of vitamin E is disturbed. Even biochemical evidence of poor vitamin E status in both adults and children is minimal. Meta-analysis of data collected within European countries indicates that optimum intakes may be implied when plasma concentrations of vitamin E exceed 25–30  $\mu\text{mol/l}$  of lipid-standardized  $\alpha$ -tocopherol (37). However, this approach should be treated with caution, as plasma vitamin E concentrations do not necessarily reflect intakes or tissue reserves because only 1% of the body tocopherol may be in the blood (38) and the amount in the circulation is strongly influenced by circulating lipid (39); nevertheless, a lipid-standardized vitamin E concentration (e.g. a tocopherol–cholesterol ratio) greater than 2.25 (calculated as  $\mu\text{mol}/\text{mmol}$ ) is believed to represent satisfactory vitamin E status (38, 39). The erythrocytes of subjects with values below this concentration of vitamin E may show evidence of an increasing tendency to haemolyse when exposed to oxidizing

agents and thus, such values should be taken as an indication of biochemical deficiency (40). However, the development of clinical evidence of vitamin E deficiency (e.g. muscle damage or neurologic lesions) can take several years of exposure to extremely low vitamin E levels (41).

Dietary intakes of PUFAs have been used to assess the adequacy of vitamin E intakes by United States and United Kingdom advisory bodies. PUFAs are very susceptible to oxidation and their increased intake, without a concomitant increase in vitamin E, can lead to a reduction in plasma vitamin E concentrations (42) and to elevations in some indexes of oxidative damage in human volunteers (43). However, diets high in PUFAs tend also to be high in vitamin E, and to set a dietary recommendation based on extremes of PUFA intake would deviate considerably from median intakes of vitamin E in most populations of industrialized countries. Hence 'safe' allowances for the United Kingdom (men 10 and women 7 mg/day) (44) and 'arbitrary' allowances for the United States (men 10 and women 8 mg/day) (45) for vitamin E intakes approximate the median intake in those countries. It is worth noting that only 11 (0.7%) out of 1629 adults in the 1986–1987 British Nutrition Survey had  $\alpha$ -tocopherol–cholesterol ratios  $<2.25$ . Furthermore, although the high intake of soybean oil, with its high content of  $\gamma$ -tocopherol, substitutes for the intake of  $\alpha$ -tocopherol in the British diet, a comparison of  $\alpha$ -tocopherol–cholesterol ratios found almost identical results in two groups of randomly-selected, middle-aged adults in Belfast (Northern Ireland) and Toulouse (France), two countries with very different intakes of  $\alpha$ -tocopherol (36) and cardiovascular risk (32).

It has been suggested that when the main PUFA in the diet is linoleic acid, a  $d$ - $\alpha$ -tocopherol–PUFA ratio of 0.4 (expressed as mg tocopherol per g PUFA) is adequate for adult humans (46, 47). This ratio has been recommended in the United Kingdom for infant formulas (48). Use of this ratio to calculate the vitamin E requirements of men and women with energy intakes of 2550 and 1940 kcal/day, respectively, and containing PUFAs at 6% of the energy intake (approximately 17 g and 13 g, respectively), (44) produced values of 7 and 5 mg/day of  $\alpha$ -TEs, respectively. In both the United States and the United Kingdom, median intakes of  $\alpha$ -TE are in excess of these amounts and the  $\alpha$ -tocopherol–PUFA ratio is approximately 0.6 (49), which is well above the value of 0.4 that would be considered adequate for this ratio. The Nutrition Working Group of the International Life Sciences Institute Europe (50) has suggested an intake of 12 mg  $\alpha$ -tocopherol for a daily intake of 14 g PUFAs to compensate for the high consumption of soybean oil in certain countries, where over 50% of vitamin E intake is accounted for by the less

biologically active  $\gamma$  form. As indicated above, however, plasma concentrations of  $\alpha$ -tocopherol in subjects from Toulouse and Belfast suggest that an increased amount of dietary vitamin E is not necessary to maintain satisfactory plasma concentrations (32).

At present, data are not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. There is some indication that newborn infants, particularly if born prematurely, are vulnerable to oxidative stress because of low body stores of vitamin E, impaired absorption, and reduced transport capacity resulting from low concentrations of circulating low-density lipoproteins at birth (51). However, term infants nearly achieve adult plasma vitamin E concentrations in the first week (52) and although the concentration of vitamin E in early human milk can be variable, after 12 days it remains fairly constant at 0.32 mg  $\alpha$ -TE/100 ml milk (53). Thus a human-milk-fed infant consuming 850 ml would have an intake of 2.7 mg  $\alpha$ -TE. It seems reasonable that formula milk should not contain less than 0.3 mg  $\alpha$ -TE/100 ml of reconstituted feed and not less than 0.4 mg  $\alpha$ -TE/g PUFA.

No specific recommendations concerning the vitamin E requirements in pregnancy and lactation have been made by other advisory bodies (44, 45) mainly because there is no evidence of vitamin E requirements different from those of other adults and, presumably, also because the increased energy intake during these periods would compensate for the increased needs for infant growth and milk synthesis.

## 5.5 Toxicity

Vitamin E appears to have very low toxicity, and amounts of 100–200 mg of the synthetic all-*rac*- $\alpha$ -tocopherol are consumed widely as supplements (29, 30). Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g. >1000 mg/day) (34). Nevertheless, the recent report from The Netherlands of increased severity of respiratory tract infections in persons over 60 years who received 200 mg vitamin E per day for 15 months, should be noted in case that is also an indication of a pro-oxidant effect (35).

## 5.6 Recommendations for future research

More investigation is required of the role of vitamin E in biological processes which do not necessarily involve its antioxidant function. These processes include:

- structural roles in the maintenance of cell membrane integrity;
- anti-inflammatory effects by direct and regulatory interaction with the prostaglandin synthetase complex of enzymes which participate in the metabolism of arachidonic acid;
- DNA synthesis;
- interaction with the immune response;
- regulation of intercellular signalling and cell proliferation through modulation of protein kinase C.

Additionally, more investigation is required of the growing evidence that inadequate vitamin E status may increase susceptibility to infection particularly by allowing the genomes of certain relatively benign viruses to convert to more virulent strains (54).

There is an important need to define optimum vitamin E intakes for younger groups of healthy persons since supplements for people who are already ill appear ineffective and can possibly be harmful in the elderly. Intervention trials with morbidity and mortality end-points will take years to complete, although the European Prospective Investigations on Cancer which has already been underway for more than 10 years (55) may provide some relevant information. One possible approach to circumvent this delay is to assess the effects of different intakes of vitamin E on biomarkers of oxidative damage to lipids, proteins, and DNA as their occurrence in vivo is implicated in many diseases, including vascular disease and certain cancers. However, clinical studies will always remain the gold standard.

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