

Vitamin E incorporated into a very-low-fat meal is absorbed from the intestine of young rats

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Vegetable fats and oils are major sources of dietary vitamin E. Consequently the current trend to reduce fat consumption is accompanied by a reduction of the intake of vitamin E. In addition, the absorption of vitamin E is thought to be dependent on the hydrolysis of dietary lipids in the small intestine. It is therefore conceivable that a lower dietary fat intake also diminishes the intestinal absorption of vitamin E. The present 3-week feeding study in young male rats was designed to investigate whether different concentrations of vitamin E added to a very-low-fat product (0, 330 or 1350 mg DL- α -tocopheryl acetate/kg product) were absorbed. We therefore incorporated these products into a very-low-fat meal (final fat concentration: 7 g/kg) or a low-fat meal containing 52 g fat/kg. The magnitude of vitamin E absorption from these meals was compared with that from meals containing similar amounts of vitamin E, but a high fat concentration of 190 g/kg. Apparent vitamin E absorption was defined as intake of α -tocopherol equivalents (α TE) minus faecal α TE excretion over 4 d during week 3 of the experimental period. The results of this study showed that apparent absorption of vitamin E from a very-low-fat meal varied, depending on the vitamin E concentration, from 73 to 83%. The magnitude of this vitamin E absorption was not significantly different from that from meals containing a high amount of fat. Liver vitamin E status was equal in rats fed on the very-low-fat meals compared with those fed on the high-fat meals. We conclude that, when very-low-fat or low-fat products are used as a replacement for full-fat products, addition of vitamin E to these products, as DL- α -tocopheryl acetate, might be useful in meeting the vitamin E requirements.

Vitamin E absorption: Low-fat products: Rats

Dietary vitamin E is passively absorbed together with dietary lipids in the upper part of the small intestine (Hollander *et al.* 1975; Muralidhara & Hollander, 1977). A crucial step in the absorption of vitamin E is the transport from the intestinal lumen into the enterocyte. For this transport vitamin E needs to be incorporated in the mixed micelles composed of monoacylglycerols, fatty acids, bile acids and phospholipids (Kayden & Traber, 1993). An incomplete hydrolysis of dietary lipids might impair the micellar uptake of vitamin E and thus vitamin E absorption. After uptake in the intestinal cells vitamin E is secreted into chylomicrons, transported via the lymph to the bloodstream and subsequently distributed into the different lipoproteins. Vitamin E is then transported to tissues of which liver and adipose tissue are the major storage organs (Machlin & Gabriel, 1982; Kayden & Traber, 1993). At present, no information is available about the influence of a substantial reduction in fat intake on vitamin E absorption.

The mechanism described above implies, however, that it is conceivable that a lower concentration of dietary fat in the intestine may decrease vitamin E absorption. Vegetable fats and oils are major sources of dietary vitamin E (Chow, 1985). Consequently, a reduced

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fat content of products may not only lead to a reduced fat intake, but also to a decreased intake of vitamin E. This hypothesis has recently been confirmed in a study in human volunteers which investigated the long-term effects of consumption of reduced-fat v. full-fat products on health (K. H. Van het Hof, personal communication). In addition, it can be hypothesized that at relatively low concentrations of triacylglycerol in the intestinal lumen, the absorption of fat-soluble vitamins may be impaired. These two factors may eventually deplete vitamin E concentrations in the tissues. To provide sufficient vitamin E with very-low-fat or low-fat products, food industries can restore vitamin E to the concentrations found in full-fat equivalents. It might be questioned, however, whether vitamin E added to a very-low-fat or low-fat product is indeed absorbed, since the presence of dietary fat is crucial for its absorption.

The aim of the present study was to investigate whether vitamin E in the form of cold-water-soluble DL- α -tocopheryl acetate was absorbed by rats from a very-low-fat product. We therefore incorporated a very-low-fat product containing different amounts of vitamin E into a very-low-fat meal (final fat concentration: 7 g/kg) and a low-fat meal (52 g fat/kg). The magnitude of the vitamin E absorption was compared with that from a high-fat meal (190 g fat/kg).

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Welfare Officer and the Animal Experimentation Committee of Unilever Research Laboratory, Vlaardingen.

Animals and housing

Male Wistar rats (n 108; Hsd/Cpb:WU, Harlan, Zeist, The Netherlands) aged about 6 weeks and with mean initial body weight of 151 (SE 1.3) g (n 112) were used. The rats were housed individually in wire-mesh-bottomed cages in a room with controlled temperature (22–24°), relative humidity (45–65%) and light cycle (light, 07.00–19.00 hours). The animals were divided into nine dietary groups of twelve animals each and were fed on semi-purified diets for 6 weeks.

Experimental meals

During a prestudy acclimatization and meal-training period the animals consumed a semi-purified meal containing 190 g dietary fat/kg (Table 1). Vitamin E was added to this meal as D- α -tocopherol (Sigma, St Louis, MO, USA, purity 67%) at a concentration of 35 mg/kg (35 α -tocopherol equivalents (α -TE)/kg). During the experimental period all rats received this meal as their morning meal, but the evening meals varied for the different experimental groups. The composition of the evening meals is given in Table 2. Three groups of rats received a high-fat evening meal containing 190 g fat/kg as lard. Three other groups of rats received a very-low-fat evening meal in which the total amount of fat was replaced by a very-low-fat product. This resulted in a fat concentration of 7 g/kg. The composition of this very-low-fat product was as follows (g/kg): monoacylglycerols 29.9, Admul Datem 1935 (Quest International, Naarden, The Netherlands; stearic or palmitic acid monoacylglycerol esterified with tartaric acid) 2.0; NaCl 14.3; β -carotene (1% cold-water soluble) 0.3, gelatin 16.8, starch 12.6, potassium sorbate 1.1, water 923. Finally, three groups of rats received a low-fat evening meal in which the total amount of fat was about 52 g/kg. The fat source of the low-fat meals consisted of the very-low-fat product (190 g/kg of the total meal) and lard (45 g/kg of the total meal). To achieve different vitamin E concentrations the very-low-fat product was incorporated into the experimental diets either as such (i.e. without vitamin E) or supplemented with 660 or 2700 mg DL- α -tocopheryl

Table 1. *Composition of the meals used during the pre-experimental period and morning meal*

Ingredient	g/kg
Ca caseinate	256
Mineral mixture*	15.0
Vitamin mixture*	3.5
Maize starch	474
Sunflowerseed oil	47
Lard	142
Solka Floc (cellulose)	62.9
D- α -Tocopherol† (mg)	21

* Composition of the mineral mixture (mg/g mineral mixture): KCl 96.72, MgHPO₄·3H₂O 264.20, KH₂PO₄ 131.27, KH₂CO₃ 198.70, CaCO₃ 81.52, C₆H₅Na₃O₇·2H₂O 196.49, MnSO₄·H₂O 14.20, C₆H₅FeO₇·5H₂O 12.13, Cu₂C₆H₆O₈·xH₂O 1.30, Zn₂C₆H₆O₈·xH₂O 3.45, KIO₃ 0.02. Composition of the vitamin mixture (mg/g vitamin mixture): choline chloride 597.5, myoinositol 29.9, cyanocobalamin 15.5, thiamin 1.8, calcium pantothenate 6.0, nicotinamide 6.0, riboflavin 1.8, pyridoxine 1.9, pteroylmonoglutamic acid 0.3, menadione 1.2, biotin 0.06, retinyl palmitate 9.2, cholecalciferol 3.0, calcium silicate 59.75, sucrose 266.09.

† Sigma, St Louis, MO, USA, purity 67%.

Table 2. *Composition of the evening meals (g/kg)*

Vitamin E (α -TE/kg)...	0			41.5			169.9		
	7	52	190	7	52	190	7	52	190
Dietary fat (g/kg)...	7	52	190	7	52	190	7	52	190
Ingredient									
Ca caseinate	256	244	256	256	244	256	256	244	256
Mineral mixture*	15.0	14.4	15.0	15.0	14.4	15.0	15.0	14.4	15.0
Vitamin mixture*	3.5	3.3	3.5	3.5	3.3	3.5	3.5	3.3	3.5
Maize starch	474	452	474	474	452	474	474	452	474
Lard	0	45	189	0	45	189	0	45	189
Solka Floc (cellulose)	62.9	60.1	62.9	62.9	60.1	62.9	62.9	60.1	62.9
Lipogel, 0 α -TE†	189	181	0	0	0	0	0	0	0
Lipogel, 220 α -TE†	0	0	0	189	181	0	0	0	0
Lipogel, 900 α -TE†	0	0	0	0	0	0	189	181	0
DL- α -Tocopheryl acetate‡ (mg)	0	0	0	0	0	25	0	0	510
Chemical analysis									
Fat	7	53	189	7	52	190	7	51	190
α -Tocopheryl acetate (mg)	0.00	0.00	0.00	61.61	61.23	60.96	232.15	230.67	234.05

* For composition, see Table 1.

† Lipogel refers to the very-low-fat product to which different amounts of DL- α -tocopheryl acetate were added: 0 α -TE: 0 g DL- α -tocopheryl acetate per kg product, 220 α -TE: 0.66 g DL- α -tocopheryl acetate per kg product, 900 α -TE: 2.70 g DL- α -tocopheryl acetate per kg product.

‡ Roche, Basel, Switzerland, type CWS/F 50%.

acetate/kg product (Roche, Basel, Switzerland, type cold-water-soluble/F 50%, 2 mg = approximately 0.67 α -TE). This resulted in final vitamin E concentrations of 0, 41.5 or 169.9 α -TE/kg respectively. DL- α -tocopheryl acetate was added to the high-fat evening meals to reach similar final vitamin E concentrations.

Experimental design

During a 7 d acclimatization period the rats consumed the semi-purified meals *ad libitum* (Table 1). After this period the animals were trained to consume two equal, restricted meals daily for 2 weeks. In the fourth week after arrival the experimental period started. The animals were meal-fed twice daily (07.00–07.30 hours and 19.00–19.30 hours) with two different meals. All rats received the same morning meal containing 190 g fat/kg and 35 mg D- α -tocopherol/kg (35 α -TE/kg) in order to prevent deficiencies of essential fatty acids and vitamin E during the experimental period. The evening meals were different for the nine experimental groups with respect to the fat and vitamin E concentrations (Table 2). Rat studies previously carried out in our laboratory have shown that emptying of the stomach is a linear process in time, and that 12 h after a morning meal the stomach is totally empty (results not shown). Since transport of chyme from the stomach to the caecum is a process which, in rats, takes maximally 2 h, it was assumed that the presence of fat in the morning meal did not influence the absorption of vitamin E from the evening meal. In the third week of the experimental period (week 6 after arrival), faeces from each rat were collected for 4 d. Throughout the experiment, food consumption was recorded for each meal and body-weight measurement and clinical observation occurred once weekly. At the end of the experiment the animals were killed by decapitation after food deprivation for 12–14 h. Macroscopic inspection was carried out on all animals. Livers were removed and weighed. A sample of the left part of the median lobe was taken for analysis of tocopherol. The right part of the median lobe was fixed in 100 ml/l phosphate-buffered formalin for pathological inspection of symptoms of vitamin E deficiency.

Determination of vitamin E in very-low-fat products, meals, faeces and liver

Very-low-fat products and meals. The very-low-fat products and the semi-purified meals were treated with an aqueous solution of trypsin (EC 3.4.21.4; Merck, Darmstadt, Germany, 200 Fédération Internationale Pharmaceutique (FIP) U/g) and pepsin (EC 3.4.23.1; Merck, 2500 FIP U/g). Following incubation, ethanol was added in order to prevent gel-formation. Subsequently the products and meals were extracted with *n*-heptane–diethyl ether (1:1 v/v). After evaporation of the solvents the residues were dissolved in chloroform–2-propanol (1:3, v/v) to a standard volume. Thereafter 20 μ l of each sample was analysed by reversed phase HPLC (Applied Biosystems, Foster City, CA, USA) with a RP-C18 column (Merck, 250 mm \times 4 mm, 5 μ m). α -Tocopheryl acetate was detected at 285 nm and calculated by an external calibration procedure.

Faeces. Faeces were collected from each animal separately for four consecutive days at 07.30, 11.00, 16.00, 20.00 and 23.00 hours. The faeces from each animal were combined in 25 ml methanol–water (1:1 v/v) containing 0.1 g butylated hydroxytoluene/l to prevent oxidation of vitamin E, and stored at 0–4° during the collection period. The combined faeces were stored at –20° until analysis. The faeces were homogenized and extracted with *n*-heptane–ethyl acetate (3:1 v/v) after addition of β -tocopherol in ethanol as internal standard. The heptane extracts were analysed by HPLC (Shimadzu, Kyoto, Japan) with a Lichrospher 100 Diol column (Merck, 250 mm \times 4 mm, 5 μ m). The eluent was monitored by fluorimetric detection at an emission wavelength of 330 nm and excitation wavelengths of 294 and 296 nm for α - and β -tocopherol respectively. For α -tocopheryl acetate the emission wavelength was 318 nm and the excitation wavelength 285 nm. The concentrations of α -tocopherol, α -tocopheryl acetate and β -tocopherol were calculated from the relative peak areas of α - and β -tocopherol, and α -tocopheryl acetate using external standard curves.

Liver. Immediately after killing the animals a piece of liver was homogenized in

ethanol-water (90:10, v/v) containing 0.1 g ascorbic acid/l and 0.002 g pyrogallol/l. The homogenate was extracted with internal standard solution (α -tocopheryl acetate in *n*-heptane containing 1 g butylated hydroxytoluene/l). Immediately before HPLC the heptane was evaporated under a stream of N₂, the residue was dissolved in chloroform-methanol (2:1 v/v), and was analysed by reverse-phase HPLC with a RP-C18 column (Merck, 250 mm × 4 mm, 5 μ m). The eluent was monitored with a u.v.-visible detector set at 294 nm for α -tocopherol and 285 nm for α -tocopheryl acetate. The concentration of α -tocopherol was calculated from the relative peak areas using extinction coefficients (E^{1%}(1 cm)) of 75.8 and 43.6 for α -tocopherol and α -tocopheryl acetate respectively.

Pathology

The livers of the rats fed on the high-fat evening meals containing 190 g fat/kg with no vitamin E and 169.9 α -TE/kg as well as those of rats fed on the very-low-fat evening meals containing 7 g fat/kg with no vitamin E and 169.9 α -TE/kg were processed and stained with Harris's haematoxylin-azophloxin for microscopic examination with special attention to vitamin E-deficiency symptoms. Additionally, unstained 3.5 μ m sections of the liver were cut. They were mounted with fluoromount (Gurr, BDH Chemicals Ltd, Poole, Dorset) and studied with a Zeiss (Oberkochen, Germany) incidence fluorescence microscope using a HBO-50W lamp with BP 485, FT 510 and LP 515 filters to detect autofluorescent lipofuscin.

Calculations and statistics

Apparent vitamin E absorption was calculated as total vitamin E intake (D- α -tocopherol and DL- α -tocopheryl acetate as mg α -tocopherol) minus faecal vitamin E excretion during the 4 d collection period. Absorption was expressed as such and as a percentage of vitamin E intake. Statistical analysis was performed with the SAS statistical package, release 6.04 (Statistical Analysis Systems Institute, Cary, NC, USA). Body-weight gain, food consumption and absorption and liver concentrations of vitamin E were evaluated by two-way ANOVA with dietary fat concentration and vitamin E concentration as main effects. For each dietary α -tocopherol concentration the Student-Newman-Keuls test was used to compare group means. The level of significance was preset at $P < 0.05$.

RESULTS

Feed consumption, body-weight gain and clinical observation

As expected with a restricted feeding regimen, the different treatments did not result in significant differences in feed consumption. The feed consumption varied from 269 (SE 6.3) to 280 (SE 3.9) g/21 d (n 12). Body-weight gain during the 3-week experimental period was 43 (SE 2) g/12 d (n 72) in rats fed on the very-low-fat meals and the low-fat meals. This was significantly lower than in rats fed on the high-fat evening meals. Body-weight gain of the latter groups was 62 (SE 4) g/21 d (n 36). This was due to the lower energy content of these meals. Vitamin E concentrations of the meals did not significantly affect body-weight gain. No clinical abnormalities were found.

Intake and apparent absorption of vitamin E

Table 3 shows no significant differences in vitamin E intake in rats given meals containing different amounts of fat at vitamin E concentrations of 0 and 41.5 α -TE/kg diet. When rats were fed on diets containing 169.9 α -TE vitamin E there was a significantly higher intake

Table 3. *Intake, absorption and liver concentration of vitamin E in rats given evening meals containing different amounts of fat and vitamin E**

(Mean values with their standard errors for twelve rats per dietary group)

Vitamin E (α -TE/kg)	Fat (g/kg)	Intake† (α -TE/4 d)		Absorption† (α -TE/4 d)		Absorption‡ (%)		Liver (μ g/g)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	7	0.889 ^A	0.019	0.776 ^A	0.020	87.2 ^{abA}	0.70	17.3 ^A	0.49
	52	0.872 ^A	0.020	0.750 ^A	0.024	85.9 ^{abA}	1.00	17.6 ^A	0.49
	190	0.893 ^A	0.021	0.807 ^A	0.022	90.4 ^{abA}	0.74	17.0 ^A	0.55
41.5	7	2.414 ^B	0.047	1.853 ^B	0.039	76.8 ^{abB}	0.69	26.9 ^B	0.58
	52	2.399 ^B	0.049	1.993 ^B	0.045	83.1 ^{bbB}	0.87	25.7 ^B	0.75
	190	2.342 ^B	0.057	1.946 ^B	0.060	82.9 ^{bbB}	0.83	27.8 ^B	0.78
169.9	7	6.789 ^{ac}	0.084	4.966 ^C	0.116	73.1 ^C	1.19	51.5 ^C	2.0
	52	6.650 ^{abc}	0.158	4.935 ^C	0.216	73.9 ^C	1.75	45.5 ^C	3.2
	190	6.259 ^{bc}	0.183	4.593 ^C	0.159	73.4 ^C	1.21	47.0 ^C	2.4

^{a, b} Mean values within a column for a particular vitamin E concentration not sharing a common superscript letter were significantly different ($P < 0.05$, Student–Newman–Keuls).

^{A, B, C} Mean values within a column for a particular fat concentration not sharing a common superscript letter were significantly different ($P < 0.05$, Student–Newman–Keuls).

* Besides the evening meals rats received similar morning meals containing 190 g fat and 35 α -TE vitamin E (D - α -tocopherol) per kg to prevent deficiency of essential fatty acids and vitamin E.

† Since it was not possible to distinguish between vitamin E excretion from the morning meal and the evening meal, total vitamin E intake and absorption values are given in this Table. When no vitamin E is present in the evening meal, intake, absorption and liver concentrations of vitamin E are the basal values from the morning meal. The increased intake and differences in absorption and liver concentrations of vitamin E at the higher vitamin E concentrations are due to variations in the composition of the evening meals.

‡ Percentage of intake.

of vitamin E at the very-low-fat concentration compared with the high-fat concentration. Vitamin E intake was higher in rats given meals containing a higher vitamin E concentration ($P < 0.001$). For each particular vitamin E concentration, apparent absorption of vitamin E, expressed in absolute figures, was not significantly influenced by dietary fat concentration (Table 3). However, when expressed as a percentage of intake, vitamin E absorption was lower in rats given the very-low-fat and low-fat evening meals compared with rats given the high-fat evening meals at a vitamin E concentration of 0 α -TE/kg (Table 3). At a vitamin E concentration of 41.5 α -TE/kg the percentage absorption in rats fed on the very-low-fat evening meals was lower than in rats fed on the low- and high-fat evening meals. At a vitamin E concentration of 169.9 α -TE/kg fat, concentration in the evening meals did not affect percentage absorption of vitamin E. For each particular fat concentration a higher intake of vitamin E resulted in a higher absolute absorption of vitamin E ($P < 0.001$). Absorption expressed as percentage of intake was lower at higher vitamin E concentrations ($P < 0.01$), indicating a decreased absorption efficiency.

Liver concentrations of vitamin E

The amount of fat did not have a significant effect on the α -tocopherol concentration in the liver. As expected, higher vitamin E concentrations, and the consequently higher absolute vitamin E absorption, resulted in a significantly higher α -tocopherol concentration in the liver ($P < 0.001$, Table 3).

Pathology

Macroscopic and microscopic examination of the liver did not indicate treatment-related pathological changes.

DISCUSSION

Based on the absorption mechanism of vitamin E, it is generally accepted that dietary fat is needed for the intestinal absorption of this fat-soluble vitamin. The first question of the present study was whether different concentrations of vitamin E (DL- α -tocopheryl acetate) were absorbed by rats when incorporated into a very-low-fat evening meal (final fat concentration 7 g monoacylglycerol/kg). Second, we wished to determine if an increase of fat concentration in an evening meal from 7 g/kg to 52 g/kg or 190 g/kg influenced the absorption of different concentrations of vitamin E. Based on the absorption mechanism of vitamin E it would be expected that vitamin E was not or was only marginally absorbed from the very-low-fat evening meals (7 g fat/kg). The results of the present study were surprising. Table 3 shows that vitamin E was adequately absorbed from a very-low-fat meal by young rats. The magnitude of this absorption was not significantly different from the absorption from low-fat meals (52 g fat/kg) or high-fat meals (190 g fat/kg). This effect was found at all levels of vitamin E. Apparent absorption of vitamin E varied from 86–90%, when no DL- α -tocopheryl acetate was added to the evening meal, to 77–83% when 41.5 α -TE/kg was added to the evening meal, and 73% for all dietary fat concentrations when 169.9 α -TE/kg was added to the evening meal. Results of mechanistic studies both *in vitro* (Hollander *et al.* 1975) and *in vivo* (Muralidhara & Hollander, 1977) suggest that the absorption of vitamin E by the enterocyte takes place by passive diffusion. In the rat, maximal absorption takes place at the junction between the upper and middle thirds of the small intestine (Gallo-Torres, 1970; Hollander *et al.* 1975). Vitamin E is a lipid-soluble, non-swelling amphiphile, as are triacylglycerols and cholesterol. As a consequence, many of the factors and processes necessary for the absorption of dietary lipids also hold for absorption of vitamin E (Carey & Small, 1970). Thus, following the enzymic degradation of triacylglycerol to monoacylglycerol and free fatty acids, vitamin E needs to be incorporated into mixed micelles for subsequent uptake by intestinal cells. The relative importance of the individual pancreatic and biliary factors necessary for the efficient absorption of vitamin E has been demonstrated in bile-duct-ligated rats (Gallo-Torres, 1970) and in patients with specific defects in fat absorption. Studies in children with cholestatic liver disease demonstrated that vitamin E is not absorbed in the absence of bile in the intestine (Sokol *et al.* 1983; Traber *et al.* 1986). Pancreatic enzymes facilitate uptake of vitamin E into enterocytes (Mathias *et al.* 1981). Patients with pancreatic insufficiency, as in cystic fibrosis, have greatly decreased amounts of pancreatic enzymes in the intestinal lumen accompanied by impaired absorption of vitamin E (Sokol *et al.* 1989). This is presumably caused by a failure to hydrolyse triacylglycerols, resulting in a decreased capacity for vitamin E solubilization in mixed micelles (Stead *et al.* 1986). *In vitro* studies using a human intestinal cell line indeed demonstrated that uptake of vitamin E occurred in the presence of bile acids and fatty acids, but was not further enhanced by the addition of bile-activated lipase (Traber *et al.* 1990). Thus, pancreatic enzymes are necessary for lipid hydrolysis, but not specifically to facilitate vitamin E absorption. However, according to the mechanism described previously, vitamin E absorption must be dependent on the amount of other fats present (Cohn *et al.* 1992).

Several potential explanations can be given for the observation that vitamin E was absorbed from a very-low-fat evening meal by young rats. First, it might be questioned whether the fat present in the morning meal was responsible for the vitamin E absorption

from the evening meal. Rat studies previously carried out in our laboratory with comparable meals have shown that emptying of the stomach is a linear process in time (results not shown). At 3 h after a meal the stomach only contained two-thirds of a meal consumed; after 6 h, this was one-third, and at 9 h after a meal the stomach contained less than 10% of the meal consumed. In another study carried out in our laboratory we showed that at 12 h after a morning meal the stomach was totally empty in seven out of eight rats (results not shown). Since transport of chyme from the stomach to the caecum is a process which in rats maximally takes 2 h, it is unlikely that in the current study fat present in the morning meal influenced the absorption of vitamin E from the evening meal.

A second possible explanation for the observed vitamin E absorption from the very-low-fat evening meal might be that this meal still contained 7 g fat/kg diet as monoacylglycerol. It is likely that the presence of this low concentration of a fat-hydrolysis product was sufficient for solubilization of vitamin E in micelles and, thus, for its transport and absorption. In addition, rats do not have a gall bladder and as a consequence bile enters the intestine directly. Although it has been suggested that the bile duct can be closed by Oddi's sphincter (Remie *et al.* 1990), previous results suggest that the tonus of the sphincter of Oddi is almost negligible in rats (Mann, 1920). Therefore, rats might have a more-or-less constant bile flow into the intestinal lumen facilitating formation of micelles for vitamin E absorption.

Third, vitamin E was added as cold-water-soluble tocopheryl acetate. If aqueous pathways for vitamin E exist, there might have been vitamin E absorption from the very-low-fat meals via such a water-soluble process. However, to the best of our knowledge there is no evidence to support such a mechanism.

As expected in the case of a passive absorption process, higher vitamin E intake resulted in higher absolute vitamin E absorption. Addition of vitamin E as DL- α -tocopheryl acetate decreased absorption efficiency from about 87 to 74%. This is probably not due to a difference in absorption efficiency of α -tocopherol and α -tocopheryl acetate. Because DL- α -tocopheryl acetate must be hydrolysed before absorption (Nakamura *et al.* 1975; Muller *et al.* 1976), it might be that the absorption of this form of vitamin E is less efficient than that of free α -tocopherol. However, Burton *et al.* (1988) showed no significant differences in absorption of deuterated α -tocopherol and α -tocopheryl acetate. Other literature results confirm the finding that absorption efficiency of vitamin E decreases with higher vitamin E intakes (Bjorneboe *et al.* 1990). The absorption efficiency of vitamin E as measured in the present experiment is relatively high (73–90%) compared with literature values. Depending on the method, absorption efficiencies from 21 to 72% have been reported in humans (Blomstad & Forsgren, 1968; MacMahon & Neale, 1970). In rats with cannulated thoracic ducts vitamin E absorptions from 15 to 65% were reported (Bjorneboe *et al.* 1986; Traber *et al.* 1986). It should be noted that the efficiency of vitamin E absorption is difficult to establish because of the number of uncontrolled variables involved in this measurement (Traber *et al.* 1993). The young animals used in the present study generally have high absorption efficiencies, and the rather low vitamin E concentrations might be responsible for the observed high absorption efficiency.

Compared with other organs, the liver is most sensitive to changes in vitamin E intake or absorption (Machlin & Gabriel, 1982). At each vitamin E concentration, intake and absorption of vitamin E were similar in rats fed on different dietary fat concentrations (Table 3). Consequently, there were no significant effects of fat concentration on the liver status of vitamin E (Table 3). As expected, a higher vitamin E intake resulted in a higher liver status of vitamin E (r 0.998, see Table 3).

Vegetable oils and fats, and food products containing them, provide about 50% of the dietary vitamin E intake in The Netherlands (Voorlichtingsbureau voor de Voeding, 1993).

Replacement of these products by very-low-fat or low-fat substitutes might, therefore, seriously impair the supply of vitamin E. Despite the hypothesis that dietary fat is needed for adequate absorption of vitamin E, the present study shows that it is useful to supplement very-low-fat or low-fat replacements with vitamin E. Our results clearly demonstrate that DL- α -tocopheryl acetate, when added to a very-low-fat product, is absorbed by rats from a very-low-fat meal (7 g fat/kg) in the same magnitude as when added to a high-fat meal (190 g fat/kg). We conclude that addition of vitamin E, as DL- α -tocopheryl acetate, to very-low-fat and low-fat products might be useful in meeting the vitamin E requirements when these products are used to replace full-fat products.

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