The Nutritional Effects of Olestra

Olestra Affects Serum Concentrations of α-Tocopherol and Carotenoids but not Vitamin D or Vitamin K Status in Free-Living Subjects

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ABSTRACT Normal, healthy, free-living adults ingested either 18 g/d olestra, with or without 1.1 mg tocopheryl acetate/g olestra, or 18 g/d triglyceride placebo, for 16 wk in a double-blind, placebo-controlled study. Serum concentrations of α-tocopherol, α-carotene, β-carotene, lycopene, lutein/zeaxanthin, retinol and cholesterol were measured biweekly. Serum 25-hydroxyvitamin D concentration, prothrombin time, partial thromboplastin time and plasma concentration of functional prothrombin (Simplastin-Ecarin assay) were measured at wk 0, 8 and 16. Relative to the placebo group, serum α-tocopherol concentration was reduced 8% for the group given 18 g/d olestra. Addition of tocopheryl acetate to olestra partially offset the effect of olestra. For the group given 18 g/d olestra plus 1.1 mg tocopheryl acetate/g olestra, serum α-tocopherol concentration was reduced 4% less than the placebo value. Olestra reduced serum concentration of β-carotene by 27%; the other carotenoids were similarly affected. Serum cholesterol concentration was reduced 4.5% in the olestra groups, relative to placebo, but the differences were not significant. Serum triglycerides, serum 25-hydroxyvitamin D, prothrombin time, partial thromboplastin time or the plasma concentration of under-carboxylated prothrombin were unaffected by olestra. Clinical observations and laboratory measures indicated no health-related effects of olestra; mild-to-moderate transient gastrointestinal symptoms such as bloating, cramping, loose stools and diarrhea were reported by all groups. J. Nutr. 127: 1636S–1645S, 1997.

KEY WORDS: • β-carotene • carotenoids • olestra • vitamin E • vitamin K • vitamin D • humans

Olestra (Olean, Procter & Gamble, Cincinnati, OH), a mixture of hexa-, hepta- and octaesters of sucrose formed from long-chain fatty acids from vegetable oils, has the organoleptic and thermal properties of regular fats (Kester 1993). However, olestra is not hydrolyzed by gastric and pancreatic lipases (Mattson and Volpenhein 1972) and is not absorbed (Miller 1982). The degree to which this interference might occur depends on several factors. A primary one is the degree of lipophilicity of the molecule. The more lipophilic the molecule, the more it will partition into the olestra. Water-soluble substances will not partition into olestra; therefore olestra has no effect on their absorption, a fact substantiated in a number of studies presented elsewhere in this supplement (Cooper et al. 1997a and 1997b, Schlageck et al. 1997a and 1997b). Another important factor influencing the degree to which olestra can affect the absorption of fat-soluble molecules is the time between the consumption of olestra and the substance. The two must be present in the GI tract simultaneously for fat-soluble substances to interfere with the absorption of fat-soluble substances. A plausible mechanism proposed to explain how olestra interferes with fat-soluble substances is that the olestra in the GI tract competes with the intestinal mixed micelles for the fat-soluble molecules, making them unavailable for absorption (Jandacek 1992). The degree to which this interference might occur depends on several factors. A primary one is the degree of lipophilicity of the molecule. The more lipophilic the molecule, the more it will partition into the olestra. Water-soluble substances will not partition into olestra; therefore olestra has no effect on their absorption, a fact substantiated in a number of studies presented elsewhere in this supplement (Cooper et al. 1997a and 1997b, Schlageck et al. 1997a and 1997b). Another important factor influencing the degree to which olestra can affect the absorption of fat-soluble molecules is the time between the consumption of olestra and the substance. The two must be present in the GI tract simultaneously for the interaction to occur. Finally, the amount of olestra eaten also affects the interaction.

Evidence that olestra can affect the absorption of lipophilic molecules comes from a number of animal and human studies. Olestra reduced serum cholesterol concentration in humans (Crouse and Grundy 1979, Fallat et al. 1976, Glueck et al. 1979) and increased cholesterol excretion in rats (Mattson et al. 1976) and in humans (Jandacek et al. 1980 and 1990). Reductions in plasma tocopherol concentration (Glueck et al. 1982, Mellies et al. 1983 and 1985) and serum 25-hydroxycholecalciferol concentration (Jones et al. 1991b) have been ob-
served in humans consuming olestra. In a study in which free-
living subjects ate 20 g/d olestra for 6 wk, olestra did not affect
vitamin K function, as measured by prothrombin time (PTT),
partial thromboplastin time (PTT) or functional prothrombin
concentration [Simplessin:Ecarin (S:E) ratio], although it had
an effect on serum phyloquinone concentration (Jones et al.
1991a).

Results from short-term feeding studies and long-term safety
studies in animals indicate that the effects of olestra on the
absorption of fat-soluble vitamins can be offset by adding extra
amounts of the vitamins to the diet. Mattson et al. (1979)
measured the liver vitamin A content of rats fed 0, 677 or
1331 RE (µg retinol equivalents) of vitamin A total after feeding
the rats a vitamin A–free diet containing 15% (wt/ wt)
olestra for 3 d. The liver contents of vitamin A were
<50, 180 and 343 RE, respectively, indicating that addition
of vitamin A offset the effect of olestra, in a roughly linear
manner. In another study, mice were fed 0, 2.5, 5 or 10% Olestra
in diets supplemented with 2500 IU (1375 RE)/kg of vitamin D,
750 IU (18.8 µg)/kg of vitamin D, and from 160 to
640 IU [107 to 429 µg α-tocopherol equivalents (α-TE)]
kg of vitamin E for 2 y (Lafranconi et al. 1994). The liver
concentrations of vitamins A and E for mice in the olestra
fed groups, measured periodically throughout the study, were
comparable to control values. Serum 25-hydroxyvitamin D
[25(OH)D] concentrations for the groups fed 2.5 or 5% olestra
were comparable to control values; the concentration for the
group fed 10% olestra was ~77% of control.

The purposes of this study were as follows: 1) to determine
the effects of olestra on serum concentrations of cholesterol,
α-tocopherol, 25-hydroxyvitamin D [25(OH)D], carotenoids
and retinol; and 2) to determine whether addition of an extra
amount of vitamin E (1.1 mg vitamin E/g olestra) would offset
the effect of olestra on serum α-tocopherol concentration in a
free-living population consuming olestra in foods.

SUBJECTS AND METHODS

Study design and procedures. This study was a parallel, placebo-
controlled, double-blind design. The protocol was approved by the
institutional review board of Walker Clinical Evaluations, Indiana-
polis, IN, the study site. Signed informed consent was obtained from
each subject before admission. The study was conducted during late
winter and early spring.

The study consisted of two treatment groups and a placebo control
group. The treatment groups were given either 18 g/d olestra or 18 g/d olestra supplemented with 1.1 mg d-α-tocopheryl acetate (α-TA)/g olestra. The olestra was delivered in cookies and a frozen dessert. The placebo groups were given the same food items prepared with triglyceride. The olestra-containing test foods were prepared by substituting olestra for triglyceride in the recipes and were identical in appearance and taste to the placebo foods. The frozen dessert contained 9 g olestra or placebo per serving; each cookie contained 3 g olestra or placebo. The subjects were instructed to consume one serving of the dessert and three cookies per day to yield the 18 g/d dose of olestra. The placebo groups was instructed to consume the same number of placebo food items. The items were to be consumed with meals at the subject’s discretion throughout the day. The subjects were not specifically requested to divide the daily allocation among the meals, and there was no restriction on what other foods they could eat between meals. All other food items were self-selected and freely consumed.

The daily olestra intake, 18 g/d, was exaggerated relative to ex-
pected intake from savory snacks to invoke any measurable effects on nutrient absorption, if they exist. The estimated chronic intake of olestra from savory snacks by the average snack consumer (all ages, both sexes) is 3.1 g/d; for the 90th-percentile consumer, it is 6.9 g/d (Webb et al. 1997). The olestra, prepared as described by Rizi and Taylor (1978), consisted of >99% octa- and heptaeasters. The relative composition of the fatty acids making up the ester groups was 11% palmitic, 49% stearic, 31% oleic, 7% linoleic and 2% others.

The amount of vitamin E (Covitol 1360, Henkel, Cincinnati,
OH) added to the olestra, 1.1 mg d-α-tocopheryl acetate/g olestra,
was estimated from preliminary animal studies (data not shown). It was added to the olestra before the foods were prepared.

Two hundred and nineteen subjects (67 males and 152 females,
18–65 y of age) were admitted to the study. The subjects were in
good health as determined by medical history, physical examination
and clinical laboratory data. To be admitted, the subjects had to have
a serum α-tocopherol concentration > 11.6 µmol/L, a fasting serum
triglyceride value < 3.1 mmol/L and a fasting serum cholesterol value
< 7.1 mmol/L. Subjects who were pregnant or nursing, using vita-
min supplements or regularly using oral contraceptives were excluded.
The subjects were assigned randomly within strata of age, sex, body
mass index (BMI) and smoking status. Within each stratum, treatment
groups were balanced so that there were no significant differ-
ences in serum α-tocopherol concentrations, normalized with respect to
serum lipids, among the groups.

Urinalysis, clinical chemistry and hematology profiles were ob-
tained at the beginning, midpoint (wk 8) and end of the study by
standard accredited methods (The Medical Laboratory, Indianapolis,
IN). Fasting blood samples were collected biweekly by venipuncture
for analyses of tocopherol, carotenoids, 25(OH)D and lipid (chol-
erol and triglyceride) concentrations. Prothrombin time, PTT and
functional prothrombin concentration were measured at baseline,
wk 8 and wk 16. Serum was prepared from fresh blood. The samples
were immediately frozen and stored at ~20°C until analyzed.

Body weights were monitored weekly to ensure that the subjects
maintained their weight within ±2.25 kg of their base-line value. Any
subject whose body weight changed beyond the ±2.25 kg limit was
dropped from the study. An assessment of dietary intake was made
at wk 2 and wk 14 using a self-administered food-frequency question-
naire, which assesses food intake over the past year (Willett et al.
1985).

Subject compliance with respect to olestra consumption was moni-
tored by means of diaries in which the subjects recorded the number
of food items consumed and the time the items were consumed
each day. These diaries were reviewed on a biweekly basis by study person-
nel to determine the daily amount of olestra test food item eaten.
To be included in the database, a subject had to consume the test
foods for 101 of the 112 study days, 90% of the total dose of olestra.

Analytical methods. Serum concentrations of α-tocopherol, γ-
tocopherol, retinol (a-carotene, b-carotene, lycopene and lutein/exanthan) were determined simultaneously by HPLC. After denaturation of serum proteins with ethanol, the fat-
soluble vitamins and carotenoids were extracted with hexane, recon-
stituted in acetone/methylene chloride/methanol (4:1.67:1), and
separated and quantified using a reverse-phase column (Beckman
TN) was added before the extraction step as an internal standard.
The calibration curves were prepared with retinol (Kodak no. 1178722; Eastman Chemicals, Kingsport, TN) and separated and quantified using a reverse-phase column (Beckman UltraspHERE ODS 5-µm) and UV detection (Waters 490E, Waters, Milford, MA), following the method of Miller and Yang (1985).

Retinyl acetate (Kodak no. 1178722; Eastman Chemicals, Kingspor-
to, TN) was added before the extraction step as an internal standard.
Calibration curves were prepared with retinol (Kodak no. 1178617),
α-tocopherol (Kodak no. 1184175), γ-tocopherol (Kodak no. 1187962), lycopene (Sigma L-9879; Sigma Chemical, St. Louis, MO),
lutein (Sigma X-6250), a-carotene (Sigma C-0251) and b-carotene
(Sigma C-0216) standards. Aliquots of a pooled normal human serum
(Pel-Freeze Clinical Systems, Brown Deer, WI) were analyzed with
each batch of test samples to monitor the reproducibility of the method.

To determine the serum concentration of total 25-hydroxyvitamin D [25(OH)D], the sum of 25-hydroxycholecalciferol and 25-hydroxy
g ergocalciferol, the hydroxylated metabolites were extracted from se-
rum with acetoniitrile, and the lipids were removed with a Sep-Pak C-
column (Waters) using acetonitrile as the solvent. The metabolites
were further purified by eluting the samples from a Sep-Pak silica column (Waters) with 96:4 hexane/isopropyl alcohol. The purified
samples were assayed for 25(OH)D by a radio-binding assay (Nichols
Institute, San Juan Capistrano, CA) using rat intestinal extract as the
tracer and [3H]-25(OH)D as the ligand.

Vitamin K function was measured by the Simplessin(E)-Ecarin(E)
assay (Suttie et al. 1988). This assay provides an indirect measure of
TABLE 1
Base-line demographics for subjects consuming 18 g/d olestra or placebo for 16 wk

<table>
<thead>
<tr>
<th>Number of subjects per group¹</th>
<th>O + TA</th>
<th>O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enrolled</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>21</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Females</td>
<td>53</td>
<td>51</td>
<td>48</td>
</tr>
<tr>
<td><strong>Completed study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>16</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Females</td>
<td>46</td>
<td>45</td>
<td>44</td>
</tr>
</tbody>
</table>

¹O + TA = olestra plus tocopheryl acetate group, O = olestra group, P = placebo group.

RESULTS

Of the 219 subjects who enrolled, 194 completed the study, 67 in the placebo (P) group, 65 in the olestra (O) group and 62 in the olestra plus tocopheryl acetate (O + TA) group. Base-line demographics of the subjects who completed the study are shown in Table 1. The mean (± SD) daily consumption of olestra was 17.6 ± 0.1 g for both olestra groups, and the mean (± SD) daily consumption of triglyceride from the placebo test foods was 17.4 ± 0.1 g.

On the basis of the food-frequency questionnaire, there were no significant differences among the groups in daily intakes of vitamin E, vitamin D or carotenoids at wk 2 or 14 of the study with one exception. Vitamin E intake by the O + TA group at wk 14 was significantly less than that of either the O (24% less) or P (27% less) group (Table 2). At wk 2, one subject in the O + TA group had a vitamin E intake of 427 α-TE/d and one subject in the P group had an intake of 234 α-TE/d. The data for these two subjects were excluded from the data set. If these subjects are included, the mean ± SEM vitamin E intake for the O + TA group for wk 2 would be 13.7 ± 6.6 α-TE/d and the mean ± SEM intake for the P group for wk 2 would be 10.8 ± 3.4 α-TE/d. These subjects were probably taking a vitamin E supplement. If the data for these two subjects are included, there are still no significant differences among the groups at wk 2.

Group mean body weights did not change for those subjects completing the study, and no significant differences were found among the groups. Base-line and wk-16 group mean (± SD) body weights were 77 ± 2 and 77 ± 2 kg for the O + TA group, 78 ± 2 and 78 ± 2 kg, for the O group, and 76 ± 2 and 77 ± 2 kg for the P group.

Five subjects withdrew voluntarily because of illnesses unrelated to treatment. Seven subjects, four in the O + TA group, two in the O group, and one in the P group, withdrew voluntarily because of mild or moderate GI symptoms such as bloating, cramping, nausea and loose stools or diarrhea. Thirteen subjects were dropped or excluded from the data set because they did not consume 90% of the total dose of olestra, did not provide all blood samples or had unacceptable weight changes.

All laboratory values remained within normal laboratory ranges during the study. No changes indicative of an olestra-related effect were observed in any of the measures (data not shown). Sporadic intergroup significant differences occurred but were not consistent over time or with olestra ingestion.

Serum α-tocopherol concentration remained essentially constant for the P group and decreased slightly for the O and the O + TA groups (Table 3). The decline occurred within 2 wk, after which the values remained essentially constant. Using the average of the values measured at wk 2 through 16 as the final steady-state value, the decrease was 7.3% in the O group and 5.4% in the O + TA group. Although the repeated-measures ANOVA showed no significant olestra effect, the test for parallelism indicated a significant difference in trend among the groups. ANOVA showed that serum α-tocopherol values in the O group were significantly less than those in the P group at all time points except base line and wk 6 and 8. Values in the O + TA group were not significantly different than those in the P group at any time. The only significant differences between the O and the O + TA groups occurred at wk 10 and 14, when the values in the O + TA group were significantly greater than the values in the O group (Table 3). There was no significant olestra effect on serum γ-tocopherol concentration (data not shown).

Because α-tocopherol is transported as a constituent of lipoproteins (Behrens et al. 1982, Traber et al. 1988), serum α-tocopherol concentration, normalized with respect to serum lipids, provides a more reliable measure of vitamin E status than the unnormalized concentration. This is the case especially when serum lipids change during a study, as they did here. Figure 1 shows α-tocopherol concentrations, normalized with respect to serum cholesterol plus serum triglycerides and expressed as a percentage of base line for the three study groups. The value for the placebo group was essentially un-
OLESTRA’S EFFECTS ON FAT-SOLUBLE VITAMINS

DAILY INTAKE OF VITAMIN E, VITAMIN D, CAROTENOID AND VITAMIN A FOR SUBJECTS CONSUMING 18 G/D OLESTRA OR PLACEBO FOR 16 WK

<table>
<thead>
<tr>
<th>Vitamin E</th>
<th>Vitamin D</th>
<th>Carotenoids</th>
<th>Vitamin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Wk 2</td>
<td>Wk 14</td>
<td>Wk 2</td>
</tr>
<tr>
<td>O + TA</td>
<td>α-TE/d²</td>
<td>µg/d</td>
<td>RE/d</td>
</tr>
<tr>
<td>O</td>
<td>7.1 ± 0.5</td>
<td>6.5 ± 0.4²</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>7.5 ± 0.4</td>
<td>8.9 ± 0.7³</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>O</td>
<td>8.0 ± 0.6</td>
<td>8.5 ± 0.9³</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>7.5 ± 0.4</td>
<td>8.9 ± 0.7³</td>
<td>5.3 ± 0.4</td>
</tr>
</tbody>
</table>

1. Values are means ± SEM (n = 59–67). Groups in the same column having different superscripts are significantly different (P ≤ 0.05).
2. α-TE = µg α-tocopherol equivalents. Does not include the vitamin E added to the olestra in the O + TA group. One subject in the O + TA group had a vitamin E intake of 427 α-TE/d at wk 2 and was excluded from the data set. Including this subject produces a value of 13.7 ± 6.6 α-TE/d for this group. Similarly, one subject in the P group had an intake of 234 α-TE/d for wk 2 and was excluded from the data set. Including this subject produces a value of 10.8 ± 3.4 α-TE/d for this group. These subjects may have been taking vitamin E supplements.
3. Provitamin A carotenoids.
4. Preformed vitamin A (retinol).

Serum α-tocopherol concentration for subjects consuming 18 g/d olestra or placebo for 16 wk

<table>
<thead>
<tr>
<th>Study week</th>
<th>O + TA group</th>
<th>O group</th>
<th>P group</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/L</td>
<td>O + TA group</td>
<td>O group</td>
<td>P group</td>
</tr>
<tr>
<td>Base</td>
<td>20.2 ± 0.7</td>
<td>19.1 ± 0.6</td>
<td>19.7 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>19.3 ± 0.6³</td>
<td>17.7 ± 0.6²</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>18.9 ± 0.6³</td>
<td>18.0 ± 0.6³</td>
<td>19.7 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>19.5 ± 0.6³</td>
<td>17.4 ± 0.5²</td>
<td>19.8 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>19.1 ± 0.7³</td>
<td>17.4 ± 0.5³</td>
<td>19.6 ± 0.5</td>
</tr>
<tr>
<td>12</td>
<td>19.1 ± 0.7³</td>
<td>17.8 ± 0.6³</td>
<td>19.9 ± 0.5</td>
</tr>
</tbody>
</table>

1. Values are group means ± SEM; O + TA = olestra plus tocopheryl acetate group; O = olestra group; and P = placebo group; (n = 60–67). *Significantly less than base line (P = 0.05). **Significantly different than P group (P ≤ 0.05). ***Significantly different than O group (P ≤ 0.05).

DISCUSSION

In general, the effects of olestra on the absorption of fat-soluble nutrients observed in this study agreed with findings from previously reported olestra studies. Further, the effects are consistent with the mechanism proposed to explain how olestra affects nutrient absorption, i.e., the partitioning mechanism (Jandacek 1982). The absorption of the carotenoids was...
most affected, a reduction of \( \sim 27\% \), followed by \( \alpha \)-tocopherol (\( \sim 6\% \)) and then by cholesterol (\( \sim 4.5\% \)). Of these three nutrients, the carotenoids are the most lipophilic and cholesterol the least.

The lipophilicity of a molecule can be determined from the degree to which it partitions between an oil and water; the octanol-water partition coefficient is commonly used as a measure of the lipophilicity of a molecule. This coefficient can be either measured experimentally or calculated from knowledge of molecular structure (Hansch and Leo 1979, Meylan and Howard 1995). Octanol-water partition coefficients for \( \beta \)-carotene, \( \alpha \)-tocopherol and cholesterol are 17.6, 12.2 and 8.7, respectively (Cooper et al. 1997c). Because octanol-water partition coefficients are expressed in log units, \( \beta \)-carotene is almost 100 times more lipophilic than cholesterol.

The circulating concentration of \( \alpha \)-tocopherol concentration is a reliable indicator of vitamin E status (Bieri 1990, Machlin 1991). Specifically, the ratio of serum \( \alpha \)-tocopherol concentration to the sum of serum cholesterol and serum triglyceride concentrations has been shown to be a sensitive measure of vitamin E status (Thurnham et al. 1986). In this study, serum \( \alpha \)-tocopherol concentration in both olestra groups fell to a constant percentage of control in about 2 wk. A decrease in serum \( \alpha \)-tocopherol concentration within 2 wk was found in an 8-wk study in which subjects ate 8, 20 or 32 g/d olestra as part of a controlled diet (Schlagheck et al. 1997b). These rapid changes in serum \( \alpha \)-tocopherol with olestra intake are consistent with changes that occur when intake of the vitamin is changed (Baker et al. 1986, Behrens and Madere 1990, Horwitt et al. 1972).

Although olestra reduced serum \( \alpha \)-tocopherol concentration, vitamin E status among the subjects remained adequate. The new steady-state concentrations in the placebo group and the olestra groups, 17–20 \( \mu \text{mol/L} \), were within the 11–37 \( \mu \text{mol/L} \) range indicative of sufficient vitamin E status (Farrell 1980). The vitamin E intake of the three test groups was within the normal range reported for the general U.S. population (NRC 1989), i.e., about one Recommended Dietary Allowance (RDA), which is 8 and 10 mg \( \alpha \)-tocopherol equivalents for adult females and males, respectively.

Even though the serum \( \alpha \)-tocopherol concentration responded rapidly to olestra and reached a new steady-state concentration within about 2 wk, this response reflected changes in nutritionally important vitamin E stores. In young and adult animals, vitamin E concentrations in plasma and lean tissues were found to decline rapidly when the animals were placed on vitamin E–free diets; most of the change occurred within 2 wk (Bieri 1972, Machlin et al. 1979). In contrast, the mass of vitamin E in adipose tissue changed little, although the vitamin E adipose concentration declined as the amount of adipose tissue expanded with growth. These findings indicate that adipose stores of the vitamin, although large, are not

FIGURE 1 Effect of 18 g/d olestra and 18 g/d olestra supplemented with 1.1 mg/g \( \alpha \)-tocopherol acetate on serum \( \alpha \)-tocopherol concentration normalized with respect to serum total lipids, expressed as a percentage of base line. Values are means ± SEM. □ = placebo (P) group; ▲ = olestra (O) group; and ♦ = olestra supplemented with tocopherol acetate (O + TA) group. Values in the O group indicated by asterisks were significantly different than values in the P group (\( P \leq 0.05 \)). There were no significant differences between values in the O and the O + TA group or the P and O + TA group.
mobilized in response to decreased intake and act to maintain serum concentrations of vitamin E. Similar effects occur in humans. For example, Schaefer et al. (1983) found that the amount of vitamin E per adipocyte remained constant in humans during periods of weight loss (i.e., declining adipose mass) despite a large depletion of triglyceride. These observations support the conclusion that adipose stores of vitamin E do not sustain the vitamin E nutritional needs of the body and do not mobilize to offset any decline in serum vitamin E concentration resulting from decreased availability of the vitamin.

The effect of olestra on serum α-tocopherol measured in the present study was a reduction of ~6%, less than the effect measured in a study in which subjects consumed olestra for 8 wk as part of a controlled diet (Schlagheck et al. 1997b). In that study, the subjects ate olestra foods at each of the three daily meals and were not permitted to eat between meals. Under those dietary conditions, 20 g/d olestra reduced serum α-tocopherol concentration by ~17%, almost three times the effect produced by 18 g/d in the present study. These results indicate that eating olestra every time vitamin E, or any other nutrient, is eaten exaggerates the olestra effect on absorption relative to dietary patterns in which olestra and vitamins may be consumed at different times. This is consistent with the partitioning mechanism, which requires that olestra and the vitamin be present in the GI tract at the same time for olestra to affect the absorption of the vitamin (Jandacek 1982).

Addition of 1.1 mg d-α-TA/g olestra partially restored the normalized serum α-tocopherol concentration to control concentration, an indication that the effect of olestra on vitamin E status can be offset by adding adequate vitamin E to olestra, an expected result on the basis of the partitioning mechanism. However, an amount of vitamin E greater than that used in this study is required to completely offset the olestra effect and restore serum α-tocopherol concentration to the control concentration. Studies in humans in which the vitamin E and other nutrient intakes were controlled showed that 2.1 mg d-α-TA/g olestra completely restored serum α-tocopherol to control concentration (Schlagheck et al. 1997a).

Measurement of serum 25(OH)D is an accepted indicator of overall vitamin D status (Fraser 1984). Because hepatic 25-hydroxylase is not tightly homeostatically regulated, circulating concentrations of the 25-hydroxy metabolites of vitamin D1 and vitamin D2 are indices of the supply of vitamin D from dietary and endogenous sources (Holick et al. 1991). Serum 25(OH)D concentrations at the start of the study ranged from 20.1 to 24.1 nmol/L, typical of concentrations measured in free-living populations in the midwest and the northern U.S. in winter (Brazerol et al. 1988, Haddad and Hahn 1973, Jones 1978, Jones et al. 1991b). The lack of an effect on serum 25(OH)D concentration indicates that olestra did not measurably affect overall vitamin D status. The decline in serum 25(OH)D observed in all groups at wk 8, relative to baseline, and the subsequent increase at wk 16 were seasonal effects on the 25-hydroxyvitamin D1 contribution to 25(OH)D. The study started in February; therefore, the contribution to 25(OH)D from sunlight-induced synthesis of 25-hydroxyvitamin D1 was declining through wk 8 (April) before the subsequent increase with increased sun exposure during the last few weeks of the study.

In a previous 6-wk study in free-living subjects, 20 g/d olestra reduced serum 25(OH)D by ~5% (Jones et al. 1991b). Two factors help explain why the effect on serum 25(OH)D measured in this study was smaller than that. The first is a difference in the relative contribution of dietary vitamin D1, the only portion expected to be affected by olestra, to serum 25(OH)D. That contribution probably was considerably lower in the present study than in the 6-wk study. Daily ergocalciferol intake was not measured in the 6-wk study, but 20 μg/d was given as a supplement, resulting in a contribution of dietary vitamin D2 to serum 25(OH)D of ~50%. The dietary intake of vitamin D1 in this study was 5–6 μg/d (200–250 IU/d). The dietary contribution to serum 25(OH)D, although not measured, was probably 10–15%.

The second factor that helps explain the smaller effect of olestra on serum 25(OH)D in the present study relative to the 6-wk study is the difference in the way olestra and vitamin D were eaten in the two studies. In the 6-wk study, the 20 μg/d ergocalciferol supplement was always eaten simultaneously with olestra, thus providing maximum opportunity for olestra to affect absorption. In the present study, the subjects had the

### TABLE 4

<table>
<thead>
<tr>
<th>Study group</th>
<th>Base line</th>
<th>Wk 8</th>
<th>Wk 16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/E ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O + TA</td>
<td>1.04 ± 0.01</td>
<td>1.04 ± 0.01</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>O</td>
<td>1.05 ± 0.01</td>
<td>1.04 ± 0.01</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td>P</td>
<td>1.03 ± 0.01</td>
<td>1.03 ± 0.01</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O + TA</td>
<td>11.9 ± 0.04</td>
<td>11.9 ± 0.05</td>
<td>11.9 ± 0.05</td>
</tr>
<tr>
<td>O</td>
<td>11.9 ± 0.07</td>
<td>11.9 ± 0.06</td>
<td>12.0 ± 0.08</td>
</tr>
<tr>
<td>P</td>
<td>11.8 ± 0.05</td>
<td>11.8 ± 0.06</td>
<td>11.8 ± 0.06</td>
</tr>
<tr>
<td>PTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O + TA</td>
<td>24.4 ± 0.2</td>
<td>24.9 ± 0.2a</td>
<td>24.8 ± 0.3</td>
</tr>
<tr>
<td>O</td>
<td>24.5 ± 0.2</td>
<td>24.9 ± 0.2a</td>
<td>25.0 ± 0.2a</td>
</tr>
<tr>
<td>P</td>
<td>24.4 ± 0.2</td>
<td>24.8 ± 0.2a</td>
<td>24.7 ± 0.3</td>
</tr>
</tbody>
</table>

1Values are group means ± SEM; O + TA = olestra plus tocopheryl acetate group; O = olestra group; P = placebo group; (n = 59–60).

2There were no significant differences among the groups for any parameter. Significantly greater than base line (P < 0.05).

### TABLE 5

<table>
<thead>
<tr>
<th>Study group</th>
<th>Serum 25-hydroxyvitamin D concentration2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum 25-hydroxyvitamin D concentration2</td>
</tr>
<tr>
<td></td>
<td>Base line</td>
</tr>
<tr>
<td></td>
<td>nmol/L</td>
</tr>
<tr>
<td>O + TA</td>
<td>21.5 ± 0.9</td>
</tr>
<tr>
<td>O</td>
<td>23.3 ± 1.0</td>
</tr>
<tr>
<td>P</td>
<td>22.0 ± 1.0</td>
</tr>
</tbody>
</table>

1Values are group means ± SEM; O + TA = olestra plus tocopheryl acetate group; O = olestra group; and P = placebo group; (n = 61–68).

25-Hydroxyvitamin D = 25-hydroxycholecalciferol plus 25-hydroxyergocalciferol. Significantly different than base line (P < 0.05). There were no significant differences among the groups.
FIGURE 2  Effect of 18 g/d olestra and 18 g/d olestra supplemented with 1.1 mg/g α-tocopherol acetate/g on serum β-carotene concentration.
Values are means ± SEM. ■ = placebo (P) group; ▲ = olestra (O) group; and ◆ = olestra supplemented with tocopherol acetate (O + TA) group.
Values in the O group and the O + TA group were significantly different (P ≤ 0.05) than values in the P group at all points except base line, but were not significantly different from each other at any time.

opportunity to eat vitamin D–containing foods at times other than when they ate the olestra foods.

Olestra did not affect vitamin K function as evidenced by the lack of any significant effect on the S:E ratio, PT or PTT. The ratio of the amount of des-γ-carboxylated and partially γ-carboxylated prothrombin to that of fully γ-carboxylated prothrombin, the S:E ratio, is a direct and sensitive measure of the adequacy of vitamin K for clotting factor synthesis (Suttie et al. 1988). Full vitamin K sufficiency results in a S:E value of ~1, depending on the specific batches of reagents used in the assay. When the intake of vitamin K by a vitamin K–replete subject falls below the amount needed for complete γ-carboxylation of the vitamin K–dependent proteins over a period of several days, the S:E ratio declines. The S:E ratio is more reliable than serum phylloquinone concentration as a measure of vitamin K functional status because the circulating concentration of phylloquinone fluctuates rapidly as a result of its short half-life in the plasma (~2 h) and because of the lack of tissue stores of the vitamin (Schlagheck 1997b, Shearer et al. 1974).

The absence of change in measures of vitamin K function agrees with the results of a previous 6-wk free-living study in

TABLE 6  Serum α-carotene, lycopene, lutein/zeaxanthin and total carotenoid concentrations for subjects consuming 18 g/d olestra or placebo for 16 wk

<table>
<thead>
<tr>
<th>Study group</th>
<th>α-Carotene Base line</th>
<th>α-Carotene Wk 16</th>
<th>Lycopene Base line</th>
<th>Lycopene Wk 16</th>
<th>Lutein/zeaxanthin Base line</th>
<th>Lutein/zeaxanthin Wk 16</th>
<th>Total carotenoids Base line</th>
<th>Total carotenoids Wk 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>O + TA</td>
<td>50 ± 4</td>
<td>31 ± 2</td>
<td>243 ± 12</td>
<td>166 ± 7</td>
<td>668 ± 40</td>
<td>520 ± 24</td>
<td>686 ± 32</td>
<td>492 ± 19</td>
</tr>
<tr>
<td>O</td>
<td>51 ± 4</td>
<td>30 ± 3</td>
<td>247 ± 11</td>
<td>176 ± 9</td>
<td>631 ± 31</td>
<td>517 ± 24</td>
<td>663 ± 27</td>
<td>497 ± 23</td>
</tr>
<tr>
<td>P</td>
<td>56 ± 4</td>
<td>34 ± 4</td>
<td>244 ± 10</td>
<td>229 ± 9</td>
<td>734 ± 44</td>
<td>752 ± 43</td>
<td>726 ± 32</td>
<td>731 ± 34</td>
</tr>
</tbody>
</table>

1Values are group means ± SEM; (n = 60–67). O + TA = olestra plus tocopheryl acetate group. O = olestra group, P = placebo group.
2Lutein and zeaxanthin co-elute under the HPLC conditions used and are thus quantitated and reported together.
which the same measures were unaffected by 20 g/d olestra (Jones et al. 1991a). It is also consistent with the results of studies in which olestra doses as high as 32 g/d had no effect on the urinary excretion of γ-carboxylglutamic acid or the plasma concentration of des-γ-carboxyprothrombin (Schlagheck et al. 1997a and 1997b), both sensitive indicators of vitamin K function (Suttie 1992).

The half-lives of the vitamin K–dependent proteins and clotting factors, ≤60 h, are such that any effect of olestra on vitamin K function would have been manifested during this study (Ferland et al. 1993). For example, the S/E ratio has been shown to decline within 2 wk in response to a reduction in vitamin K1 intake (Allison et al. 1987, Suttie et al. 1988). Other measures of vitamin K function, such as urinary excretion of γ-carboxylglutamic acid, also have been shown to change within 2–3 wk of restricted vitamin K intake (Allison et al. 1987, Ferland et al. 1993).

Comparison of the carotenoid intake of the present study population with values reported by others indicates that the intake was similar to that of the general population (Forman et al. 1993, Stryker et al. 1988). In addition, the base-line serum β-carotene concentrations, 0.27–0.28 μmol/L, were in agreement with values reported by others for normal, healthy adults (Forman et al. 1993, Henderson et al. 1989, Weststrate and van het Hof 1995).

Because serum carotenoid concentration is directly proportional to carotenoid intake, changes in serum carotenoid concentration are reliable indicators of changes in absorption (Forman et al. 1993, Henderson et al. 1989, Ribaya-Mercado et al. 1989, Stryker et al. 1988). Nutritionally active carotenoids provide at least 25% of the vitamin A in the U.S. diet; the remainder comes from preformed vitamin A ( Olson 1987).

Therefore, a decrease in the absorption of either dietary source can result in a decrease in total vitamin A stores. However, the 27% reduction in serum carotenoid concentration measured in this study does not translate into a 27% reduction in body vitamin A stores because olestra has no significant effect on the absorption of retinyl palmitate, the major dietary source of vitamin A stores (Daher et al. 1997b).

Because liver vitamin A stores maintain serum vitamin A concentration when dietary intake is reduced until liver stores become depleted ( Olson 1984), the lack of change in serum retinol concentration observed in this study is not surprising.

The 27% reduction in serum carotenoid concentration seen in this study was less than the 61% reduction produced by 20 g/d olestra in studies in which subjects ate olestra at every meal for 8 wk and were not permitted to eat between meals (Schlagheck et al. 1997a and 1997b). This is explained primarily by the difference between the studies in the frequency of co-consumption of olestra and carotenoid-containing foods.

In the 8-wk studies, olestra was eaten at all meals and the subjects were not permitted to eat between meals, which means that olestra and carotenoids were always eaten together. This pattern provides the maximum opportunity for olestra to affect the absorption of the carotenoids. In the present study, the subjects were requested to eat olestra at meals but were allowed to eat between meals, which meant that substantial amounts of carotenoids were probably eaten at times when olestra was not eaten. Separating the time between olestra intake and nutrient intake reduces or greatly decreases the effect of olestra on nutrient absorption ( Daher et al. 1997a).

The effect of olestra on carotenoid absorption measured in this study or in the 8-wk study is greater than the effect that would be expected from reducing carotenoid intake for 8 wk (Suttie 1992). This is supported by the fact that the reduction in serum carotenoid concentration in the present study (27%) is slightly higher in subgroups of the population such as lacto-vegetarians than in the general population (10% in carotenoid intake for the general population; Stryker et al. 1988). Thus, the effect of olestra on carotenoid absorption is likely to be greater in people with low carotenoid intake.

In the present study, the effect of olestra on carotenoid absorption was measured in subjects consuming 18 g/d olestra for 16 wk, and the results are presented in Table 7.

**Table 7**

<table>
<thead>
<tr>
<th>Week</th>
<th>O + TA group</th>
<th>O group</th>
<th>P group</th>
<th>O + TA group</th>
<th>O group</th>
<th>P group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>5.14 ± 0.05</td>
<td>5.14 ± 0.14</td>
<td>5.20 ± 0.11</td>
<td>1.21 ± 0.07</td>
<td>1.21 ± 0.06</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>4.99 ± 0.12a</td>
<td>5.02 ± 0.12a</td>
<td>5.39 ± 0.12c</td>
<td>1.21 ± 0.08</td>
<td>1.20 ± 0.07</td>
<td>1.23 ± 0.07c</td>
</tr>
<tr>
<td>4</td>
<td>5.07 ± 0.11</td>
<td>5.06 ± 0.13</td>
<td>5.29 ± 0.12</td>
<td>1.22 ± 0.09</td>
<td>1.24 ± 0.08</td>
<td>1.15 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>5.04 ± 0.11a</td>
<td>5.14 ± 0.13</td>
<td>5.34 ± 0.12</td>
<td>1.20 ± 0.09</td>
<td>1.27 ± 0.07</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>8</td>
<td>5.11 ± 0.10</td>
<td>5.09 ± 0.12</td>
<td>5.35 ± 0.13c</td>
<td>1.31 ± 0.09b</td>
<td>1.36 ± 0.10b,c</td>
<td>1.06 ± 0.06</td>
</tr>
<tr>
<td>10</td>
<td>5.03 ± 0.11a</td>
<td>4.91 ± 0.11a</td>
<td>5.17 ± 0.12</td>
<td>1.29 ± 0.11</td>
<td>1.24 ± 0.08</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>12</td>
<td>4.97 ± 0.11a</td>
<td>4.87 ± 0.11a</td>
<td>5.29 ± 0.13</td>
<td>1.20 ± 0.07</td>
<td>1.32 ± 0.09</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>14</td>
<td>4.93 ± 0.11a</td>
<td>5.01 ± 0.12a</td>
<td>5.27 ± 0.11</td>
<td>1.36 ± 0.13</td>
<td>1.22 ± 0.08</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>16</td>
<td>4.83 ± 0.10a</td>
<td>4.85 ± 0.11a</td>
<td>5.14 ± 0.11</td>
<td>1.26 ± 0.12</td>
<td>1.21 ± 0.08</td>
<td>1.18 ± 0.09</td>
</tr>
</tbody>
</table>

Values are group means ± SEM; O + TA = olestra plus tocopheryl acetate group; O = olestra group; and P = placebo group; (n = 59–67). aSignificantly less than base line (P = 0.05). bSignificantly different than P group (P = 0.05). cSignificantly greater than base line (P = 0.05).
The authors gratefully acknowledge the assistance of J. W. Suttle in the design and interpretation of this study and for making the S:E measurements, Pat Hudson for technical assistance, and K. D. Lawson for assistance in preparing the manuscript.

LITERATURE CITED

ACKNOWLEDGMENTS

The results of this study demonstrated that it is feasible to offset the effects of olestra on vitamin E status in a free-living population by adding the vitamin to olestra. This result applies to fat-soluble vitamins in general because the mechanism by which olestra affects vitamin E status is general for fat-soluble nutrients. Complete restoration of tissue concentrations of vitamin A, vitamin E and vitamin D has been demonstrated in pigs fed olestra mixed in the diet (Cooper et al. 1997a and 1997b) and, for vitamins E and D, in humans eating olestra in foods such as potato chips, biscuits and cookies (Schlagheck et al. 1997a). Extra amounts of all of these vitamins, as well as vitamin K, will be added to marketed olestra snacks.
OLESTRA’S EFFECTS ON FAT-SOLUBLE VITAMINS


