Olestra Ingestion and Retinyl Palmitate Absorption in Humans1,2,3

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ABSTRACT This study examined the effect of olestra, a zero-calorie fat replacement, on the absorption of retinyl palmitate in humans. After a 30-d adaptation period during which they consumed 10 g olestra/d in potato chips under free-living conditions, 68 healthy male subjects were housed in a metabolic ward and given a single dose of retinyl palmitate (0.33 RDA) containing a trace amount of 3H-retinyl palmitate with a breakfast that contained 0, 8, 20 or 32 g of olestra and about 38 g of triglyceride. Blood was collected at defined intervals for 48 h and plasma analyzed for 3H-retinyl esters by HPLC and liquid scintillation spectrometry. There was no significant effect on retinyl palmitate absorption as determined from the area under the plasma 3H-retinyl esters concentration-time curve. However, an area under the plasma concentration-time curve in the 32-g olestra group that was 81% (mean value) or 70% (median value) of the area under the curve for the placebo group suggested that olestra may have affected retinyl palmitate absorption. Inclusion or exclusion of 13 high responders did not change the results. J. Nutr. 127: 1686S–1693S, 1997.

KEY WORDS: • olestra • vitamin A • absorption • humans

Olestra (Olean, Procter & Gamble, Cincinnati, OH), a mixture of polyesters formed from sucrose and long-chain (>16 carbons) fatty acids from vegetable oils, is a lipid-based noncaloric replacement of dietary fat that is not hydrolyzed by gastric or pancreatic lipases (Mattson and Volpenhein 1972) or absorbed from the gastrointestinal (GI) tract (Miller et al. 1995). A lipophilic nonabsorbed substance in the GI tract has the potential to interfere with the absorption of lipophilic nutrients (Jandacek 1982). Several studies in animals and humans have shown that olestra interferes with the absorption of highly lipophilic nutrients. In a study in which free-living subjects consumed 18 g olestra/d for 16 wk, serum concentrations of α-tocopherol and carotenoids were reduced (Koonsvitsky et al. 1997). Studies in which normal healthy subjects consumed olestra at 8, 20 or 32 g/d for 8 wk, as part of a controlled diet, showed that olestra reduced serum levels of α-tocopherol, 25-hydroxyvitamin D, phylloquinone and carotenoids (Schlagheck et al. 1997a and 1997b).

Because vitamin A, usually consumed as retinyl esters, is highly lipophilic, olestra might be expected to interfere with the absorption of this vitamin. Results from animal studies support this possibility. Mattson et al. (1979) found that olestra reduced liver vitamin A stores in rats. Using a jejunum perfusion technique, Sletten et al. (1985) found that 1% olestra, in an emulsion containing retinol, sodium taurocholate, oleic acid and glyceryl monooleate, reduced the absorption of retinol in rats. Recently, Cooper et al. (1997a–c) found dose-responsive decreases in liver stores of vitamin A when domestic pigs were fed olestra and a 3:1 mixture of retinol equivalents from retinyl palmitate and β-carotene.

The concentration of retinol in the blood is homeostatically controlled in humans; therefore plasma retinol levels do not reflect short-term changes in intake of the vitamin, and can remain relatively constant over long periods of vitamin A depletion (Olson 1984, Sauberlich et al. 1974). Apparent decreases in plasma retinol concentration were observed in early olestra studies (Crouse and Grundy 1979, Fallat et al. 1976, Glueck et al. 1979 and 1982, Mellies et al. 1983). These decreases, however, probably resulted from the fluorometric assay used to measure plasma retinol, which is subject to interference from the carotenoid phytol (Thompson et al. 1971). In a study in which HPLC was used to measure plasma retinol concentration, no effect of olestra was found (Mellies et al. 1985).

There are two sources of vitamin A in the average U.S. diet. The major proportion, about 75%, comes from retinyl esters, predominately retinyl palmitate, the remainder, about 25%, comes from provitamin A carotenoids (Olson 1987). Therefore, it is necessary to examine the effect of olestra on each source to assess olestra’s effect on overall vitamin A status in humans. Because serum retinol concentration is homeostatically controlled, an effect on retinyl ester absorption is determined most accurately by measuring postprandial plasma concentration of retinyl esters after an oral dose of retinyl palmitate, the predominate retinyl ester in food. During digestion, retinyl palmitate is hydrolyzed to retinol; after the retinol has
been taken up by the enterocytes, it is re-esterified and incorporated into triglyceride-rich chylomicrons (Goodman et al. 1966). In the blood, the chylomicrons are hydrolyzed into remnants by lipoprotein lipase (Scow et al. 1976). The retinyl esters remain with the remnants during hydrolysis (Blomhoff et al. 1991), and chylomicron remnant–associated retinyl esters are the predominant postprandial plasma metabolites of ingested vitamin A (Blomhoff et al. 1992). Under fasting conditions, only minimal amounts of retinyl esters are found in the plasma (Krasinski et al. 1989).

Measurements of postprandial plasma levels of retinyl esters have been used to evaluate retinyl ester absorption in patients with gastrointestinal diseases (Johnson et al. 1992a) and in obese subjects (Lewis et al. 1990). The method also has been used to evaluate the effects of age (Krasinski et al. 1990) and gender (Johnson et al. 1992b) on retinyl ester absorption. In most of these studies, pharmacological doses of retinyl palmitate were used because of the insensitivity of the measurement methods (Kahan 1969 and 1970). More recently, HPLC methods have been developed which allow the quantification of plasma concentrations of newly absorbed retinyl esters from doses that are 2–5 times the Recommended Dietary Allowance (RDA) (Bankson et al. 1986, Johnson et al. 1992a and 1992b, NRC 1989, Ruotolo et al. 1992).

The purpose of this study was to measure the effect of olestra on the absorption of retinyl palmitate from a meal containing 0.33 RDA of vitamin A; a trace amount of [11,12-3H]-retinyl palmitate; 0, 8, 20 or 32 g of olestra; and about 38 g of digestible fat. Absorption was determined by measuring the concentration of 3H-retinyl esters in the plasma over a 48-h period after the meal. Radiolabeled retinyl palmitate was used to assure adequate analytical sensitivity for the low, but typical, dietary level of retinyl palmitate employed.

**SUBJECTS AND METHODS**

**Study design.** This study was a parallel, double-blind, placebo-controlled trial with four groups of healthy 19- to 44-y-old males, 17–19 per group. The groups were balanced with respect to age, body mass index (BMI), fasting serum triglyceride concentration and plasma retinol concentration. The subjects were enrolled in three cohorts, consisting of 23, 18 and 30 subjects, each over a 30-d period. Subjects from each cohort were randomly assigned into the test groups.

The study consisted of a 30-d free-living adaptation period followed by a 14-d period during which the subjects were housed in a metabolic ward. During the adaptation period, subjects consumed their habitual diets with either potato chips that delivered 10 g olestra/d, or potato chips prepared with corn oil (control). The purpose of this period was to allow GI functions to adapt to olestra consumption, should any adaptation occur. Subjects were assigned to treatment groups at the beginning of the adaptation period so that subjects in the olestra groups would receive olestra chips during the adaptation period, and those in the placebo group would receive placebo chips. During the metabolic ward period, the subjects were given 0, 8, 20 or 32 g olestra/d in potato chips and cookies for 3 d until the retinyl palmitate absorption study started.

During the 14 d when the subjects were in the metabolic ward, vitamin A and fat absorption were measured in two sequential tests. The results of the vitamin A absorption study are presented in this paper. The results of the fat absorption study are presented elsewhere (Dahe et al. 1997).

The study was conducted in compliance with Good Clinical Practices regulations. Signed informed consent was obtained from each subject before enrollment. The protocol was approved by the Nebraska State Radiation Safety Committee and by the Institutional Review Board of Harris Laboratories (Lincoln, NE), where the study was conducted.

A pilot study with four subjects was conducted prior to the full-scale study. This study was done to develop dosing procedures that would represent the consumption of vitamin A from the diet, to determine the appropriate dose of 3H-retinyl palmitate required to produce measurable plasma levels of 3H-retinyl esters, and to ascertain the frequency and length of blood collection required to accurately determine the 3H-plasma retinyl esters concentration-time curves.

**Subjects.** To be included in the study, a subject was required to be in good health, as determined by medical history, physical examination, and a full battery of hematology, blood chemistry, and urinalysis tests, including a urine drug screen (Harris Laboratories, Lincoln, NE). Inclusion criteria included a BMI of 19–30 kg/m², fasting serum total cholesterol concentration of <6.98 mmol/L, fasting serum triglyceride concentration of <2.71 mmol/L (as triolein), and a plasma retinol concentration of 1.40–2.79 mol/L. In addition, all other clinical laboratory values were required to be within 10% of normal limits. Other inclusion criteria relevant to the fat absorption study are described elsewhere (Dahe et al. 1997).

Exclusion criteria included chronic use of any drug that had the potential to interfere with vitamin absorption, exposure to radioactivity or X-rays within the past year, use of antibiotics within the past 2 wk, any type of dietary restriction, and a greater than average caloric need because of high activity level.

Seventy-one subjects were enrolled in the study. Table 1 shows the randomization parameters for the subject population at the beginning of the study.

**Olestra test material.** The olestra was synthesized by the method of Rizzi and Taylor (1978). It consisted of 99.7% octa- and heptaeasters and 0.3% hexa- or lower esters. The relative composition of the fatty acids making up the ester groups was 19% palmitic, 4% stearic, 33% oleic, 34% linoleic, 9% behenic, and 1% others.

**Diet.** While the subjects were in the metabolic ward, they were provided with all food items. The core menu, including the olestra or placebo potato chips, provided about 10.88 MJ (2600 kcal) of energy. The subjects were fed to maintain their enrollment weight to within ±5% by providing smaller portions of the core meal to those subjects with lower energy needs and a larger portion of the core meal to those subjects requiring more energy. The calculation of energy needs and serving sizes was accomplished using the Harris-Benedict equation which was modified to account for activity level as in previous studies (Schlagheck et al. 1997a and 1997b). If additional energy was required to maintain energy balance, it was provided as snack items that contained essentially no triglyceride or vitamin A. The diet provided 100 ± 20% of the RDA of vitamin A, about 15% of energy as protein, about 55% as carbohydrate, and about 30% as fat. The ratio of saturated:saturated:polyunsaturated fat was targeted at 1:1:1.

The level of digestible fat was kept constant across the treatment

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**TABLE 1**

Randomization parameters for subjects completing the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>8</th>
<th>20</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>19</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Age, y Mean</td>
<td>28.1</td>
<td>26.7</td>
<td>27.5</td>
<td>29.3</td>
</tr>
<tr>
<td>Range</td>
<td>19–38</td>
<td>19–44</td>
<td>19–42</td>
<td>19–43</td>
</tr>
<tr>
<td>Body mass index, kg/m² Mean</td>
<td>24.3</td>
<td>24.1</td>
<td>24.3</td>
<td>24.6</td>
</tr>
<tr>
<td>Plasma retinol, µmol/L Mean</td>
<td>1.93</td>
<td>1.91</td>
<td>1.92</td>
<td>1.88</td>
</tr>
<tr>
<td>Range</td>
<td>1.47–2.58</td>
<td>1.43–2.76</td>
<td>1.40–2.72</td>
<td>1.40–2.44</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L Mean</td>
<td>1.40</td>
<td>1.48</td>
<td>1.17</td>
<td>1.38</td>
</tr>
<tr>
<td>Range</td>
<td>0.51–2.46</td>
<td>0.70–2.65</td>
<td>0.59–2.07</td>
<td>0.26–2.55</td>
</tr>
</tbody>
</table>
groups by adding triglyceride to the diet, in the form of butter or corn oil margarine, to compensate for the fat replaced by olestra. Dietary carotenoid intakes were maintained at comparable levels across the groups. During the 3 d in the metabolic ward before the subjects were dosed with \(^3\text{H}\)-retinyl palmitate, they were given 0, 8, 20 or 32 g olestra/d in potato chips and cookies, divided evenly among the three daily meals.

On the day \(^3\text{H}\)-retinyl palmitate was administered, the subjects were only given breakfast and dinner. The breakfast consisted of a biscuit or a bagel, or some of each; jelly (28 g); apple juice (168 g); one half of a plain cake doughnut hole (the section of a doughnut removed when the hole is cut), used to deliver the retinyl palmitate; potato chips, used to deliver the olestra; and instant tea (168 g). The amounts of biscuit and bagel were varied across the groups to keep fat, protein, and carbohydrate levels constant. The bagels were essentially fat-free. The biscuits contained butter and corn oil margarine, which were used to keep digestible fat intake constant across the groups. The placebo group did not receive a biscuit; the 32-g olestra group did not receive a bagel. This breakfast provided about 4.72 MJ (1130 kcal) of energy, and about 38 g of fat (primarily corn oil), about 0.02 RDA of vitamin A, not including the dose delivered on the doughnut hole, and contained the entire daily dose of olestra or placebo.

Lunch consisted of a diet soft drink. Dinner consisted of an Italian sausage (105 g) on a white bun with mustard, French fries (1 cup), a bagel (144 g) with jelly (28 g), and instant tea (237 g). This meal provided about 5.99 MJ (1433 kcal) of energy, about 45 g of fat, and essentially no vitamin A.

The total daily food consumption was controlled and determined by dispensing amounts of preweighed food to each subject and weighing any uneaten portions. During the period the subjects were housed in the metabolic ward, they were required to consume at least 85% of the meals and at least 90% of the potato chips. On the day they were given the dose of \(^3\text{H}\)-retinyl palmitate, the subjects were required to consume all of the meals and test potato chips.

Daily intakes of total energy, protein, carbohydrate, fat (as percentage of calories and in grams) and vitamin A were calculated by the University of Minnesota Nutrient Data System, Version 2.3. Olestra intake was calculated from the amount of olestra potato chips eaten and from the analytically determined olestra content of the chips.

**Dosing procedures.** After 3 d in the metabolic ward, the subjects were given a single oral dose of \(5.55 \text{ MBq of all-trans } [11,12-^3\text{H}]\)-retinyl palmitate per kg) labeled and \(^3\text{H}\)-retinyl palmitate (specific activity = 4.27 GBq/mg, Amer sham, Arlington Heights, IL). The radiolabeled retinyl palmitate was obtained as an ethanol solution. The ethanol was evaporated under a stream of argon, and the residue was reconstituted in peanut oil containing unlabeled retinyl palmitate (Hoffman-LaRoche, Nutley, NJ). The total dose of retinyl palmitate, labeled plus unlabeled, was about 0.33 RDA (1 RDA = 1000 g retinol = 1000 retinol equivalents; NRC 1989).

The peanut oil solution of retinyl palmitate was pipetted directly onto the freshly cut surface of one half of a doughnut hole. The section of doughnut hole was then eaten with the breakfast described above and 0, 8, 20 or 32 g olestra, delivered in potato chips. The subjects were required to consume the entire meal and the entire amount of potato chips and were not allowed to eat again for 12 h. The subsequent meal did not include olestra.

**Sample collection.** Blood samples were taken by venipuncture for analysis of \(^3\text{H}\)-retinyl esters immediately before the breakfast and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24 and 48 h after breakfast. Blood was collected in tubes containing potassium ethylenediaminetetraacetic acid. The collection times were chosen to provide a complete profile of the blood concentration of \(^3\text{H}\)-retinyl esters resulting from the breakfast, based on the findings from the pilot study. The blood samples were centrifuged immediately at 4°C to obtain plasma. The plasma samples were stored in the dark, at \(-20^\circ\text{C}\), until they were analyzed. Blood and urine samples for hematology, clinical chemistry and urinalysis were collected at the beginning of the adaptation period for randomization and again at the end of the study for overall safety assessment.

**Analytical methods.** The serum concentration of retinol used to randomize the treatment groups was measured by the method of Driskell et al. (1982). Plasma \(^3\text{H}\)-retinyl esters were measured by the method of Epler et al. (1993). Briefly, the \(^3\text{H}\)-retinyl esters were extracted from deproteinized plasma with hexane and isolated by reverse-phase HPLC. The fractions, which contained primarily retinyl palmitate, stearate and oleate, were collected in scintillation vials and dried. Scintillation fluid (15 mL) (Pemfluor E Plus, Packard Instrument, Downers Grove, IL) was added and the radioactivity of each fraction determined in a liquid scintillation spectrometer (Wallac Model 1410, Wallac Oy, Turku, Finland) by counting for 20 min or until a total counts per minute of 2500 was reached. The counts per minute were converted to disintegrations per minute (DPM), and the DPM in the total volume of body plasma were calculated by using 33.45 mL/kg as the total volume of plasma in adult males (ICSH 1973, Miale 1982).

The fractional dose (FD) of \(^3\text{H}\)-retinyl esters in the plasma was calculated by dividing the total DPM in the plasma by the total DPM in the administered dose. The recovery of \(^3\text{H}\)-retinyl esters from the plasma by the extraction process, determined by using spiked plasma samples, was 93.5%. Overall recovery was 83%. The analyses were conducted by American Medical Laboratories (Chantilly, VA).

**Calculation of \(^3\text{H}\)-retinyl palmitate absorption.** The relative absorption of \(^3\text{H}\)-retinyl palmitate was determined for each subject from the area under the FD-time curve (AUC). The AUC reflects the fraction of the dose of retinyl palmitate that is absorbed into the systemic circulation (Blomhoff et al. 1991). The individual AUC was calculated by the standard trapezoidal rule and also by a recently developed method, Method 9, which has been reported to produce a better estimate of AUC for sharply peaked curves typical of vitamin A absorption profiles (Purves 1992). Method 9 fits the tops of the segmental rectangles on the ascending side of the curve with parabolas starting at the origin. On the descending side of the curve, the tops of the segmental rectangles are fitted by a log function. The AUC values provided here were calculated by using the FD\(_{0}\) data for 12 h after dosing. The use of 24-h FD\(_{0}\) data produced the same results.

The effect of olestra on \(^3\text{H}\)-retinyl palmitate absorption was determined by statistically comparing median or mean AUC values for each group. The time to reach peak plasma concentration (\(t_{\text{max}}\)) was based on the blood draw with the highest level of radioactivity, averaged for the treatment groups. No kinetic parameters were derived.

**Statistical methods.** Subjects were assigned to treatment groups by the method of Pocock and Simon (1975). This method maintains covariant balance within each group by assigning each new subject to the group on the basis of the covariate information from all previously assigned subjects. ANOVA was used to assess the effect of olestra on the AUC values. When the hypothesis of equal group means was rejected, the protected least significant difference multiple-comparisons procedure (Carter and Swanson 1973; Welch 1977) was used to identify differences among the groups. Linear trend tests were conducted on the group means, using a single degree of freedom.

### Table 2

Energy, macronutrient, olestra and vitamin A intake for each treatment group on the day \(^3\text{H}\)-retinyl palmitate was administered.

<table>
<thead>
<tr>
<th>Group (g olestra)</th>
<th>Diet component</th>
<th>0</th>
<th>8</th>
<th>20</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total energy (MJ)</strong></td>
<td>11.04</td>
<td>11.56</td>
<td>11.52</td>
<td>10.10</td>
<td></td>
</tr>
<tr>
<td><strong>Fat (% of energy)</strong></td>
<td>29.1</td>
<td>28.9</td>
<td>29.0</td>
<td>33.2</td>
<td></td>
</tr>
<tr>
<td><strong>Protein (% of energy)</strong></td>
<td>10.4</td>
<td>9.9</td>
<td>9.9</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate (% of energy)</strong></td>
<td>59.2</td>
<td>59.7</td>
<td>60.5</td>
<td>54.8</td>
<td></td>
</tr>
<tr>
<td><strong>Energy in the breakfast (MJ)</strong></td>
<td>4.84</td>
<td>4.76</td>
<td>4.87</td>
<td>4.54</td>
<td></td>
</tr>
<tr>
<td><strong>Fat in the breakfast (g)</strong></td>
<td>37.7</td>
<td>38.0</td>
<td>39.2</td>
<td>39.3</td>
<td></td>
</tr>
<tr>
<td><strong>Olestra in the breakfast (g)</strong></td>
<td>0</td>
<td>7.8</td>
<td>19.3</td>
<td>31.2</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin A in the breakfast (RE)</strong></td>
<td>&lt;1.0</td>
<td>9.0</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

1 Excludes the 333 RE (0.33 RDA) dose. RE = \(\mu\)g retinol equivalents.
OLESTRA AND VITAMIN A ABSORPTION

Residual analysis showed that the individual AUC values were not normally distributed. The data were transformed by using a log transformation (Z = ln(Y + 1)) and also by the Box-Power power transformation (Z = Y – 0.4) as described by Draper and Smith (1981). Both transformations produced normally distributed data.

All statistical tests were performed at the two-sided 0.05 significance level, using PC SAS® Version 6.04 software (SAS Institute, Cary, NC).

RESULTS

Sixty-eight of the 71 subjects completed the study. Three subjects were removed or withdrew voluntarily during the adaptation period for reasons unrelated to olestra consumption or to study procedures. These subjects were not dosed with 3H-retinyl palmitate.

Nutrient intakes during the metabolic-ward period did not differ among the groups. Daily total energy intake ranged from 10.89 to 11.56 MJ (2605–2766 kcal): 13% came from protein, 58% from carbohydrate, and 29% from fat. Table 2 shows nutrient and olestra intakes on the day when 3H-retinyl palmitate was administered. The fat and vitamin A contents of the breakfast containing the dose of 3H-retinyl palmitate did not differ significantly among the groups. Vitamin A intake increased slightly with olestra dose because of the increasing amounts of corn oil margarine added to the biscuits to keep total fat intake constant across the treatment groups.

Olestra did not affect the general shape of the 3H-retinyl esters concentration-time curves (Fig. 1) or the time to reach peak plasma concentration (Table 3). By 12 h, the concentration was approaching baseline in all groups.

The mean and median AUC values for each group are shown in Table 3. The mean AUC value for the 32-g group was 81% of the value for the placebo group and the median AUC value was 70% of the placebo value. However, statistical analysis showed no significant differences among the groups.

Figure 2 shows the distribution of AUC values in each treatment group. Several individuals in each group had AUC values that were markedly higher than the rest. Calculation of the individual AUC values by Method 9 (Purves 1992) produced essentially the same results; there were no significant differences among the groups (data not shown).

It was considered whether the presence of an unequal number of unusually high AUC values in the treatment groups might mask an olestra effect. There appear to be

FIGURE 1 Mean fractional dose of 3H-retinyl esters in plasma (Fdₚ) as a function of time after subjects ingested 0, 8, 20 and 32 g olestra.

![Graph showing the mean fractional dose of 3H-retinyl esters in plasma (Fdₚ) as a function of time after subjects ingested 0, 8, 20, and 32 g olestra.](https://example.com/figure1.png)
two populations of subjects, those with AUC values <0.65 FDp·h and those with AUC values >0.65 FDp·h (Fig. 2). The latter population consisted of 13 subjects; two in the placebo group, five in the 8-g olestra group, four in the 20-g olestra group, and two in the 32-g olestra group. The data were analyzed without these 13 high responders; ANOVA revealed no significant olestra effect. Mean and median AUC values calculated for each treatment group after the high responders were removed are shown in Table 4.

The mean 3H-retinyl palmitate concentration-time curve was qualitatively the same for the high responders as for the rest of the subjects (Fig. 3). The maximum concentration of plasma 3H-retinyl esters reached among the high responders was about twice the level reached among the other subjects. The initial rate of increase in plasma retinyl ester concentration was the same in both populations, although tmax was longer in the high responders: 3.5 versus 2.5 h for the other subjects.

DISCUSSION

In this study, the concentration of 3H-retinyl esters in the plasma of subjects who consumed about 0.33 RDA of vitamin A in a single meal was measured for up to 48 h after dosing, at which time the concentration had returned to base line. The results show that the relative absorption of retinyl palmitate can be measured at typical single-meal intakes.

The qualitative features of the plasma 3H-retinyl esters concentration-time curves were similar to those observed in other studies (Berr 1992, Cortner et al. 1987, John et al. 1992a and 1992b, Krasinski et al. 1990, Lewis et al. 1990, Wilson et al. 1983). A single peak concentration occurred between 2 and 3 h, followed by a rapid decline in concentration of 3H-retinyl esters. Negligible concentrations of 3H-retinyl esters were found in the plasma after 24 h. Olestra had no effect on tmax. The tmax values of individual subjects ranged from 1.5 to 5.0 h (data not shown). Other investigators have reported tmax values ranging from 3 to 8 h (Cortner et al. 1987, John et al. 1992a and 1992b, Krasinski et al. 1990, Lewis et al. 1990, Ooi et al. 1992, Rasmussen et al. 1991, Wilson et al. 1983). Those values were measured by using vitamin A doses that ranged from 0.5 to about 7 RDA; the vitamin A was fed in meals that varied in nutrient content, particularly fat content.

There were no significant olestra effects, within the power of the study, on the relative absorption of retinyl palmitate, using either group mean or median AUC values. Although the difference did not reach statistical significance, the lower

![FIGURE 2](https://academic.oup.com/jn/article-abstract/127/8/1686S/4728933) Distribution of the individual area-under-the-curve (AUC) values within subjects who ingested 0, 8, 20 or 32 g olestra.

AUC value for the 32-g olestra group suggested that olestra may have affected retinyl palmitate absorption, in agreement with the partitioning mechanism (Jandacek 1982). Thirty-two grams of olestra per meal is an extreme exaggeration of the estimated intake of olestra from savory snacks. If 32 g were eaten at each meal, the daily intake would be 96 g, almost 14 times the estimated 90th-percentile chronic olestra intake (6.9 g/d) by the total population of savory snack consumers (Webb et al. 1997).

Even though subjects were excluded from the study if they had fasting triglyceride levels >2.71 mmol/L or a BMI >30 kg/m² (factors associated with abnormal clearance of postprandial lipoproteins), 13 of the 68 subjects had retinyl ester AUC values ranging from 3 to 8 h (Cortner et al. 1987, John et al. 1992a and 1992b, Krasinski et al. 1990, Lewis et al. 1990, Ooi et al. 1992, Rasmussen et al. 1991, Wilson et al. 1983). Those values were measured by using vitamin A doses that ranged from 0.5 to about 7 RDA; the vitamin A was fed in meals that varied in nutrient content, particularly fat content.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>tmax (h)</th>
<th>AUC (FDp·h)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>2.6 ± 0.6</td>
<td>0.444 ± 0.188</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>2.6 ± 0.8</td>
<td>0.535 ± 0.325</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>2.8 ± 0.9</td>
<td>0.536 ± 0.315</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
<td>2.7 ± 1.0</td>
<td>0.358 ± 0.163</td>
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### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AUC (FDp·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>0.390 ± 0.097</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>0.350 ± 0.096</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>0.384 ± 0.120</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
<td>0.305 ± 0.079</td>
</tr>
</tbody>
</table>

1 FDp, fractional dose in plasma.

The qualitative features of the plasma 3H-retinyl esters concentration-time curves were similar to those observed in other studies (Berr 1992, Cortner et al. 1987, John et al. 1992a and 1992b, Krasinski et al. 1990, Lewis et al. 1990, Wilson et al. 1983). A single peak concentration occurred between 2 and 3 h, followed by a rapid decline in concentration of 3H-retinyl esters. Negligible concentrations of 3H-retinyl esters were found in the plasma after 24 h. Olestra had no effect on tmax. The tmax values of individual subjects ranged from 1.5 to 5.0 h (data not shown). Other investigators have reported tmax values ranging from 3 to 8 h (Cortner et al. 1987, John et al. 1992a and 1992b, Krasinski et al. 1990, Lewis et al. 1990, Ooi et al. 1992, Rasmussen et al. 1991, Wilson et al. 1983). Those values were measured by using vitamin A doses that ranged from 0.5 to about 7 RDA; the vitamin A was fed in meals that varied in nutrient content, particularly fat content.

There were no significant olestra effects, within the power of the study, on the relative absorption of retinyl palmitate, using either group mean or median AUC values. Although the difference did not reach statistical significance, the lower
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FIGURE 3  Fractional dose of $^3$H-retinyl esters ($F_{dp}$) in plasma as a function of time after subjects ingested 0, 8, 20 or 32 g olestra. □ = mean of 13 subjects with AUC values $>0.65 ~F_{dp} \cdot h$ (high responders); ● = mean of 55 subjects with AUC values $\leq 0.65 ~F_{dp} \cdot h$.

values substantially greater than the average value of the others. Unexpectedly high postprandial plasma responses to a dose of vitamin A were observed recently by another researcher. Schrezenmeir et al. (1992) found that in a similar proportion of subjects (2 of 13), postprandial serum and chylomicron triglyceride concentrations and chylomicron retinyl palmitate concentrations were about three times as great as levels measured in the other subjects in response to a meal containing 58 g of fat and 30,000 IU (30 RDA) of vitamin A. All 13 subjects had normal fasting triglycerides at the beginning of the study.

Other researchers also have found individuals with high postprandial triglyceride responses but normal (<2.26 mmol/L) fasting serum triglycerides (Schrezenmeir et al. 1993, Weintraub et al. 1987). Schrezenmeir et al. (1993) found that 17 of 113 subjects had high postprandial triglyceride responses after a standardized lipid load containing 58 g of fat.

The high AUC values observed in 13 subjects in this study could have been the result of greater efficiency in retinyl palmitate absorption, increased chylomicron production, or decreased clearance of chylomicrons and retinyl esters from the plasma. The data do not allow a definite choice among these different possibilities; there is evidence, however, that decreased clearance of chylomicrons was a factor. The chylomicron clearance rate can decrease when chylomicron triglycerides and VLDL triglycerides compete for lipoprotein lipase (Grundy and Mok 1976). Elevated levels of VLDL inhibit the hydrolysis of chylomicron triglycerides by the lipase, and thus delay clearance of chylomicrons and their substituents. The result is a correlation between high fasting serum triglycerides and high postprandial plasma concentrations of retinyl esters.

Other researchers have observed positive correlations between fasting serum triglycerides and postprandial plasma retinyl esters concentrations (Lewis et al. 1990, Ooi et al. 1992, Wilson et al. 1985). Similarly, this study showed a positive correlation between fasting serum triglycerides and retinyl ester AUC values. Figure 4 shows the individual AUC values plotted against the fasting serum triglyceride values measured at the end of the study. The correlation is significant ($P = 0.0001$), with an $r^2$ of 0.59. Eight of the 13 subjects with the highest AUC values had serum triglyceride concentrations $>2.26$ mmol/L at the end of the study. There was a similar
but weaker correlation with the fasting serum triglyceride concentrations measured at the beginning of the study ($P = 0.0002$, $r^2 = 0.31$, data not shown).

Even though only subjects with fasting serum triglycerides <2.71 nmol/L were enrolled in the study, the values at the end of the study ranged as high as 3.03 nmol/L in some subjects. Forty-one of the 68 subjects had higher serum triglycerides at the end of the study than at the beginning. These increases may have resulted from the diet, which was relatively high in carbohydrate (about 55% of energy) and low in fat (about 30% of energy). High carbohydrate diets have been shown to elevate serum triglyceride levels within several days (Schonfeld et al. 1976).

Regardless of the mechanism responsible for the high responders, including or excluding them from the database did not change the overall finding. Olestra did not have a statistically significant effect on retinyl palmitate absorption at any dose despite the stringent conditions of the study design which required that all of the daily olestra dose be consumed with a meal containing 0.33 RDA of vitamin A. Removing the high responders from the data set did not change this outcome.

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