

<Original Article>

## Metabolism of retinyl esters in humans after ingestion of retinol

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**Summary** Using high-performance liquid chromatography, we determined which retinyl esters predominately circulate in human serum after the ingestion of rapeseed oil and a few drops of retinyl palmitate. Fatty acids, predominately as palmitic acid followed by oleic, linoleic, and stearic acids, as well as retinyl esters, predominately as retinyl palmitate followed by retinyl stearate, oleate and linoleate, increased in serum after the ingestion of rapeseed oil and a few drops of retinyl palmitate. Re-esterification ratios (molar ratios between the concentrations of fatty acids and the corresponding retinyl esters recovered in serum after the ingestion of rapeseed oil and a retinyl palmitate) were markedly increased in saturated fatty acids (palmitic and stearic acids). Re-esterification ratios of unsaturated fatty acids (oleic and linoleic acids) were low compared with saturated fatty acids.

**Key words:** Vitamin A, Fatty acid, Palmitic acid, Stearic acid, LDL receptor

### 1. Introduction

Dietary retinol esters are cleaved to retinol and fatty acids by pancreatic and mucosal esterase and hydrolase, and are absorbed via the intestinal lymphatics. Cellular retinol-binding protein, type II, i.e., CRBP (II), present in the small intestine, may play an important role in the absorption and metabolism of retinol and  $\beta$ -carotene. Absorption of free retinol is followed by re-esterification within the mucosal enterocytes with long-chain saturated and unsaturated free fatty acids. The composition of newly formed retinyl esters is independent of the fatty acid composition of dietary lipid during absorption and

re-esterification<sup>1)</sup>. On the other hand, dietary supplementations of retinol and lipid resulted in higher serum levels of retinyl esters. The dominant ester here was retinyl stearate followed by retinyl palmitate and retinyl oleate in ferrets<sup>2,3)</sup>, dogs and silver foxes<sup>4)</sup>, while, in the serum of raccoon dogs<sup>4)</sup> and humans<sup>5)</sup>, retinyl palmitate dominated over retinyl stearate. There were no reports on the relationship between the composition of fatty acids in dietary lipids and the composition of retinyl esters recovered in serum. Therefore, the present study was designed to investigate which fatty acid is attributable to the newly formed retinyl esters in human serum.

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## 2. Materials and methods

### 1. Subjects

Seven healthy volunteers, five men (age 30 to 49 years) and two women (age 52 and 55 years), all with a normal body mass index (21 to 25), were enrolled in this study. Venous serum specimens were collected from these volunteers before and 1, 2, 3, 4, 6 and 8 hours after the ingestion of 37 g of rapeseed oil and a few drops of retinyl palmitate (Chocola A, Eisai Co., Ltd. Tokyo, Japan) equivalent to 32.7 mg of retinol (0.114 mmol). All subjects had fasted overnight for 12 hours, remaining in a fasting state except for water and a light lunch (soba, i.e., buckwheat noodles) for 4 hours until the end of the study. They reported no side effects other than occasional mild headaches and gastric upset. From all volunteers, informed written consent for these studies was obtained, and studies were approved by the guidelines established by the Protection of Human Subjects Committee of the Japanese Society of Nutrition and Food Science.

### 2. Assays

Serum concentrations of retinyl esters were measured by high-performance liquid chromatography (HPLC) as described previously<sup>6, 7, 8)</sup> on C<sub>18</sub> column 150 x 4.6 mm inner diameter, containing 5  $\mu$  m particles (STR-II, Shimadzu Techno-Research Inc., Kyoto, Japan). The mobile phase was comprised of 10 g/L of AgNO<sub>3</sub> dissolved in ethanol/H<sub>2</sub>O, 97:3 (v/v). The elution rate was 1.0 mL/min, and the eluent was monitored at 325 nm. To calibrate the chromatogram, we used retinol and retinyl palmitate (from Wako Pure Chemical Industries, Ltd., Osaka, Japan). Retinyl oleate, stearate, and linoleate were synthesized by reacting retinol with their corresponding acyl chlorides. Concentrations in the eluent were calculated based on the molar absorptivity of retinol<sup>9)</sup>. This was possible because retinol and several retinyl esters have nearly identical molar absorptivities at 325 nm owing to their retinol moiety<sup>10)</sup>. The fatty acid compositions of rape oil and serum specimens were determined by gas chromatography (GC) after a modified Folch extraction and transmethylation<sup>11)</sup>.

An incremental area under the response curve

(IAUC) was calculated by plotting the concentrations of fatty acids, retinyl esters and their molar ratios vs. time. The IAUC was the sum of the trapezoids fitting under the curve.

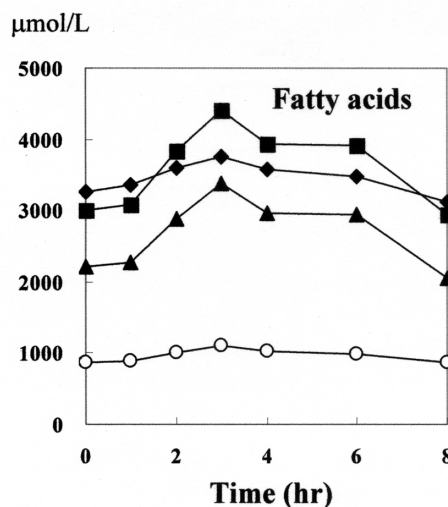


Fig. 1 Fatty acid levels in serum from zero to 8 hours after ingestion of 37 g of rapeseed oil and a few drops of retinyl palmitate. ■, palmitic acid; ◆, linoleic acid; ▲, oleic acid; ○, stearic acid.

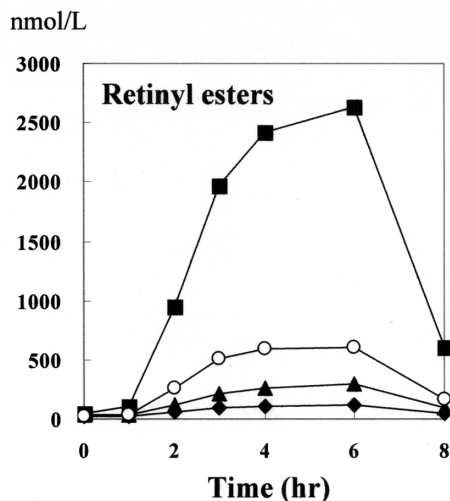


Fig. 2 Retinyl ester levels in serum from zero to 8 hours after ingestion of 37 g of rapeseed oil and a few drops of retinyl palmitate. ■, retinyl palmitate; ◆, retinyl linoleate; ▲, retinyl oleate; ○, retinyl stearate.

### 3. Results

#### 1. Fatty acid composition of rape oil and serum

By GC analysis, 37 g of rapeseed oil predominantly contained 62.4 mmol (16.0 g) palmitic acid, 57.4 mmol (16.2 g) oleic acid, 8.2 mmol (2.3 g) linoleic acid, and 8.1 mmol (2.3 g) stearic acid. A few drops of retinyl palmitate contained palmitic acid as low as 0.114 mmol. In fasting serum specimens, fatty acids (mainly as linoleic acid) dominated with  $3264 \pm 596 \mu\text{mol/L}$  (Table 1), followed by palmitic acid ( $2997 \pm 718 \mu\text{mol/L}$ ), oleic acid ( $2218 \pm 741 \mu\text{mol/L}$ ) and stearic acid ( $861 \pm 122 \mu\text{mol/L}$ ). Among these fatty acids, palmitic acid (IAUC= $5639 \pm 3807 \mu\text{mol/L} \times \text{hr}$ ) and oleic acid (IAUC= $4469 \pm 3495 \mu\text{mol/L} \times \text{hr}$ ) increased markedly following the ingestion of rapeseed oil and a few drops of retinyl palmitate in accord with the fatty acid composition of the rapeseed oil (Fig. 1).

#### 2. Re-esterification of retinol

In fasting serum specimens, retinyl palmitate dominated with  $46 \pm 13 \text{ nmol/L}$  (Table 1), followed by retinyl oleate ( $35 \pm 14 \text{ nmol/L}$ ), retinyl stearate ( $21 \pm 6 \text{ nmol/L}$ ) and retinyl linoleate ( $19 \pm 21 \text{ nmol/L}$ ). Serum concentrations of these four retinyl esters increased after the ingestion of 37 g rapeseed oil and

a few drops of retinyl palmitate (Fig. 2). Compared to the concentrations in a fasting state, retinyl palmitate increased maximally to 57-fold (IAUC= $12159 \pm 3455 \text{ nmol/L} \times \text{hr}$ ) in accord with the increased serum concentration of palmitic acid. Contrary to the lower

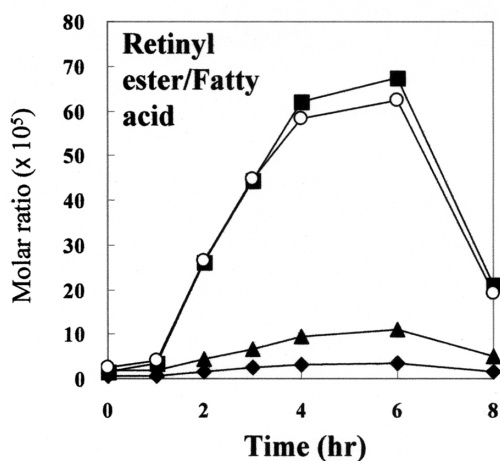


Fig. 3 Re-esterification ratios (molar ratios) between concentrations of fatty acids and the corresponding retinyl esters recovered in serum from zero to 8 hours after ingestion of 37 g of rapeseed oil and a few drops of retinyl palmitate. ■, retinyl palmitate/palmitic acid; ◆, retinyl linoleate/linoleic acid; ▲, retinyl oleate/oleic acid; ○, retinyl stearate/stearic acid.

Table 1 Concentrations of fatty acids and retinyl esters, and their molar ratios before and after ingestion of rapeseed oil and retinyl palmitate

Time after ingestion (hr)	0	1	2	3	4	6	8	IAUC
Fatty acids ( $\mu\text{mol/L}$ )								
Linoleic acid	3264 $\pm$ 596	3353 $\pm$ 626	3592 $\pm$ 797	3747 $\pm$ 800	3577 $\pm$ 729	3477 $\pm$ 780	3129 $\pm$ 573	1694 $\pm$ 2065
Oleic acid	2218 $\pm$ 741	2276 $\pm$ 788	2894 $\pm$ 1154	3388 $\pm$ 1593	2963 $\pm$ 1056	2945 $\pm$ 1450	2064 $\pm$ 761	4469 $\pm$ 3495
Palmitic acid	2997 $\pm$ 718	3082 $\pm$ 777	3834 $\pm$ 1214	4397 $\pm$ 1661	3932 $\pm$ 1182	3906 $\pm$ 1566	2943 $\pm$ 782	5639 $\pm$ 3807
Stearic acid	861 $\pm$ 122	887 $\pm$ 145	1013 $\pm$ 218	1102 $\pm$ 258	1020 $\pm$ 203	984 $\pm$ 237	864 $\pm$ 183	930 $\pm$ 800
Retinyl esters (nmol/L)								
Retinyl linoleate	19 $\pm$ 21	22 $\pm$ 16	60 $\pm$ 21	94 $\pm$ 53	111 $\pm$ 53	116 $\pm$ 31	47 $\pm$ 13	474 $\pm$ 219
Retinyl oleate	35 $\pm$ 14	36 $\pm$ 12	114 $\pm$ 45	217 $\pm$ 114	264 $\pm$ 109	296 $\pm$ 104	94 $\pm$ 12	1188 $\pm$ 428
Retinyl palmitate	46 $\pm$ 13	107 $\pm$ 59	952 $\pm$ 394	1968 $\pm$ 965	2424 $\pm$ 935	2627 $\pm$ 1209	603 $\pm$ 129	12159 $\pm$ 3455
Retinyl stearate	21 $\pm$ 6	36 $\pm$ 15	263 $\pm$ 128	508 $\pm$ 258	592 $\pm$ 222	607 $\pm$ 206	164 $\pm$ 28	2917 $\pm$ 717
Retinyl ester/Fatty acid (molar ratio, $\times 10^5$ )								
Retinyl linoleate/Linoleic acid	0.6 $\pm$ 0.8	0.6 $\pm$ 0.5	1.7 $\pm$ 0.6	2.4 $\pm$ 1.1	3.1 $\pm$ 1.2	3.4 $\pm$ 0.8	1.5 $\pm$ 0.3	13 $\pm$ 7
Retinyl oleate/Oleic acid	1.8 $\pm$ 1.2	1.8 $\pm$ 0.8	4.4 $\pm$ 2.4	6.6 $\pm$ 2.5	9.4 $\pm$ 4.1	11.0 $\pm$ 3.5	5.0 $\pm$ 1.6	40 $\pm$ 10
Retinyl palmitate/Palmitic acid	1.6 $\pm$ 0.6	3.5 $\pm$ 1.7	26.3 $\pm$ 12.4	44.4 $\pm$ 14.2	62.2 $\pm$ 18.9	67.5 $\pm$ 20.5	21.0 $\pm$ 4.1	311 $\pm$ 44
Retinyl stearate/Stearic acid	2.5 $\pm$ 0.9	4.2 $\pm$ 1.9	26.3 $\pm$ 12.3	44.8 $\pm$ 16.8	58.4 $\pm$ 21.3	62.3 $\pm$ 20.6	19.3 $\pm$ 3.7	288 $\pm$ 61

The values are means  $\pm$  SD for seven healthy subjects.  
IAUC: incremental area under response curve.

increase of stearic acid (IAUC=  $930 \pm 800 \mu\text{mol/L} \times \text{hr}$ ) and the higher increase of oleic acid (IAUC=  $4469 \pm 3495 \mu\text{mol/L} \times \text{hr}$ ), the corresponding increase of retinyl ester was high in retinyl stearate (maximally, 29-fold: IAUC=  $2917 \pm 717 \text{nmol/L} \times \text{hr}$ ) and lower in retinyl oleate (maximally, 9-fold: IAUC=  $1188 \pm 428 \text{nmol/L} \times \text{hr}$ ), respectively. The increase of retinyl linoleate was as low as 6-fold (IAUC=  $474 \pm 219 \text{nmol/L} \times \text{hr}$ ).

We calculated the re-esterification ratios (molar ratios) between concentrations of fatty acids and their corresponding retinyl esters recovered in serum after the ingestion of rapeseed oil and a retinyl palmitate (Table 1). A marked increase was observed in the re-esterification ratios of retinyl palmitate to palmitic acid, and retinyl stearate to stearic acid (Fig. 3). The ratios of retinyl stearate to stearic acid were identical near to the same ratios of retinyl palmitate to palmitic acid. The re-esterification of fatty acids with retinol was revealed to be low in both oleic and linoleic acids when compared with those of palmitic acid and stearic acid.

#### 4. Discussion

In humans, the dietary sources of retinol were retinyl esters and carotenoids, i.e.,  $\beta$ -carotene. Retinyl esters were hydrolyzed to retinol, which was re-esterified and packaged in the core of chylomicrons as retinyl ester (i.e., retinyl palmitate and retinyl stearate). Some of the  $\beta$ -carotene was cleaved to form two moles of retinal, which when formed was reduced to retinol, that was then taken up by chylomicrons also as retinyl esters<sup>12</sup>.

It is natural to consider why saturated fatty acids rather than unsaturated fatty acids were preferentially esterified with retinol. We hypothesize that retinyl esters together with esterified cholesterol present in the core of chylomicron remnants would be released into the liver cells via the LDL receptor, wherein saturated fatty acids regulate the LDL receptor pathway largely at the mRNA level<sup>13</sup>. Once the cell has satisfied its requirement for retinol, saturated fatty acids inhibit receptor activity, so that the receptor is no longer able to internalize retinyl esters. When the liver cells

need more retinol, the receptors again come into play<sup>14, 15</sup>. In response to cellular requirements, the liver releases retinol in the form of a retinol-RBP4 complex bound to the transthyretin in circulation. In target cells, a cell-surface receptor for retinol-RBP4 removes retinol from RBP4. Thus, we conclude that sufficient intake of saturated fatty acids is required to maintain the nutritional status of retinol. Although unsaturated fatty acids, i.e., oleic acid and linoleic acid (essential fatty acid) are needed by the human body, they may provide only a minor poor contribution to retinol absorption. While among animal species other than humans, retinyl stearate as a dominating form of retinyl ester in circulation is unknown, but it appears to be among the essential physiological conditions in these animals.

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