Determination of Retinyl Esters and Retinol in Serum or Plasma by Normal-Phase Liquid Chromatography: Method and Applications

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Retinol and retinyl esters are measured in serum or plasma samples by gradient, normal-phase, adsorption "high-performance" liquid chromatography, with ultraviolet detection at 325 nm. The four major circulating retinyl esters in humans (esters of palmitate, stearate, oleate, and linoleate) are co-eluted as a single peak. Retinyl acetate is included as an internal standard, to correct for variable recovery. Retinol values so measured correlated well (r = 0.88) with those by a widely used reversed-phase chromatographic technique (Clin Chem 1983;29:708-12). The mean retinol concentration was 570 (SEM 17) μg/L and the mean for retinyl esters was 33 (SEM 4) μg/L as determined in samples from 88 fasting young adults. Concentrations of retinol in plasma as low as 50 μg/L can be detected in 100-μL samples, as can 10 μg of retinyl esters per liter. Using this method, we measured absorption of low doses of vitamin A, which may provide a more physiological approach to assessment of fat malabsorption. Additionally, the procedure proved useful for quickly screening for vitamin A toxicity. Major advantages include small sample size, direct injection of the extracted sample without evaporation, rapid elution pattern, co-elution of major retinyl esters as a single peak, and low limit of detection.

Additional Keyphrases: fat malabsorption · screening for vitamin A toxicity · steatorrhea · reference values · liver disease · measuring absorption of vitamin A · measurements in tissue

Concentrations of retinyl esters in the circulation increase after ingestion of vitamin A or in vitamin A toxicity. However, symptoms of toxicity can occur, although the total vitamin A (retinyl esters plus retinol) or retinol in serum may remain within the normal range (1). Concentrations of retinyl esters in the serum of a fasting individual are at or below the limits of detection by most methods if sample volumes of 200 μL or less are used. Sensitivity limitations of older techniques have hindered the development and use of the vitamin A tolerance test, in which pharmacological oral doses of vitamin A (2250 retinol equivalents [RE]kg body weight to a maximum total dose of 10 000 RE or 350 000 int. units) were used, rather than physiological doses, as an aid in evaluating the intestinal absorption of fat (2). However, the specificity of the test has been questioned because, especially in mild steatorrhea, the increase in total serum vitamin A often is not correlated with fecal fat concentrations. In addition, the classical tolerance test has been associated with reports of transient symptoms of vitamin A toxicity.

Retinol and retinyl esters can be separated by conventional methods involving column chromatography (alumina, silicic acid, and Sephadex LH-20), and thin-layer chromatography. For reasons of sensitivity, specificity, and adaptability to automation, "high-performance" liquid chromatography (HPLC) is acknowledged to have replaced these techniques for most applications (3). Reversed-phase HPLC for the measurement of retinol (4) currently is a popular technique, and recent reports describe conditions for its use in the complete separation of individual retinyl esters (5, 6). However, reported isocratic and gradient reversed-phase methods require 20 to 80 min for complete ester separation and, as in the conventional chromatographic techniques, the small concentrations of retinyl esters in the circulation during the post-absorptive state are difficult to detect (7).

To detect the low concentrations of retinyl esters (in blood of the fasting subject or after dosing with small amounts of vitamin A) and to screen for vitamin A toxicity, we developed a 10-min normal-phase HPLC technique in which the major retinyl esters are co-eluted as a single peak. This simplifies the chromatography and enables the routine use of small sample volumes, 100 or 200 μL.

Materials and Methods

Procedures

Reagents. Hexane (Fisher Scientific, Medford, MA 02155), and dioxane (Burdick and Jackson Laboratories Inc., Muskegon, MI 49442) were both "HPLC grade." Pyridine was from Fisher. Absolute ethanol (AAPE Alcohol and Chemical Co., Shelbyville, KY 40065) was reagent grade. Fatty acyl chlorides (of arachidonate, stearate, linoleate, oleate, palmitate, heptadecanoate, laurate, myristate, and caprylate), 11-cis retinaldehyde, beta-carotene and all-trans isomers of retinol, retinyl acetate, retinyl palmitate, retinol, and retinoic acid were from Sigma Chemical Co., St. Louis, MO 63178. We synthesized retinyl esters by reacting retinol with these respective fatty acyl chlorides (6, 8). [15-

¹H]Retinol (1 Ci/L; New England Nuclear, Boston, MA 02118) was purified by HPLC and diluted to a specific activity of 85.9 Ci/g for use in analytical-recovery experiments.

We purified 100 μg of injected retinol acetate by collecting liquid-chromatographic eluate corresponding to the middle part of the peak. Other standards were so purified as needed. Weekly, we made working standard solutions by diluting the purified retinoid in hexane or ethanol. Absorptivities at 325 nm were: retinol 1835, retinyl acetate 1550, and retinyl palmitate 975 (9). All solid standards were kept at 70 °C, but working standards could be validly stored for as long as two weeks at 20 °C, in the dark.

Instrumentation. The HPLC system consisted of a Model 421 controller with two Model 112 pumps and a Model 340 organizer/mixing chamber (all from Beckman Instrument Co., Wakefield, MA 01880). For sample injection we used either a Model 7125 manual injector (Rhodeyne, Cotati, CA 94928) or an ISS-100 autosampler (Perkin-Elmer Corp.,

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Norwalk, CT 06850), both equipped with 200-μL sample loops. A Rhodyne Model 7000 switching valve allowed easy use of either injection route. The detection wavelength was set at 325 nm (Perkin-Elmer LC-85, with a high-speed 2.4-μL flow cell). Signal output was monitored with a Model 3390A integrator (Hewlett-Packard, Andover, MA 01810) with chart speed at 0.5 cm/min. Other equipment included an ultrasonic bath (Branson, Shelton, CT 06484), a bench top centrifuge (Sorvall RT6000, E.I. Du Pont, Wilmington, DE 19810), and a UV/visible spectrophotometer (Lambda 3, Perkin-Elmer).

**Chromatography.** For the two-solvent, gradient elution of the retinyl esters, retinyl acetate, and retinol we initially pumped solvent A (dioxane/hexane, 5/1000 by vol) and, starting at 1 min after injection, linearly increased the proportion of solvent B (100% dioxane). As Figure 1 (a and b) illustrates, during the first 5 min after sample injection, the proportion of solvent B had increased to 20% of the total. One minute later, solvent B had decreased to 0% during 0.5 min, and the column was re-equilibrated until the next injection at 10.5 min. The flow rate through the column was constant, 2.5 mL/min, which produced a back-pressure of 5.17 to 8.3 MPa (750 to 1200 psi).

For the analyses we used pre-packed 12.5 cm × 4 mm (i.d.) columns filled with LiChrosorb Si 60 of 5-μm particle size (EM Science, Cherry Hill, NJ 08034). A 3 cm × 4 mm (i.d.) guard column slurry-packed with LiChrosorb Si 60 (10-μm particle size), preceded the analytical column. An SSI 0.5-μm high-pressure column prefilter (Rainin, Woburn, MA 01801) was inserted between the injector and guard-column. Immediately before use, we purified the HPLC solvents by passage through a 0.45-μm (pore size) "nylon-66" filter (Rainin) and de-gassed them by sonication and vacuum treatment. Samples were injected with either a 250-μL syringe (Hamilton, Reno, NY 89510) or the autosampler. All runs were performed at ambient temperature.

For comparison studies we used an isocratic reversed-phase method of retinol analysis (4).

**Standard curves and calculations.** To correct for differences in analytical recovery between samples, we used retinyl acetate, dissolved in hexane, as an internal standard. The standard curves were generated by injecting constant volumes of increasingly concentrated retinoid in hexane. Computed areas were regressed against the amount of standard injected. Retinoid standards gave a linear calibration curve over the range of concentrations encountered in serum (0 to 22 ng for retinyl palmitate, 5 to 130 ng for retinol). For all determinations, calculated values lay within the extreme limits of a standard curve. Peak-area measurements were preferred for quantification, because the gradient solvent system generated peaks of inconsistent height. After generating a mean standard curve, we evaluated system performance daily by the reproducibility of a standard serum pool and the response factor from the retinyl acetate internal standard.

**Sample extraction.** A 100-μL aliquot of plasma or serum (a 1:10 dilution if vitamin A intoxication was suspected) was transferred to a 10 mm × 75 mm glass culture tube by micropipet. Subsequently, 100 μL of ethanol and 70 ng of retinyl acetate in 15 μL of hexane were added and the tube's contents were vortex-mixed for 10s. Then we added 150 μL of mobile-phase solvent A (hexane/dioxane, 5/1000 by vol) and vortex-mixed intermittently for 30 s. The mixture was centrifuged (2 min, 350 × g, 4 °C). The hexane layer was then drawn off by syringe and placed in a 0.4-mL autosampler vial. Owing to the small volumes of hexane used, the extraction process was repeated a second time, starting with the addition of hexane. For serum volumes of 200 μL, we used 200 μL of ethanol and continued with the above extraction procedure.

**Clinical Applications**

Blood sampling from human volunteers conformed to the guidelines of the Human Investigation Review Committee of Tufts University and the New England Medical Center. In addition, blood was also obtained from two groups of weaning Sprague-Dawley rats (Charles River Breeding Labs., N. Wilmington, MA 01897) fed a semi-purified diet with vitamin A (16 000 int. units/kg of diet) or deficient in vitamin A. Because of retinoid instability, all sample processing was done under dim red light (General Electric, G140R). Samples were stored at −70 °C until analyzed, unless otherwise stated.

I. We performed modified human vitamin A tolerance tests, using physiological doses of vitamin A to assess fat malabsorption indirectly. Experiments were performed on four fasting individuals, who took oral doses of 3000, 4500, or 6000 RE of oil-soluble vitamin A (1). Hoffman-La Roche, Nutley, NJ 07110), which represented two, three, four and a half, or six times the human recommended dietary allowance (10). These tests were performed while the subjects were fasting, and with no fat load. Blood was obtained by venipuncture 0, 0.5, 1, 2, 3, 4, 5, and 6 h later from three normal control subjects and, in addition, 7 and 12 h later from a documented fat-malabsorbing patient.

II. We obtained plasma from a child suspected of having vitamin A toxicity. Serial samples were obtained over a four-month period.

**Results**

The chromatogram of an extracted 100-μL serum sample, with added internal standard, is shown in Figure 1 (left: fasting; right: 5 h after dosing with 6000 RE). Retinol and retinyl esters were identified by comparison of retention times and by co-elution with standards. In addition, to confirm identity and purity, the retinyl ester and retinol fractions, extracted from human serum, were collected separately as they were eluted from the normal-phase system and rechromatographed on the reversed-phase system (4). Further proof of their retinoid character was obtained by scanning, between 210 and 500 nm, the absorption spectra of the fractions corresponding to collected peaks. The susceptibility of retinoids to photo-oxidation was also used to indirectly identify retinol and retinyl ester peaks. Extracted serum and retinoid standards were inactivated by exposure to ultraviolet (280 nm) light. After 30 min, retinol was completely photo-oxidized, while 48% of the more-stable retinyl ester remained.

Using plasma samples of 100 to 200 μL, we observed a single peak corresponding to total retinyl esters, which was resolved from unidentified material eluting near the solvent front. The second column of Table 1 summarizes the relative retention times (k') for various vitamin A-active compounds. Beta-carotene standard was not retained under these conditions; it was eluted with unidentified early-eluting compound(s). All retinyl esters evaluated had capacity ratios that varied between 1.25 and 1.31 as indicated by the (+) symbol. On the right of Table 1, the columns of shared asterisks (*) designate retinyl esters that co-eluted—co-elution being defined as two or more compounds eluting without evidence of a shoulder on the corresponding peak of the chromatogram. Each pure retinyl ester standard was mixed with one or more pure retinyl ester and chromatographed. For example, retinyl arachidonate (C:20) co-eluted with all esters having a carbon chain length C:16 or longer, whereas retinyl caprylate (C:8) co-eluted only with retinyl...
Fig. 1. Chromatogram from 100 \( \mu \)L of (left) serum from a fasting human subject and (right) a non-fasting subject. In a, retinyl esters are 50 \( \mu \)g/mL. Peak 1: retinyl esters; peak 2: retinyl acetate (internal standard); peak 3: retinol. The two-solvent gradient profile is shown superimposed as a dashed line (—). Solvent A is dioxane/hexane, 8:100 by vol; solvent B is 100% dioxane. Absorbance units full scale at 325 nm: 0.01.

In b retinyl esters are 170 \( \mu \)g/mL. Blood was obtained 5 h after oral dosing with 6000 RE of oil-soluble P1MO (approximately 50 RE/kg body weight).

Table 1. Capacity Ratios (\( k' \)) of Vitamin A-Active Compounds and Co-elution Characteristics of Retinyl Esters on a 5-\( \mu \)m LiChrosorb Si 60 Column

<table>
<thead>
<tr>
<th>Compound</th>
<th>( k' )</th>
<th>Co-elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-carotene</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Retinyl arachidionate (C:20:4)</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Retinyl estearate (C:18:0)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl oleate (C:18:1)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl linoleate (C:18:2)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl heptadecanoate (C:17:0)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl palmitate (C:16:0)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl margarate (C:14:0)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl laurate (C:12:0)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinyl caprylate (C:8:0)</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>Retinyl acetate</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td>11-cis Retinolaldehyde</td>
<td>4.19</td>
<td></td>
</tr>
<tr>
<td>All-trans retinolaidhyde</td>
<td>4.83</td>
<td></td>
</tr>
<tr>
<td>All-trans retinol</td>
<td>7.25</td>
<td></td>
</tr>
<tr>
<td>All-trans retinoic acid</td>
<td>retained</td>
<td></td>
</tr>
</tbody>
</table>

\( k' = (t - t_0)/t_0 \) for peak a, where \( t_0 \) = elution time (min) for solvent front or unretained compound, and \( t \) = retention time for compound a. The exact \( k' \) for retinyl esters between 1.25 and 1.31 varies, as indicated by the (+), owing to the gradient-solvent program. *Co-eluting retinyl esters share an asterisk (*) in the same column (for example, retinyl palmitate: C:16:0 co-elutes with retinyl stearate: C:18:0) as well as retinyl laurate: C:12:0, but retinyl stearate and retinyl laurate do not co-elute with each other.

Table 2. Analytical Recovery of Exogenous Retinoid after Extraction from Serum

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovered, %</th>
<th>SD, %</th>
<th>CV, %</th>
<th>n</th>
<th>Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>90.9</td>
<td>3.5</td>
<td>3.9</td>
<td>8</td>
<td>84–96</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>99.8</td>
<td>4.0</td>
<td>4.0</td>
<td>8</td>
<td>92–104</td>
</tr>
<tr>
<td>Retinyl acetate</td>
<td>92.1</td>
<td>1.9</td>
<td>2.1</td>
<td>10</td>
<td>90–96</td>
</tr>
</tbody>
</table>

Table 3. Analytical Precision of Serum Retinol and Retinyl Esters by HPLC

<table>
<thead>
<tr>
<th></th>
<th>Mean ( \mu g/L )</th>
<th>SD</th>
<th>CV, %</th>
<th>n</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Within day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>10</td>
<td>550</td>
<td>17</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Retinyl esters</td>
<td>10</td>
<td>180</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Between day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>10</td>
<td>500</td>
<td>30</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Retinyl esters</td>
<td>10</td>
<td>170</td>
<td>17</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Laetate (C:12). Simultaneous injection of all of the retinyl esters produced two main peaks. The 11-cis and all-trans isomers of retinoldehyde were well resolved and were quantified in the same run with retinyl esters, retinyl acetate, and retinol. Before the aldehydes were eluted the detection wavelength was changed to 340 nm, which is closer to their absorption maxima. All-trans retinoic acid was retained on the column, even with dioxane concentrations increased to 40%.

Table 2 shows the analytical recovery from serum of added retinyl palmitate and retinol standards; Table 3 shows the method precision. Retinol was detected in amounts as low as 50 \( \mu g/L \) in a 100-\( \mu L \) sample of plasma; the detection limit for retinyl palmitate was 10 \( \mu g/L \). In other experiments, the off-column recovery (n = 6) of 80 ng of [15-\(^3\)H]retinol was 95.6%. Therefore, 4.4% of the injected radioactive retinol was retained on the column.

We analyzed plasma samples that had been stored at -20 \(^\circ\)C for two months to two years; the samples were from 88 young men and women volunteers. Using our normal-phase technique, the mean (and SEM) for retinol during fasting was 570 (17) \( \mu g/L \) (range: 240 to 1000 \( \mu g/L \)) while that for retinyl esters was 33 (4) \( \mu g/L \) (range: 0 to 180 \( \mu g/L \)). Using these 88 plasma samples, we compared the normal-phase retinol results with those previously obtained by reversed-phase methodology (4), finding a good correlation (\( r = 0.88 \)). However, the latter results for retinol were consistently lower (mean ± SEM: normal-phase = 570 ± 17; reversed-phase = 680 ± 16 \( \mu g/L \)). Because this difference may have been due to losses related to storage, we obtained fresh plasma samples from 20 young fasting adults. A good
correlation \( r = 0.883 \) was again obtained and the two methods were not significantly different as determined by paired \( t \)-test \( p = 0.16 \). The mean (and SEM) results were: normal-phase = 586 (4); reversed-phase = 619 (4) \( \mu \)g/L.

As another indicator of technique specificity we analyzed serum from weanling rats deficient in vitamin A. Retinyl esters were not detectable, but the retinol concentration averaged 40 \( \mu \)g/L as detected in 200 \( \mu \)L of serum (data not shown).

Clinical Applications

I. Physiological vitamin A tolerance tests. Figure 2 shows the appearance of retinyl esters in the serum of human volunteers over a 6-h period. For the 3000-RE dose the concentration of retinyl esters was highest at 5 h. With doses of 4500 and 6000 RE the maximum circulating ester concentration may not have been observed, because sampling was terminated at 6 h, except for the fat-malabsorbing patient (chronic pancreatitis, with fecal fat >10 g/day). For this patient, after an oral dose of 6000 RE, the concentration of retinyl esters in serum declined from a high of 78 \( \mu \)g/L at 5 h to 50 \( \mu \)g/L at 7 h and 14 \( \mu \)g/L at 12 h (data beyond 6 h are not shown in Figure 2). Note that significant maximal increases in circulating retinyl esters occurred in all the normal subjects but not in the malabsorber. Maximum increases in retinyl ester concentration from baseline were 350, 270, and 180 \( \mu \)g/L for the normal individuals, as compared with 60 \( \mu \)g/L for the fat-malabsorbing patient.

II. Vitamin A intoxication. A child on a "normal" intake of vitamin A presented with scaling skin, pseudo-tumor, and increased alanine aminotransferase and aspartate aminotransferase activities. Suspecting possible vitamin A toxicity, we analyzed the plasma and found it to contain above-normal concentrations of retinyl ester (Figure 3), and we confirmed this by separating the extracted plasma on a reversed-phase system (4). A diet low in vitamin A was initiated and after two weeks plasma retinyl esters had declined from the initial 4500 \( \mu \)g/L to 500 \( \mu \)g/L. Clinical symptoms also gradually disappeared. Seven weeks after the first sample was analyzed, the child was given halothane anesthesia for a surgical procedure. After this surgery, the initial symptoms returned and the retinyl ester concentration in plasma had increased to over 15 000 \( \mu \)g/L. Concentrations of ester gradually declined during the following 10 weeks. Plasma retinol concentrations behaved similarly, varying between 1500 and 300 \( \mu \)g/L (data not shown).

Discussion

In a 10-min run we can quantify total retinyl esters in serum, separate from retinol. Unlike other normal-phase HPLC methods designed to resolve individual retinyl esters (11-13), in our technique more than 94% of the circulating esters co-eluted. Also, our method uses ultraviolet/visible detection, lessening the risk (7) of interference from non-retinoid compounds such as phytofluene (14), which is detected by some fluorimetric methods (15). Although an isocratic reversed-phase method has been used to measure retinyl ester and carotene, retinol appears to be obscured by the solvent front (16).

For plasma, the use of ethanol/hexane as the precipitating/extracting solvents has generally been reported to enable reproducible and high extraction recovery for retinol and the retinyl esters (1, 4, 6, 15). However, others have reported poorer analytical recovery and recommend the use of chloroform/methanol, especially in analysis for retinyl esters (7). This discrepancy may be ascribed in part to the vitamin A not always remaining in solution and whether or not evaporation/resuspension is required. Those methods that involve an evaporation step to transfer the retinol and retinyl esters from hexane to, for example, the reversed-phase mobile-phase solvent methanol require a strongly lipophilic solvent, such as ethyl ether or chloroform, to resolubilize the vitamin A (4). It is generally agreed that
extraction with ethanol/hexane is not recommended for tissues.

Our procedure is designed so that the HPLC mobile phase is used for the extraction, and it can then be directly injected without need for an evaporation and resuspension step, the stage at which losses are likely. In addition, the dioxane included in the mobile phase may improve our extraction efficiency. We, like others (4), use retinyl acetate as an internal standard and find that it compensates for sample preparation and column adsorption losses. For convenience we added a hexane solution of retinyl acetate to the samples, rather than an ethanol solution, after observing that extraction recoveries were the same with either solvent. A compromise is made in the calculation when we use the absorption coefficient of the major circulating ester, retinyl palmitate, to approximate the coefficient for the total co-eluting pool of retinyl esters. In practice, the molar absorption coefficients of the retinyl esters are the same, because it is the retinol moiety that contributes the characteristic absorbance at 325 nm (6).

Our system is not designed for simultaneous separation of carotene, because the carotenes have little affinity for the column and are eluted at the solvent front. Neither are we able to measure alpha-tocopherol simultaneously, without baseline-correction techniques, because dioxane absorbance interferes at 292 nm (but not at 325 nm). However, concurrent, in-line fluorimetric detection of alpha-tocopherol (with negligible interference from dioxane) may prove successful and increase the versatility of our method. The chromatographic method has also been used to quantify vitamin A in tissues: a recent abstract reports separate quantification of total retinyl esters and retinol in liver and eye (17). Before chromatography, tissue samples were extracted with chloroform/methanol and resuspended with the mobile-phase solvent (18).

In the present survey of 88 young adults, retinyl ester concentrations after a short fast were 33 (SEM 4) μg/L, averaging 6% of the total vitamin A, with a range from 0 to 18%. Others have reported similar concentrations, 16 (SEM 4) μg/L for 14 control subjects (1). For our population, retinol values by normal-phase HPLC (570 ± 17 μg/L) agree with reported values for well-nourished individuals (19). The correlation (r = 0.88) between results for retinol by the normal-phase and reversed-phase methods is similar to correlations reported for other such comparisons (4).

We have reported here our results for two applications of our normal-phase technique. In a fat-malabsorbing human, after a 6000 RE (20 000 int. units) oral dose of vitamin A, a lower maximal retinyl ester concentration was found as compared with normal subjects taking the same or lower doses. This provides evidence that, after oral administration of a physiological dose of vitamin A, retinyl ester measurements may differentiate between normal and abnormal fat absorbers. We are currently conducting tests on other subjects and patients, using 3000-RE doses.

In the second application, the child had clinical symptoms of vitamin A toxicity, which disappeared coincident with a progressive decline in plasma retinyl ester concentrations. Hypervitaminosis A is usually reported as the primary event that precedes and induces liver damage. However, halothane anesthesia presumably produced an increased concentration of retinyl esters in plasma and a return of hypervitaminosis A symptoms via release of vitamin A from the damaged liver. The unmasking of hypervitaminosis A through liver damage has been observed before (20).

Our normal-phase technique has been used (21) to measure dietary vitamin A supplement-dependent increases in fasting retinyl esters in the elderly. Fasting retinyl esters increased in proportion to the supplement in the elderly but not in young adults. The physiological significance of this as it may relate to intestinal vitamin A absorption, hepatic vitamin A clearance, and incidence of vitamin A toxicity in the elderly is currently being investigated. Additionally, our method was used (22) to document that the elderly have greater maximal retinyl ester concentrations in plasma and that the area under the absorption curve after dosing with low physiological amounts of oil-soluble vitamin A is greater than is the case for young adults.

The clinical applications we have outlined show the importance of differentiating between retinyl esters and retinol. The present method for quantifying total retinyl esters of retinol is rapid, sensitive, fast, and can be automated to allow for unattended operation. The chromatographic profile is simplified by the elution of the major circulating retinyl esters as a single peak, which, when combined with superior integrator signal processing, improves the apparent lower detection limit as compared with previously reported methods. The ability to directly inject the extracting solvent makes the method applicable for quick screening of plasma should vitamin A toxicity be suspected. A less-common application, for which the method is uniquely suited, is in measurement of the absorption of small amounts of vitamin A as retinyl ester, which may prove to be a more sensitive physiological indicator of fat absorption than are previously described vitamin A tolerance tests. Finally, using a Bligh-Dyer type of extraction, we have found that our technique is adaptable to measurement of total retinyl esters and retinol in tissue.

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References