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Original Article

A simple and robust quantitative analysis of retinol and retinyl palmitate using a liquid chromatographic isocratic method

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ABSTRACT

Vitamin A is a vital nutritional substances that regulates biological activities including development, but is also associated with disease onset. The extent of vitamin A intake influences the retinoid content in the liver, the most important organ for the storage of vitamin A. Measurement of endogenous retinoid in biological samples is important to understand retinoid homeostasis. Here we present a reliable, highly sensitive, and robust method for the quantification of retinol and retinyl palmitate using a reverse-phase HPLC/UV isocratic method. We determined the impact of chronic dietary vitamin A on retinoid levels in livers of mice fed an AIN-93G semi-purified diet (4 IU/g) compared with an excess vitamin A diet (1000 IU/g) over a period from birth to 10 weeks of age. Coefficients of variation for intra-assays for both retinoids were less than 5%, suggesting a higher reproducibility than any other HPLC/UV gradient method. Limits of detection and quantification for retinol were 0.08 pmol, and 0.27 pmol, respectively, which are remarkably higher than previous results. Supplementation with higher doses of vitamin A over the study period significantly increased liver retinol and retinyl palmitate concentrations in adult mice. The assays described here provide a sensitive and rigorous quantification of endogenous retinol and retinyl palmitate, which can be used to help determine retinoid homeostasis in disease states, such as toxic hepatitis and liver cancer.

1. Introduction

Vitamin A is essential for maintaining normal growth, development, vision, immunity, and neurogenesis [1]. Both retinoid excess and deficiency can have adverse effects, such as abnormal fetal development and disruption to energy balance regulation [2,3].

Some studies have examined the effects of excess vitamin A intake on serum and tissue retinoid levels in rodents, which were often fed standard laboratory rodent chow or were treated by injection [4–8]. However, vitamin A

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injection paradigms independent of ingestion are difficult for study compared with ingesting pellets containing high concentrations of vitamin A because vitamin A is easily absorbed during or after a meal. Standard laboratory rodent chow comprises natural ingredients, which vary in lot-to-lot reproducibility, and also contains copious amounts of nutrients, including vitamins. In contrast, a semi-purified diet provides a consistent nutrient composition in amounts that meet National Research Council recommendations for rodent nutrient intake, avoiding the delivery of excessive vitamins that can confound studies of nutrient functions. The American Institute of Nutrition (AIN) has determined that the minimum amount of vitamin A necessary for rodents is 2.9 IU/g diet, and recommends an intake of 4 IU/g diet to provide a margin of safety, such as the AIN-93G diet [9,10]. The upper limit of vitamin A consumption in humans is suggested as being no more than three times the recommended dietary allowance [11]. The amount of vitamin A in standard rodent chow diet is approximately five times (20 IU/g diet) that of the semi-purified AIN-93G diet recommended for rodents.

Quantifying circulating retinoid concentrations reflects whole-body vitamin A status, and can provide insights into retinoid homeostasis and metabolism. Being able to measure endogenous retinoid levels in serum and tissues is pivotal to elucidate the regulatory mechanisms that maintain retinoid homeostasis and, eventually, to overcome many pathological conditions and diseases associated with alterations in retinoid metabolism [12–15].

HPLC is one of the best analytical techniques available to assess and characterize retinoid concentrations in biological samples. However, the different chemical properties of retinoid metabolites do not allow for accurate quantification of each retinoid in a single chromatographic run [16]. In addition, levels of retinoic acid in biological samples are extremely low, thus requiring sophisticated methods for their accurate quantification [17,18].

Many assays have been developed to analyze retinol and retinyl ester levels in biological samples. Some previously described HPLC gradient methods focus on separation of the different molecular species [7,16,17,19,20].

In this study, we describe a reverse-phase HPLC isocratic method that allows for the simple separation and quantitation of retinol and retinyl palmitate and has high reproducibility and sensitivity. As an example of the application of the method, we analyzed the effects of chronic intake of excess dietary vitamin A on retinoid accumulation in the mouse liver, the most important organ for the storage of vitamin A.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals used, their purity grade, and the source of purchase were: methanol-HPLC grade (Cat. No. 21929-23), ethanol-HPLC grade (Cat. No. 14741-41), hexane-HPLC grade (Cat. No. 17929-53) from Nacalai Tesque (Kyoto, Japan); retinol (Cat. No. R7632-100MG), retinyl acetate (Cat. No. 46958) from Sigma Aldrich (St. Louis, MO, USA); and distilled water-HPLC grade (Cat. No. 042-16973) and retinyl palmitate (Cat. No. 188-01331) from Wako Chemicals (Osaka, Japan).

2.2. Animal and vitamin A supplementation experiments

Five pregnant ICR mice were obtained from CLEA (CLEA Japan, Inc., Tokyo, Japan). On the day of birth (day 0), the litter size was standardized to eight pups to avoid fluctuations in the pups' dietary intake. From gestation until weaning, all dams and their pups were fed AIN93G semi-purified diet (Oriental Yeast Co., Ltd., Osaka, Japan) containing sufficient vitamin A (4 IU/g diet) in the form of retinyl palmitate. After weaning on postnatal day 21, male mice were randomly assigned to AIN-93G diet (control) or modified excess vitamin A diet groups. The control group (two litters per dam) was maintained on the AIN93G semi-purified diet. The excess vitamin A group (two litters per dam) was changed to the AIN93G semi-purified diet containing modified excess vitamin A (1000 IU/g diet). The doses used were part of a study aimed to observe the effects of high vitamin A intake on leptin expression in mice adipose tissue samples [4] or on fatty acid composition of phospholipids in mice [21], as previously reported. Both groups were fed each diet for a period from 3 to 10 weeks of age in home cages at 23 ± 2 °C on a 12-h light/dark cycle (lights on from 8:00 to 20:00). Food and water were provided *ad libitum* during the experiment.

All experiments were performed in accordance with National Institutes of Health (USA) guidelines for animal experiments and were approved by the Animal Care Committee of Ohu University (approval No. 2015-64).

At postnatal days 70 and 72, tissue samples were obtained under sodium pentobarbital euthanasia. All efforts were made to minimize suffering. At the time of euthanasia, each mouse was weighed, blood was taken from the inferior vena cava, and the remnant liver was removed under a dim yellow safety light to prevent photoisomerization and photodegradation of retinoid [16,22–24]. Dissected livers were rapidly frozen in liquid nitrogen and stored at −80 °C. Tissues were stored continuously without thawing at −80 °C under nitrogen gas until analysis.

2.3. Preparation of standard stock solutions

All laboratory manipulations involving retinoids were performed in dark rooms under dim yellow light. Stock solutions of retinoids were prepared by dissolving 1 mg (for retinol and retinyl acetate) and 400 μg (for retinyl palmitate) in 1 mL ethanol. After degassing using nitrogen gas, solutions were kept in brown sample vials at −30 °C until use.

2.4. Analytical performance for calibration and system validation

Samples were directly injected into a HPLC/UV apparatus (PU-2080 plus chromatography pump, UV-2075 plus ultraviolet detector, and 807-IT integrator; Jasco, Tokyo, Japan) equipped with a Mightysil RP-18 GP column (Cat. No. 25416-96, 150 × 4.6 mm, 5-μm particle size; Kanto Chemical Co., Inc., Tokyo, Japan). The column was protected by a guard column

(Cat. No. 25418-96; Kanto Chemical Co.). The injection volume was 20 μL for all samples using a universal loop injector (Rheodyne 7725i). The mobile phase was 100% methanol delivered at a flow rate of 1.0 mL/min. Evaluation of chromatograms was performed using a detection wavelength of 325 nm. For peak assignment, each retinoid alone and/or mixtures were injected to determine an analysis sequence as the retention time-control sample. The analytical performance of each assay was evaluated for the limit of detection (LOD), limit of quantification (LOQ), linearity, reproducibility, and extraction efficiency. LOD is defined as a signal-to-noise ratio of 3:1, whereas LOQ is defined as a signal-to-noise ratio of 10:1. After the addition of the appropriate amount of internal standard, retinol and retinyl palmitate stock solutions were appropriately diluted and used as calibration standards, yielding various concentrations for retinol and retinyl palmitate in ethanol. The internal standard was used as retinyl acetate [16], of which the concentration was 4 μg/mL. These standards were independently prepared and entered into the calibration graph for each concentration level. Linearity was evaluated using coefficients of correlation R^2 and by examining whether the intercepts of the calibration curves were significantly different from zero. Reproducibility was assessed according to relative standard deviations in intra-assay (same day) coefficients of variation (n = 10) and in inter-assay (consecutive days) coefficients of variation (n = 5). Recovery of the retinoids was examined by spiking mouse liver homogenate with known amounts of three different retinoid concentrations (retinol: 0.25, 2, and 4 μg/mL and retinyl palmitate: 2.5, 12.5, and 25 μg/mL) together with the internal standard (retinyl acetate: 4 μg/mL). For each concentration, three independently spiked samples (n = 3) were prepared from the same mouse liver homogenate. After extraction and analysis, the peak area for retinol and retinyl palmitate in a blank unspiked sample was subtracted from the peak area of a spiked sample. Recovery was calculated as the percentage by comparing the corrected peak areas with the peak areas obtained by direct injection of liver homogenate free standard samples with the corresponding amount of retinol and retinyl palmitate.

2.5. Sample preparation and quantification of retinoid concentrations in the mouse liver, brain, and serum

Retinol and retinyl palmitate were extracted from liver, brain tissue and serum under a dim yellow safety light. Retinol and retinyl palmitate quantifications in liver, brain (nmol/g tissue), and serum (nmol/mL serum) were determined by reverse-phase HPLC using a procedure previously described with some modification [23–25]. Briefly, liver was thawed, weighed (20–80 mg), kept on ice, and homogenized in phosphate buffered saline (pH 7.4) using a disposable homogenizer. An equal volume of absolute ethanol containing a known amount of the internal standard was added to an aliquot of liver or brain homogenate or serum. The mixture was briefly vortexed. Hexane was then added and the mixture vortexed twice so that the internal standard and endogenous retinol/retinyl palmitate were extracted into hexane. Solutions were centrifuged at 1500× *g* for 5 min at 4 °C. Thereafter, supernatants were treated with sterile water and centrifuged at 1500× *g* for 5 min at 4 °C for purification of analytes. Hexane extracts (supernatants) were evaporated until dry under a gentle stream of nitrogen gas. Immediately upon drying completely, analytes were dissolved in ethanol for injection into the HPLC system. On the basis of the calibration lines obtained for each retinoid standard solution, intrinsic retinol and retinyl palmitate levels in the mouse liver, brain, and serum were determined.

2.6. Experimental applicability

The method was applied for the determination of endogenous retinoids in the liver, brain, and serum of mice fed the AIN93G semi-purified diet with sufficient vitamin A (4 IU/g diet) or the AIN93G semi-purified diet with modified excess vitamin A (1000 IU/g diet).

2.7. Statistical analysis

Results from retinoid concentrations in the liver, brain, and serum are expressed as mean ± S.E. To compare differences in retinol and retinyl palmitate concentrations in the liver, brain, and serum between groups of AIN-93G diet and vitamin A-excess diet, the Student's *t*-test was performed. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Chromatogram of each standard working solution (retinol, retinyl acetate, retinyl palmitate)

To confirm retinoid chromatographic separation profiles, retinoid was dissolved in ethanol and analyzed using the HPLC/UV system. Fig. 1 showed a typical chromatogram of retinoid dissolved in ethanol (standard working solution). Each standard working solution was injected into the HPLC system. Although the retention times changed depending on column batch, flow speed, and consistency of the mobile phase, each peak was detected at almost similar retention times and was completely separate from the others in this method. Retention times of retinol, retinyl acetate, and retinyl palmitate were approximately 3.2 min, 4.1 min, and 30.9 min, respectively. Chromatograms of mixtures were separated and also showed similar retention times compared with those of each standard working solution (Fig. 1).

3.2. Stability of internal standard

Retinyl acetate was used as the internal standard. We evaluated its stability using coefficient of variation. There was little degradation in absorbance units (mAU) of the internal standard until 43 days after preparation of the standard internal stock solution (Fig. 2). These results were obtained from groups of 10 samples prepared separately from 1, 3, 9, 34, and 43 days after preparation of the same internal stock solution. Reproducibility was assessed according to relative standard deviations (%) in intra-assay coefficients of variation and inter-assay coefficients of variation. Relative standard deviations in intra-assay

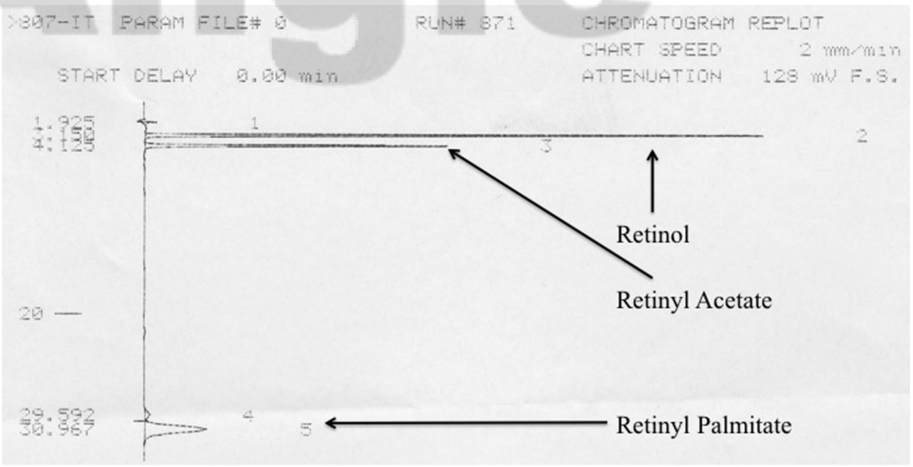

Fig. 1 – Reverse-phase HPLC chromatograms of each standard solution. A mixture of typical chromatograms of retinol, retinyl acetate, and retinyl palmitate are shown. The retention time was 3.2 min for retinol, 4.1 min for retinyl acetate, and 30.9 min for retinyl palmitate.

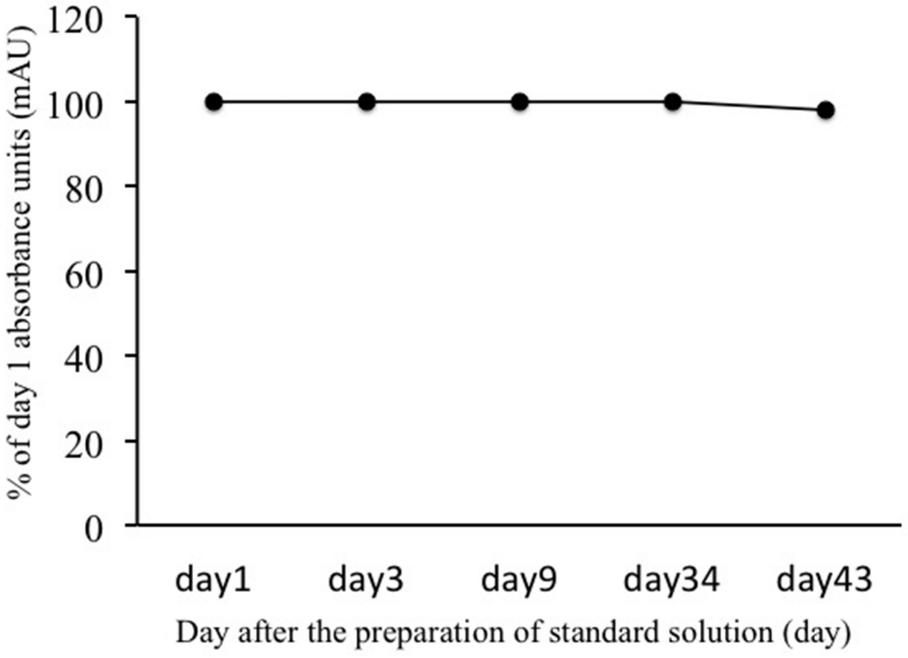

Fig. 2 – Relative standard deviations in inter-assay coefficients of variation of the internal standard. Days (day 1, 3, 9, 34, and 43) of analysis after the preparation of the internal standard solution are shown on the x-axis. The relative absorbance unit (mAU) to percentage at day 1 (100%) is shown on the y-axis.

coefficients of variation ranged from 0.72% to 0.84%. The relative standard deviation in the inter-assay coefficient of variation was 0.89%.

3.3. *Calibration curve*

The standard curve for retinyl acetate was evaluated. A representative standard curve for retinol:retinyl acetate and retinyl palmitate:retinyl acetate was also evaluated. All standard curves were linear ($R^2 = 1.000$) for the concentrations used for each retinoid.

3.4. *Limits of detection, limits of quantification, and reproducibility*

Sensitivity was obtained by measuring standard solutions prepared on the day of use with spectrophotometrically verified concentrations. LOD was defined as a signal-to-noise ratio of 3:1, whereas LOQ was defined as a signal-to-noise ratio of 10:1. The results showed sub pmol detection limits (0.08 pmol for retinol and 4.78 pmol for retinyl palmitate) and quantification limits (0.27 pmol for retinol and 15.92 pmol for retinyl palmitate) per 20-μL injection assay. For each retinol

concentration, relative standard deviations in intra-assay coefficient of variation ranged from 1.02% to 3.22% (n = 10). For LOQ of retinol, the relative standard deviation in intra-assay coefficient of variation was 3.22%. For each retinyl palmitate concentration, relative standard deviations in intra-assay coefficient of variation ranged from 0.97% to 4.52%. For LOQ of retinyl palmitate, the relative standard deviation in intra-assay coefficient of variation was 4.52%.

3.5. *Extraction efficiency*

Retinyl acetate was used as an internal standard to assess handling losses and extraction efficiency for total retinol and retinyl palmitate. The recoveries ranged from spiked mouse livers with known amounts of three different retinol concentrations (low [0.02 μg/mL], moderate [0.2 μg/mL], and high [4 μg/mL]) and were 102.14%, 100.31%, and 105.79%, respectively. Relative standard deviations in intra-assay coefficient of variation of three different retinol concentrations (low, moderate, high) were 1.14%, 1.16%, and 1.10%, respectively. The recoveries ranged from spiked mouse livers with known amounts of three different retinyl palmitate concentrations (low [2 μg/mL], moderate [10 μg/mL], and high [25 μg/mL]) and were 91.96%, 88.77%, and 84.77%, respectively. Relative standard deviations in intra-assay coefficient of variation of three different retinyl palmitate concentrations (low, moderate, and high) were 4.17%, 4.26%, and 4.53%, respectively.

3.6. *Application*

This method was used to quantify retinol and retinyl palmitate in the livers of mice fed the AIN-93G diet or the AIN-93G modified with high concentrations of vitamin A diet. Fig. 3A shows a representative chromatogram of a liver sample from a mouse that had ingested the excess vitamin A diet. The liver retinol concentration of mice fed the excess vitamin A diet was significantly higher than that of mice fed the AIN-93G diet (8.04 ± 0.63 *vs.* 136.46 ± 12.72 nmol/g tissue) (Fig. 3B). The liver retinyl palmitate concentration of mice fed the excess vitamin A diet also significantly increased compared with that of mice fed the AIN-93G diet (1008.89 ± 80.54 *vs.* 101,239.25 ± 15,641.06 nmol/g tissue) (Fig. 3C).

This method was also used to quantify retinol and retinyl palmitate in the brain and sera of mice fed the AIN-93G diet or the AIN-93G diet modified with high concentrations of vitamin A. The whole brain retinol concentrations of mice fed the excess vitamin A diet were significantly higher than those of mice fed the AIN-93G diet (41.04 ± 2.41 *vs.* 5997.54 ± 682.48 pmol/g tissue) (Fig. 4A). However, the brain retinyl palmitate concentrations of mice fed the AIN-93G diet could not be quantified because of less than LOQ. The mean brain retinyl palmitate concentrations of mice fed the excess vitamin A diet was 21.89 ± 6.57 nmol/g tissue. The serum retinol concentrations of mice fed the excess vitamin A diet were significantly higher than those of mice fed the AIN-93G diet (1.07 ± 0.06 *vs.* 2.21 ± 0.22 nmol/mL serum) (Fig. 4B). However, the serum retinyl palmitate concentrations of mice fed the AIN-93G diet could not be quantified because of less than LOQ. The mean serum retinyl palmitate concentrations of mice fed the excess vitamin A diet was 39.56 ± 7.20 nmol/mL serum.

4. Discussion

This study focused on characterizing a simple chromatographic method for retinoid determination and quantification without the use of sophisticated gradient elution, and without employing an expensive mobile phase such as acetonitrile. Many previous assays focused on the separation of retinoid but did not rigorously characterize the analytical performance [23,26,27]. Other assays have focused on the identification of retinoid species and were non-quantitative [28,29]. Here, we aimed to provide an analytically robust method to identify and quantify key retinoids of interest, such as retinol and retinyl palmitate.

The linear range was determined to clarify the range of concentrations where the relationship between the concentrations and signals was linear. This method was verified as being linear for retinol and retinyl palmitate concentrations ranging between 0.27 and 558.46 pmol and 15.92 and 7621.08 pmol, respectively.

The reproducibility was tested at linear ranges of retinol and retinyl palmitate concentrations. At any concentrations in the linear range, relative standard deviations in intra-assay coefficient of variation were less than 5%, which suggested more acceptable values for method precision than previously described [16]. Based on the signal-to-noise ratio, LOD and LOQ for retinol were 0.08 pmol and 0.27 pmol, respectively. The relative standard deviation in intra-assay coefficient of variation was 3.22% for LOQ for retinol. These results are markedly higher than previous results of 0.64 pmol [20] for LOD, and modestly higher than 0.20 pmol for LOD and 0.40 pmol for LOQ [16] using the HPLC/UV gradient method.

LOQ for retinyl palmitate was 15.92 pmol, with a relative standard deviation in intra-assay coefficient of variation of 4.52%. The results showed high reproducibility and similar sensitivity to that of a previous study at 15 pmol for LOQ for retinyl palmitate using the HPLC isocratic method [26].

Overall recoveries from spiked mouse livers with known amounts of retinol and retinyl palmitate concentrations ranged between 80% and 110%.

Retinol and retinyl palmitate quantification are relative to the value of the internal standard (internal standard method). Thus, evaluating the stability of the internal standard is important for reliable quantification. Stability of the internal standard in our method was evaluated according to relative standard deviations in inter-assay coefficients of variation and was confirmed as not being degraded until 43 days after preparation of the internal standard. Therefore, all results in the current study were obtained within 43 days after preparation of the internal standard solution, although retinoid in tissues is usually safe for several years if stored at below −80 °C [22].

Taken together, there were no problems with quantifying retinoid via the extraction method and the corresponding internal standard was established with high reproducibility.

The HPLC isocratic method used in this study was applied to evaluate the effects of a chronic intake of a high vitamin A diet on retinol and retinyl palmitate levels in the livers, brains, and sera of adult male mice. It should be noted that diet had a profound effect on retinoid levels and the diet of the dam

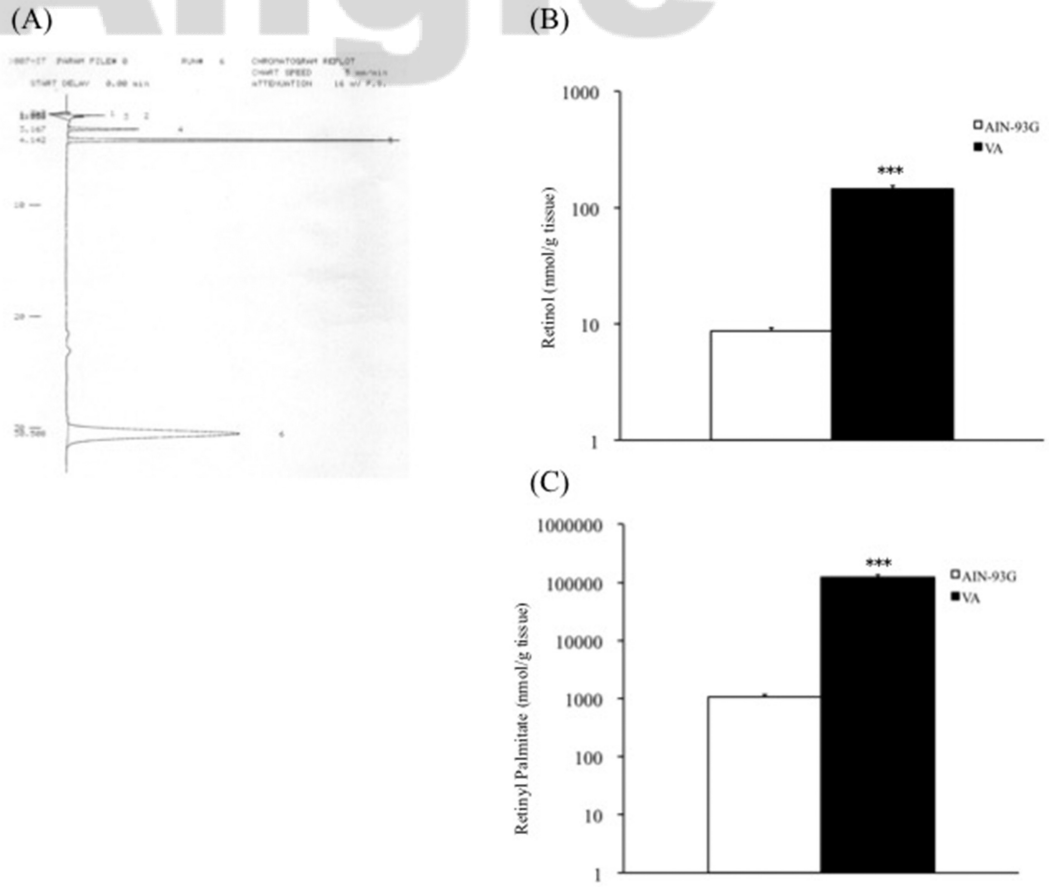

Fig. 3 – Effect of chronic feeding of the excess vitamin A diet on the accumulation of retinoid in the mouse liver. Graphs are in a single logarithmic chart. (A) Representative chromatogram from reverse-phase HPLC analysis of retinol and retinyl palmitate in the liver of a mouse chronically fed a high vitamin A diet. (B) Each column shows the concentrations of retinol in the liver (nmol/g tissue). White column shows data from a mouse that had ingested the AIN-93G purified diet (n = 10). Black column shows data from a mouse that had ingested the high vitamin A diet (n = 7). (C) Each column shows the concentrations of retinyl palmitate in the liver (μmol/g tissue). White column shows data from a mouse that had ingested the AIN-93G purified diet (n = 10). Black column shows data from a mouse that had ingested the high vitamin A diet (n = 7). Values are mean ± S.E. Asterisk shows significant differences in each retinoid concentration between the AIN-93G group and the high vitamin A diet group ($p < 0.05$).

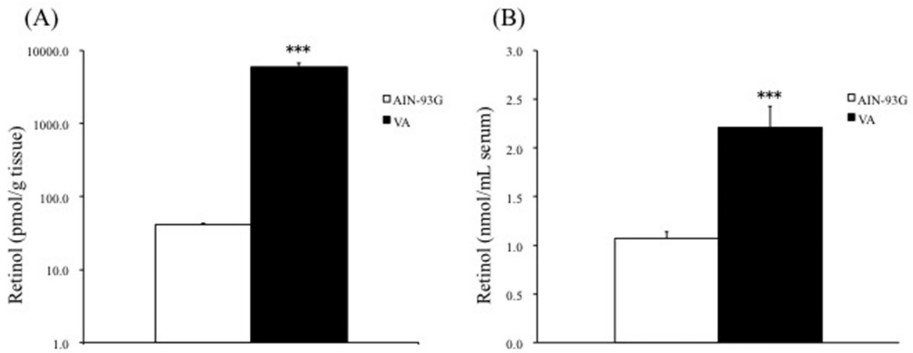

Fig. 4 – Effect of chronic feeding of the excess vitamin A diet on the accumulation of retinol in the mouse brain and serum. (A) Each column shows the concentrations of retinol in the whole brain (pmol/g tissue). White column shows data from a mouse that had ingested the AIN-93G purified diet (n = 10). Black column shows data from a mouse that had ingested the high vitamin A diet (n = 7). (B) Each column shows the concentrations of retinol in the serum (nmol/mL serum). White column shows data from mice that ingested the AIN-93G purified diet ($n = 10$). Black column shows data from mice that ingested the high vitamin A diet ($n = 7$). Values are means ± S.E. Asterisks show significant differences in each retinoid concentration between the AIN-93G group and the high vitamin A diet group ($p < 0.05$).

influenced the diet of the offspring. Mice fed standard rodent chow diet or bred from mothers fed standard rodent chow diet (approximately 20 IU vitamin A/g diet) had substantially higher retinol and higher retinyl palmitate levels than mice bred from dams fed the 4 IU vitamin A/g purified diet, which can often complicate and obscure effects on retinoid metabolism [11,16]. A key point in the current study was the establishment of an absolute control group, which allowed for the relatively accurate quantification of retinol and retinyl palmitate in the livers, brains, and sera of mice fed excess vitamin A compared with that of control mice fed the AIN-93G purified diet.

The obtained values for retinol and retinyl palmitate quantification in the current study were similar to studies that used other methods of analysis. Liver retinol levels in mice fed the AIN-93G purified diet (8.0 nmol/g tissue) (Fig. 3B) were similar to those fed a comparable vitamin A diet, ranging between 2.8 and 13.0 nmol/g tissue depending on mouse strain differences [11,16]. The concentration of liver retinol in mice fed the 4 IU vitamin A/g diet was less than half (20.7 nmol/g tissue) that of mice fed the 30 IU vitamin A/g standard rodent diet, which reflected a lower level of vitamin A in the diet than that of a previous study [16]. Liver retinyl palmitate levels in mice fed the AIN-93G purified diet (1008 nmol/g tissue) (Fig. 3C) were also similar to those fed a comparable vitamin A diet, ranging between 500 and 1000 nmol/g, depending on mouse strain differences [11,16]. The concentration of liver retinyl palmitate in mice fed the 4 IU vitamin A/g diet was less than one third (3600 nmol/g tissue) that of mice fed the 25 IU vitamin A/g standard rodent diet, which reflected a lower level of vitamin A in the diet than that of a previous study [6]. Serum retinol levels in mice fed the AIN-93G purified diet (1.07 nmol/mL serum) (Fig. 4B) were similar to those fed a comparable vitamin A diet, ranging between 0.8 and 1.3 nmol/mL serum depending on mouse strain differences [11,16]. The concentration of brain retinol in mice fed the 4 IU vitamin A/g diet (41.0 pmol/g tissue) (Fig. 4A) was approximately one-tenth (680.0 pmol/g tissue) that of mice fed the 30 IU vitamin A/g standard rodent diet, which reflected a lower level of vitamin A in the diet than that of a previous study [16].

A novel finding of the current study is that mice fed the 1000 IU vitamin A/g diet had 17-fold higher concentrations of retinol in the liver compared with mice fed the 4 IU vitamin A/g diet, while mice fed the 1000 IU vitamin A/g diet had 100-fold higher concentrations of retinyl palmitate in the liver compared with mice fed the 4 IU vitamin A/g diet. These results indicate that the retinoid status of mice fed the 1000 IU vitamin A/g diet are suggestive of hypervitaminosis A.

Vitamin A is a fat-soluble vitamin. Hypervitaminosis A is caused by excessive consumption of vitamin A, typically as a vitamin concentrate, supplement, or vitamin-rich liver. Both acute and chronic forms of hypervitaminosis A occur naturally in mammals. The incidence of chronic hypervitaminosis A is becoming a more frequent problem worldwide because of the increasing use of vitamin A supplements in children in developing countries where vitamin A deficiency is commonplace. There are numerous reports about the health effects of excess vitamin A using vitamin A injection paradigms. It is reported that vitamin A administration in large doses can be toxic [2,4,5].

In the current study, we designed a mouse model of chronic dietary intake of excess vitamin A. Mice that ingested excess vitamin A showed a significant accumulation of retinol and retinyl palmitate in the liver compared with control mice using the HPLC/UV isocratic method, the performance of which is well characterized.

Even though hypervitaminosis A is a global problem, the effects on various organs such as the brain and testes are not well understood. The mouse is an excellent model for genetically modification to help determine the molecular mechanisms behind retinoid signals, such as the lecithin:retinol acyltransferase gene knockout mouse [6,24]. Further studies using mouse models are required to understand the precise mechanisms behind the toxicity of retinoids and the relationship between toxicological findings and retinoid metabolism.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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