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A single dose of c9,t11 or t10,c12 conjugated linoleic acid isomers perturbs vitamin A metabolism in mice

Elena Giordano^{1,2}, Sebastiano Banni², and Loredana Quadro^{1,*}

¹Department of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ, 08901

²Department of Experimental Biology, University of Cagliari, Cagliari, 09042, Italy.

Abstract

Conjugated linoleic acid (CLA) is a polyunsaturated fatty acid that has numerous biological activities. Previous studies in rodents demonstrated that chronic intake of CLA t10,c12 or CLA c9,t11 isomers perturbs the metabolism of retinoids (vitamin A and its derivatives). Specifically, while both isomers increased liver retinoid levels, only CLA t10,c12 also stimulated hepatic retinol secretion into the bloodstream. Given that retinoid homeostasis in mammalian serum and tissues is crucial to maintain health, it is important to gain more insights into the mode of action of this nutrient-nutrient interaction. Here we hypothesized that an acute administration of either CLA isomer may also influence vitamin A metabolism. By gavaging wild-type and retinol-binding protein (RBP) knockout mice with an oral bolus of radiolabeled retinol containing one of these two isomers, we showed that both CLA t10,c12 and CLA c9,t11 rapidly enhance hepatic uptake of dietary vitamin A and its re-secretion from the liver in the form of retinol bound to RBP. Indeed, in mice lacking this protein, the sole specific carrier for retinol in the circulation, this latter effect was blunted. Also, by using a pharmacological inhibitor of the clearance of chylomicrons, that distribute recently ingested vitamin A and lipids throughout the body, we provided evidence that CLA intake might rapidly enhance intestinal absorption of dietary vitamin A. These data demonstrate the existence of multiple levels of interaction between dietary CLA and retinoid metabolism and warrant further studies to understand the molecular mechanisms underlying these effects-and their implications for human health.

Keywords

vitamin A; retinoid metabolism; conjugated linoleic acid; dietary fats; mouse model

1. INTRODUCTION

Adequate levels of retinoids (vitamin A and its derivatives) in mammalian serum and tissues are crucial to maintain health. Retinoid homeostasis is achieved by regulating absorption, storage, transport and metabolism of this essential nutrient. Mammals obtain vitamin A from animal products, mostly in the form of retinyl esters and retinol, or as pro-vitamin A

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*Address correspondence to: Department of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ, 08901, Tel. +1 732 932 9611, Fax +1 732 932 6776, quadro@aesop.rutgers.edu .

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carotenoids, mainly β -carotene, from vegetables and fruits [1, 2]. Within the intestinal mucosa the majority of retinol, regardless of its dietary origin, is enzymatically re-esterified with long-chain fatty acids primarily by the action of the enzyme lecithin: retinol acyltransferase (LRAT), which is widely expressed in tissues [2-5]. Newly formed retinyl esters are then packaged in lipoprotein particles called chylomicrons [2, 6]. Through the lymphatic system, chylomicrons reach the general circulation to be distributed to various organs and tissues upon hydrolysis by lipoprotein lipase (LPL) [6].

By acting on the surface of capillary endothelial cells, this enzyme converts chylomicrons into smaller particles called chylomicron remnants, which still contain retinyl esters [7, 8]. A large portion of the retinyl ester-containing chylomicron remnants is rapidly cleared by the liver, the major site of vitamin A storage in the body. However, approximately 25% of chylomicron remnants are taken up by extrahepatic tissues [9]. In the fasting state, retinol, secreted from the liver bound to its sole specific carrier retinol-binding protein (RBP), is the most abundant retinoid form in the circulation [10, 11].

Apart from the influence of dietary fats on intestinal absorption [7, 12, 13], examples of dietary components able to influence retinoid metabolism significantly are rare. Interestingly, previous studies from our laboratory have demonstrated that chronic intake of conjugated linoleic acid (CLA) interferes with the maintenance of serum, liver and adipose retinoid homeostasis [14].

The term CLA refers to a group of positional and geometric isomers of the omega-6 essential fatty acid linoleic acid, which are mostly found in the meat of ruminants and dairy products. Out the 28 known isomers of CLA, the c9,t11 and t10,c12 isomers appear to be the most biologically active [15]. CLA c9,t11 comprises approximately 90% of CLA found in food, while the t10,c12 isomer is present in trace amount and the dietary source is mainly from supplements [16]. In animal studies, CLA has been shown to protect against cancer and atherosclerosis, stimulate immune functions, normalize impaired glucose tolerance in type-2 diabetes and induce changes in body mass composition [17]. Overall, there are considerable variations among studies, and the beneficial effects observed in some animal models have not been fully reproduced in humans. Adverse effects of CLA intake, such as liver steatosis in mice [18] and insulin resistance in animal models and humans [19], have been reported.

We hypothesized that dietary CLA may have short-term effects on retinoid metabolism, specifically we on hepatic retinoid uptake and secretion. By using a gavage dose of radiolabeled retinol containing either one of two CLA isomers, i.e. CLA t10,c12 or c9,t11, we show that, in contrast to chronic CLA intake [14], both isomers rapidly stimulate hepatic retinoid accumulation as well as hepatic secretion of retinol likely bound to RBP. We also provide some evidence suggesting that dietary CLA intake enhances intestinal absorption of recently ingested vitamin A.

2. METHODS AND MATERIALS

2.1 CLA Isomers

CLA c9,t11 and t10,c12 (purity greater than 98%) were purchased from Matreya (Pleasant Gap, PA).

2.2 Oil for gavage

Commercially available olive oil was used.

2.3 Mouse strains

Wild-type (WT) and RBP^{-/-} [20] used for this study have a mixed genetic background (129sv/C57Bl6). Since its initial generation [20], the RBP^{-/-} mouse strain was maintained through mating of the heterozygous siblings from one generation to the next. Our colony of RBP^{-/-} mice has never been backcrossed. Experimental cohorts were generated from breeding pairs of RBP or WT littermates that were first generation of offspring from mice that were heterozygous for the RBP gene. Mice were maintained on a regular chow diet manufactured by LabDiet (ProLab Isopro RMH 3000) from weaning. This diet provided 25.0%, 12.0% and 63.0% calories from protein, fat and carbohydrates, respectively. The diet also contained 29 IU of vitamin A /g. For all the studies, both diet and water were available to the animals on an *ad libitum* basis. Mice were maintained on a 12:12 light/dark cycle, with the period of darkness between 7:00 PM and 7:00 AM. All mice used for these studies were euthanized by CO₂ inhalation between 9:30 and 11:30 AM. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals [21] and were approved by the Rutgers University Institutional Committee on Animal Care.

2.4 Gavage experiment

14 week-old WT and RBP^{-/-} male mice maintained on a regular chow diet were given a gavage dose containing one of the followings: A. 2×10^6 cpm all-trans-[³H]-retinol and 6 μ g of unlabeled all-trans-retinol in olive oil vehicle; B. 2×10^6 cpm all-trans-[³H]-retinol and 6 μ g of unlabeled all-trans-retinol in olive oil vehicle + 30 mg of CLA c9,t11; or C. 2×10^6 cpm all-trans-[³H]-retinol and 6 μ g of unlabeled all-trans-retinol in olive oil vehicle + 30 mg of CLA t10,c12. This amount of retinol corresponds to the amount of vitamin A consumed daily by our mice on a standard chow diet, as previously reported [14]. The CLA dose corresponds to the amount of CLA consumed daily by mice receiving a diet supplemented with 1% CLA, as previously published [14] [$1\% (10\text{mg/g diet}) \times 3 (\text{g of diet eaten in average by mice}) = 30\text{mg}$]. Finally, olive oil was chosen as vehicle due to the similar metabolic fate of CLA and oleic acid [22]. Three hours after administration of the bolus dose of [³H]-retinol, when the concentration of newly absorbed dietary retinoids secreted in the bloodstream is at its peak [23, 24], mice were euthanized. Serum, liver and adipose were collected to measure their retinol and retinyl ester concentration by HPLC analysis followed by fraction collection (see below) to assess the [³H]-cpm content of the individual fractions by scintillation counting. In an additional experiment, groups of 14 week-old WT male mice maintained on a regular chow diet were intraperitoneally injected with P-407 (1 mg/g body weight) the evening before the experiment began. P-407 is a total lipase inhibitor that prevents hydrolysis and clearance of chylomicrons for 4-5 days [25], thus optimizing the chance of detecting vitamin A of intestinal origin in the serum of these mice. The following morning, mice were given a gavage dose containing the same mixtures of retinol and CLA described above and euthanized, as describe in the previous section 2.3, three hours after administration of the bolus dose to collect their serum.

2.5 HPLC analysis of retinoids

Reverse-phase HPLC analysis was performed to measure serum and tissue retinol and retinyl esters levels [26]. Tissues (50-100 mg) were homogenized in PBS using a PRO200 homogenizer (Oxford, CT). Retinoids were separated on a 4.6×250 mm Ultrasphere C18 column (Beckman, Fullerton, CA) preceded by a C18 guard column (Supelco Inc., Bellefonte, PA) using acetonitrile, methanol, and methylene chloride (70:15:15, v/v) as the mobile phase flowing at 1.8 ml/min. A Dionex Ultimate 3000 HPLC system and a computerized data analysis workstation with Chromeleon software were used. Retinol and retinyl esters were identified by comparing retention times and spectral data of experimental compounds with those of authentic standards. The concentrations of retinoids were

determined by comparing peak integrated areas for unknowns against those of known amounts of purified standards. Loss during extraction was accounted for by adjusting for the recovery of retinyl acetate, the internal standard added immediately following homogenization of the tissues. In addition, fractions corresponding to retinol, retinyl linoleate, palmitate and stearate HPLC peaks were collected in scintillation vials and samples were evaporated in a fume hood overnight. After evaporation of the mobile phase, 20 ml of EcoScint H liquid scintillation counting solution (National Diagnostics, Atlanta, GA) was added to the vial. [^3H]-cpm associated with retinol and retinyl ester fractions present in serum and tissues samples were measured in a Beckman LS 1800 liquid scintillation counter.

2.6 Analysis of triglyceride levels

Triglyceride levels were assayed by an enzymatic procedure using a commercial kit according to the accompanying instructions (Infinity Triglyceride, Thermo Electron, Melbourne, Australia).

2.7 Western blot analysis

Serum levels of RBP were analyzed by Western blot [27]. A rabbit polyclonal anti-rat RBP antiserum [28] was used for immunodetection. Signals were detected by using a Biorad Chemidoc XRS Molecular Imager System. Albumin, detected by a rabbit polyclonal anti-Albumin antibody (Abcam) was used as a loading control for tissue samples. The quantification of the membranes was completed by densitometry analysis with Quantity One Program (Biorad).

2.8 Statistical analyses

Normality of the data was determined using the Kolmogorov Smirnov test. Normally distributed data were analyzed by t test or ANOVA test with a correction for multiple comparisons using the Fisher's least significant test. Analyses were performed with SPSS statistical software (SPSS 11.0 for Windows Student Version, 2001; SPSS Inc IL). A p-value <0.05 was used to establish statistical significance. Data were expressed as means $\pm$ SD.

3. RESULTS

3.1 Effects of CLA intake on tissue distribution of recently ingested vitamin A

To investigate the effects of dietary CLA on tissue distribution of recently ingested vitamin A, we followed the fate of a gavage dose of radiolabeled retinol containing either one of two CLA isomers, i.e. CLA t10,c12 or c9,t11. Wild-type (WT) and RBP knockout (RBP $^{-/-}$) mice were used. Steady state serum retinol levels within the same genotype were not affected by a single acute administration of CLA, as assessed by reverse phase HPLC analysis (Table 1). When compared to WT, RBP $^{-/-}$ mice displayed reduced serum retinol levels, as previously reported [20] (Table 1). In agreement with these results, serum protein levels of RBP were not different among the WT groups, as assessed by western blot analysis (Figure 1). The distribution of [^3H]-retinoid in the serum retinol and retinyl ester fractions of these mice was then analyzed by reverse phase HPLC coupled with fraction collection. We observed that [^3H]-cpm increased in both the retinol and retinyl ester fraction in WT mice fed either CLA isomer (Figure 2A and B). In contrast, acute administration of CLA did not affect serum [^3H]-cpm neither in the retinol nor in the retinyl ester fraction of the RBP $^{-/-}$ animals, regardless of the isomer given (Figure 2A and B). In the knockout strain, [^3H]-retinol cpm levels were significantly reduced compared to WT (Figure 2A), as expected, due to the lack of RBP [20] [23]. It is noteworthy that these values represent both the rate of clearance and

secretion into plasma of the ingested [^3H]-retinoid. Therefore, the fact that serum triglyceride (TAG) levels were not different among groups, despite an apparent reduction in the groups dosed with CLA isomers (Table 2), suggests that the chylomicron retinoid clearance rates were similar between genotypes.

Next, we analyzed retinoid levels in the liver, which is the major site of clearance of dietary vitamin A and the main body storage of retinoids. Reverse phase HPLC analysis showed that a single administration of CLA did not affect steady state hepatic retinyl ester levels within the same genotype (data not shown). Similarly, hepatic retinol levels were not affected, with the exception of a significant increase in WT mice upon gavage with CLA t10,c12 ($15.5 \pm 4.7 \mu\text{g/g}$ in CLA t10,c12 group vs. $3.5 \pm 0.3 \mu\text{g/g}$ in olive oil group; $p < 0.05$). As shown in Figure 3, [^3H]-cpm associated with the fractions corresponding to retinol (Figure 3A) and retinyl esters (Figure 3B) were significantly increased in the liver of WT mice administered with either CLA isomer. In contrast, while [^3H]-cpm associated with the retinol fraction of the RBP-/- mice remained unchanged upon CLA administration, irrespective of the isomer given (Figure 3A), [^3H]-cpm corresponding to the retinyl esters fraction were significantly reduced upon administration of CLA t10,c12 (Figure 3B). Overall, these data indicate that a single dose of either CLA c9,t11 or t10,c12 administered to WT mice stimulated a rapid accumulation of hepatic retinoids which were rapidly re-secreted as retinol likely bound to RBP.

To address whether an acute CLA administration also alters retinoid distribution to the periphery of the body, [^3H]-retinoid levels were measured in adipose tissue (visceral fat), which is another important site of retinoid storage that expresses RBP [10, 29]. In this tissue, [^3H]-cpm associated with the fractions corresponding to retinol and retinyl esters did not show any significant difference upon gavage with either CLA isomer (data not shown).

3.2 Effect of CLA intake on intestinal absorption of vitamin A

To start gaining insight into the origin of the retinoids that rapidly accumulate in the liver of the mice administered with a single bolus dose of CLA, WT male mice were administered the total lipase inhibitor P-407 [25], before given a gavage dose of radiolabeled retinol containing either one of two CLA isomers. A group of WT mice injected with P-407 and not orally administered oil was used as a control ("no gavage"). One of the short-term effects of olive oil administration is a transient elevation of serum TAG levels [30]. In agreement with the literature, serum TAG levels were elevated in mice given olive oil gavage ($3618.1 \pm 448.8 \text{ mg/dL}$) compared to the "no gavage" group ($1921.7 \pm 299.6 \text{ mg/dL}$). In contrast, oral bolus of olive oil + either CLA isomer did not result in such elevation ($1960.7 \pm 339.9 \text{ mg/dL}$ for CLA t10,c12; and $1780.2 \pm 238.5 \text{ mg/dL}$ for CLA c9,t11), confirming the notion that the greater the degree of unsaturation of the fatty acids presented in a meal, the lower the postprandial TAG response [31]. Therefore, we normalized serum [^3H]-cpm by triglyceride levels. As shown in Figure 4, serum total [^3H]-retinoid levels were significantly elevated upon administration of either CLA isomer. These data suggest that intestinal absorption of dietary vitamin A might be rapidly increased by CLA intake.

4. DISCUSSION

This study is a follow-up of our previously published report in which we investigated the effects of a chronic supplementation of dietary CLA on retinoid metabolism [14], in an attempt to clarify some of the questions left unanswered as well as some unexplored aspects of the interaction between these two nutrients (i.e. CLA and vitamin A). CLA alters retinoid metabolism in rats [32] and mice [14]. We previously demonstrated that chronic CLA c9,t11 and CLA t10,c12 intake have different effects on retinoid metabolism. Specifically, while both isomers induced hepatic retinoid accumulation, only CLA t10,c12 enhanced hepatic

retinol secretion, resulting in increased serum levels of retinol and its specific carrier RBP [14]. These and other evidence led us to hypothesize that the hepatic retinol secretion induced specifically by chronic CLA t10,c12 intake was a secondary effect to counter the hepatic accumulation of lipids, including retinoids, driven by this isomer [14]. Therefore, we sought to examine the short-term effects of dietary CLA administration to establish how rapidly hepatic retinoid accumulation and secretion of retinol bound to RBP could be stimulated.

By administering a bolus dose of radiolabeled retinol in combination with either CLA c9,t11 or t10,c12 to both WT and RBP knockout mice, we showed that hepatic accumulation of retinoids takes place rapidly (only three hours after gavage) and that recently ingested vitamin A is also rapidly re-secreted from the liver mainly in the form of retinol likely bound to RBP. Indeed, in WT mice administration of both CLA isomers resulted in increased [³H]-cpm levels in both the retinol and retinyl ester fractions. Also, the majority of serum [³H]-cpm was detected in the retinol fraction of the WT mice gavaged with either CLA isomer, and the serum response to the acute CLA administration was blunted in mice lacking RBP. This effect of CLA on hepatic retinoid secretion, unlike the chronic feeding [14], is induced by both CLA isomers. The discrepancy between the elevated levels of serum and liver [³H]-retinoid in WT mice given CLA and the absence of changes in the steady state hepatic retinoid and serum retinol and RBP levels is only apparent, because the amount of recently ingested [³H]-retinol is very small compared to the amount of retinoid already present in tissues and bloodstream. Given that the mechanisms regulating the secretion of the complex retinol-RBP from the liver have yet to be fully elucidated, our data are intriguing and certainly warrant further study to establish the exact mode of action of CLA in modulating the hepatic secretion of the complex retinol-RBP. Furthermore, it has been reported that CLA might affect hepatic very low-density lipoprotein (VLDL) assembly and secretion [33]. In agreement, both CLA t10,c12 and c9,t11 increased [³H]-cpm associated with the retinyl ester fractions in WT mice, suggesting a short-term effect of dietary CLA on recently ingested vitamin A circulating within lipoproteins. Surprisingly, this effect was blunted in RBP^{-/-} mice, suggesting some possible impairment of the production of VLDL lipoproteins by the liver in the mutant mice.

[³H]-cpm levels associated with the retinol (p=0.04) and retinyl ester fraction (p=0.02) were higher in the liver of RBP^{-/-} animals in the control group (olive oil gavage) compared to WT control mice. These data are consistent with some of our earlier studies showing significantly elevated hepatic [³H]-total retinol levels in RBP^{-/-} mice compared to WT, 4 hours after a gavage dose of radiolabeled retinol in peanut oil [23]. However, unlike WT mice, in the absence of RBP, CLA c9,t11 administration did not alter hepatic [³H]-cpm neither in the retinol nor in the retinyl ester fraction. Furthermore, in this strain a significant decrease in [³H]-cpm associated with the retinyl ester fraction was observed upon administration of a gavage dose of CLA t10,c12. We previously reported that chronic feeding of RBP^{-/-} mice with either CLA isomers has similar effects on hepatic retinoid levels due to CLA-stimulation of hepatic retinoid catabolism [14]. Our data therefore seem to suggest that recently ingested vitamin A can also be rapidly turned over in this strain, likely through increased retinoid oxidation, as proposed in the case of chronic CLA feeding [14]. Additional experiments are needed to unequivocally confirm this hypothesis.

The origin of the excess retinoids that accumulate in the liver of mice fed CLA, either acutely or chronically [14] remains unclear. Ingested vitamin A, regardless of its form, must first be emulsified to form micelles, then cross the brush-border membranes, subsequently be esterified and finally be incorporated in chylomicrons and secreted into the lymph [34]. Literature data suggest that vitamin A absorption depends mainly on assembly and secretion of chylomicrons [7, 12]. Ingestion of dietary fat is the major determinant in the absorption of

the vitamin A [7, 12]. Since CLA is a polyunsaturated fatty acid, as such it may increase the intestinal absorption of retinoids. To start gaining insight on the effect of CLA intake on intestinal absorption of vitamin A we administered P-407 to the mice to optimize the chance of detecting vitamin A of intestinal origin in their serum. The elevation of serum total [^3H]-cpm levels induced by either CLA isomer, seems to support our hypothesis of increased absorption of retinoids by CLA, although further experiments are needed to confirm it and establish whether assembly and/or secretion of retinyl ester containing chylomicrons are affected.

We also speculated that the difference in number of double bonds between CLA isomers (18:2) and oleic acid (18:1), the major fatty acid present in olive oil, could be responsible for the effect of CLA on [^3H]-retinoid content of the postprandial circulation. Preliminary studies conducted with sunflower oil (high content of linoleic acid, 18:2) showed the same [^3H]-retinyl ester content (cpm: 282.5 ± 106.7) of the postprandial circulation as olive oil (cpm: 281.8 ± 83.6), while flaxseed oil (high content of alpha-linolenic acid, 18:3), showed significantly increased serum [^3H]-cpm associated with the retinyl ester fraction, compared to olive oil, with values similar to those observed upon CLA t10,c12 gavage ([^3H]-retinyl ester cpm: 442.9 ± 151.6 and 562.6 ± 221.4 for flaxseed oil and CLA t10,c12, respectively; $p=0.355$). These preliminary data seem to rule out an influence of the number of double bonds on retinoid absorption. However, further studies are necessary to better clarify which are the discriminates in fatty acid structure that could influence vitamin A absorption likely by providing a good lipidic milieu for intestinal chylomicron assembly.

In conclusion, the results of this study support our hypothesis that a short-term intake of CLA alters retinoid metabolism. This action of CLA is rather rapid, especially on liver and possibly intestine. Although CLA is a minor fatty acid in a typical diet, its intake can dramatically increase due to ingestion of food that is fortified with CLA and/or of large doses of CLA supplements, now widely used. Therefore, the nutritional implication of this interaction can potentially be substantial. The similar effect of the two CLA isomers on serum retinol levels upon an acute administration (in contrast to their differential action upon chronic feeding [14]), strongly suggests that the mechanisms underlying the interference of dietary CLA on retinoid metabolism are more complex than previously hypothesized. One limitation of this study is the large variability mainly due to relative low number of animals and to the high sensitivity of the gavage technique used to trace the radiolabeled retinol. Further studies are needed to elucidate these mechanisms and thus understand the implication of this nutrient-nutrient interaction on human health and nutrition.

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ABBREVIATIONS

CLA	conjugated linoleic acid
RBP	retinol binding protein
LRAT	lecithin: retinol acyltransferase
LPL	lipoprotein lipase
WT	Wild-type

TAG	triglyceride
VLDL	very low-density lipoprotein

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Figure 1. Western Blot analysis of serum RBP protein levels in WT mice

(A) Representative Western blot analysis of serum RBP levels in wild-type mice given an oral gavage of [^3H]-retinol + olive oil (olive oil) or either CLA isomer (olive oil + t10,c12 or olive oil + c9,t11). A rabbit polyclonal anti-rat RBP antiserum [28] was used for RBP immunodetection. Albumin (Alb) detected by a rabbit polyclonal anti-Albumin antibody (Abcam) was used as a loading control for tissue samples. The molecular weight of each detected protein is indicated on the left side of the panels. Sera from a RBP knockout mouse (RBP $^{-/-}$) and from a wild-type mouse (WT), were used as controls. (B) Quantification of Western blots performed by densitometry of the bands detected upon Western analysis. Results presented as means $\pm$ SD are shown as a fold change. $n=3-4$ mice per group.

Figure 2. Serum [^3H]-cpm associated with retinol and retinyl ester fractions in WT and RBP $-/-$ mice

Serum samples from male mice given an oral gavage of [^3H]-retinol + olive oil or either CLA isomer and analyzed by reverse phase HPLC followed by fraction collection (see Methods and materials). [^3H]-cpm associated with retinol (A) and retinyl ester (B) fractions are shown. Data presented as means $\pm$ SD. *, $p < 0.05$ *versus* WT olive oil gavage (control group); #, $p < 0.05$ *versus* WT within the same treatment group. n= 4 mice per group. RE, retinyl ester; ROH, retinol.

Figure 3. Hepatic [^3H]-cpm associated with retinol and retinyl ester fractions in WT and RBP $-/-$ mice

Liver samples were analyzed by reverse phase HPLC followed by fraction collection (see Methods and materials). [^3H]-cpm associated with retinol (A) and retinyl ester (B) fractions are shown. Data presented as means $\pm$ SD. *, $p < 0.05$ *versus* WT olive oil gavage (control group); #, $p < 0.05$ *versus* RBP $-/-$ olive oil gavage (control group). $n = 4-5$ mice per group. §, $p < 0.05$ *versus* WT olive oil gavage (control group). $n = 4-5$ mice per group. RE, retinyl ester; ROH, retinol.

Figure 4. Serum [^3H]-cpm associated with retinol and retinyl ester fractions in WT mice
 Serum samples from male mice given P-407 (a total lipase inhibitor that prevents hydrolysis and clearance of chylomicrons for 4-5 days) the evening prior to sacrifice, followed by an oral gavage of [^3H]-retinol + olive oil or either CLA isomer. Samples were analyzed by reverse phase HPLC (see Methods and materials). Total retinoid [^3H]-cpm. Data presented as means $\pm$ SD. *, $p < 0.05$ versus olive oil gavage (control group). $n = 4-5$ mice per group.

Table 1Serum retinol levels ($\mu\text{g/dL}$) upon a single dietary administration of CLA isomers

Genotype	Gavage ^a		
	OLIVE OIL	CLA t10,c12	CLA c9,t11
WT	35.9 $\pm$ 2.9	35.9 $\pm$ 5.9	46.1 $\pm$ 3.4
RBP ^{-/-}	1.7 $\pm$ 0.4 [#]	2.1 $\pm$ 0.1 [#]	2.4 $\pm$ 0.4 [#]

WT, wild type mice; RBP ^{-/-}, retinol-binding protein knockout mice;[#], p<0.05 vs. WT within the same treatment group. Values expressed as means $\pm$ SD. n= 4 mice per group.^a Mice were given an oral bolus of all-*trans*-[³H] retinol (2×10^6 cpm) and unlabelled all-*trans*-retinol (6 μg) in 100 μl of olive oil or olive oil + either CLA isomer (70:30, v:v) and euthanized three hours later. The amount of CLA given, i.e. 30 mg, corresponds to the amount of CLA daily eaten by a mouse during our previously published chronic study [14].

Table 2

Triglyceride serum levels of mice gavaged with olive oil and either CLA isomer (mg/dL)

Genotype	Gavage ^a			
	NO GAVAGE	OLIVE OIL	CLA t10,c12	CLA c9,t11
WT	202.3 ± 17.3	210.3 ± 11.7	164.2 ± 11.8	170.1 ± 10.2

WT, wild type mice. Values expressed as means ± SD; 3-5 mice per group.

^aMice were given an oral bolus of all-*trans*-[³H] retinol (2×10^6 cpm) and unlabelled all-*trans*-retinol (6 µg) in 100 µl of olive oil or olive oil + either CLA isomer (70:30, v:v) and euthanized three hours later. The amount of CLA given, i.e. 30 mg, corresponds to the amount of CLA daily eaten by a mouse during our previously published chronic study [14].