Cellular Retinol-binding Protein Type III Is Needed for Retinoid Incorporation into Milk

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The physiologic role(s) of cellular retinol-binding protein (CRBP)-III, an intracellular retinol-binding protein that is expressed solely in heart, muscle, adipose, and mammary tissue, remains to be elucidated. To address this, we have generated and characterized CRBP-III-deficient (CRBP-III−/−) mice. Mice that lack CRBP-III were viable and healthy but displayed a marked impairment in retinoid incorporation into milk. Milk obtained from CRBP-III−/− dams contains significantly less retinyl ester, especially retinyl palmitate, than milk obtained from wild type dams. We demonstrated that retinol bound to CRBP-III is an excellent substrate for lecithin-retinol acyltransferase, the enzyme responsible for catalyzing retinyl ester formation from retinol. Our data indicated that the diminished milk retinyl ester levels arise from impaired utilization of retinol by lecithin-retinol acyltransferase in CRBP-III−/− mice. Interestingly, CRBP-I and CRBP-III each appeared to compensate for the absence of the other, specifically in mammary tissue, adipose tissue, muscle, and heart. For CRBP-III−/− mice, CRBP-I protein levels were markedly elevated in adipose tissue and mammary gland. In addition, in CRBP-1−/− mice, CRBP-III protein levels were elevated in tissues that normally express CRBP-III but were not elevated in other tissues that do not normally express CRBP-III. Our data suggested that CRBP-I and CRBP-III share some physiologic actions within tissues and that each can compensate for the absence of the other to help maintain normal retinoid homeostasis. However, under conditions of high demand for retinoid, such as those experienced during lactation, this compensation was incomplete.

Retinoids (vitamin A, its metabolites, and synthetic analogs) are important for facilitating normal vision, reproduction, immune function, and cell proliferation and differentiation (1–3). Retinol is a precursor for the synthesis of retinoic acid, the active retinoid form for regulating transcription, and for the synthesis of retinyl esters, the cellular retinoid storage forms. Retinol is very hydrophobic and is normally found within cells bound specifically to one of several cellular retinol-binding proteins (CRBPs) (4, 5). Because free retinol can become nonspecifically incorporated into membranes and disrupt normal cellular activities, it has been proposed that one of the physiologic roles of the CRBPs is to protect the cell against these potentially detrimental effects (4, 6). Another physiologic role attributed to CRBPs is to facilitate channeling of retinol toward specific enzymes required either for its oxidation to retinoic acid or for its esterification to retinyl esters (1, 5, 7, 8).

Currently, there are three known murine CRBP's termed CRBP-I, CRBP-II, and CRBP-III. The best characterized of these are CRBP-I and CRBP-II. CRBP-I is expressed at high levels in the liver, kidney, testes, and eye and is present to a lesser extent in most other tissues except the small intestine (5, 9). CRBP-II is highly but solely expressed in the small intestine of the adult (5, 10, 11). Both CRBP-I and CRBP-II have been demonstrated to have important actions for mediating intracellular retinoid transport and metabolism (12–14). In the liver, CRBP-I is needed for storage of retinoid as retinyl ester because mice deficient in CRBP-I (CRBP-I−/− mice) show a 50% reduction in retinoid storage and higher rates of hepatic retinoid turnover (12). Because CRBP-I−/− mice are not able to store retinoid properly in the liver, they more readily develop symptoms of retinoid deficiency than do wild type mice when they are maintained on a retinoid-insufficient diet (12). It is well established that CRBP-II binds both retinol and retinaldehyde in the enterocyte and that retinol bound to CRBP-II is a good substrate for the enzyme that esterifies newly absorbed dietary retinol, lecithin:retinol acyltransferase (LRAT). This action of CRBP-II facilitates dietary retinoid incorporation into nascent chylomicrons. Mice deficient in CRBP-II (CRBP-II−/− mice) show a decreased capacity for retinoid absorption, especially when dietary retinoid levels are low, and reduced hepatic retinyl ester levels. In addition, marginal dietary retinoid intake during the perinatal period leads to adverse effects on neonatal survival arising from impaired heart and lung development in CRBP-II−/− mice (14).

A third member of the murine CRBP family, CRBP-III, has been described, but its physiologic role(s) has not been established (15, 16). In addition to the three murine CRBPs, another CRBP that is expressed solely in the human has been identified (17). Although this human CRBP was originally referred to as CRBP-III, the human protein is not homologous to the mouse CRBP-III being considered here. The murine CRBP-III shows a different tissue expression pattern than CRBP-I and CRBP-II. It is solely expressed in heart, muscle, adipose, and mammary tissue (15, 16, 18). Moreover, CRBP-III binds retinol but not retinaldehyde, unlike CRBP-I and CRBP-II. There is presently

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24286
no information available regarding the physiologic role(s) of CRBP-III. To gain more insight into the role of CRBP-III in vivo, we have generated and characterized a mouse strain that lacks CRBP-III. Findings from our study of this strain are presented here.

**MATERIALS AND METHODS**

*Generation of CRBP-III−/− Mice*—The locus of CRBP-III was cloned by PCR using genomic DNA obtained from 129/SvJ mice and inserted into a vector containing a neomycin resistance gene cassette and a thymidine kinase cassette. A 2-kb region of the CRBP-III locus entails the promoter region, exon 1, and part of intron 1 and a 3.5-kb region encompassing intron 3 were cloned 5′ and 3′ of the neomycin resistance cassette, respectively. The neomycin cassette replaced a 1.8-kb DNA piece of the CRBP-III locus including part of exon 1, exon 2, intron 2, exon 3, and part of intron 3. A thymidine kinase cassette was present 3′ of the genomic DNA.

**RNAS Analysis**—RNA levels were analyzed using Northern blot procedures. Total RNA from tissues was extracted using RNA-Bee (Tel-Test). Twenty μg of total RNA was resolved on a 1% agarose gel containing 5% formaldehyde and transferred overnight to a positively charged membrane (Bio-Rad). A probe composed of exons 2 and 3 of CRBP-III cDNA was 32P labeled using a random labeling kit (Amersham Biosciences) and hybridized overnight. The signals were detected using a Storm PhosphorImager (Amersham Biosciences).

**Western Blot Analysis of CRBP-I and CRBP-III**—For detection of CRBP-I and CRBP-III in mouse tissues, polyclonal antibodies were raised in rabbits against the N terminus of CRBP-I (ICGVEGICAc-m/KQVFKKH) and CRBP-III (CEGQVC(Acm)KQTFQRA), respectively (NeoMPS). The rabbit antisera were then further purified by immunoaffinity chromatography employing either recombinant CRBP-I or CRBP-III that had been linked to CNBr-activated Sepharose according to the manufacturer’s instructions at a ratio of 5 mg of CRBP per 1 ml of activated Sepharose gel (Amersham Biosciences). After immunopurification, the antibody directed against mouse CRBP-I specifically reacted solely with CRBP-I protein and did not cross-react with CRBP-III. Similarly, the antibody directed against CRBP-III did not cross-react with CRBP-I protein and recognized only CRBP-III protein (see Fig. 2A). Cytosolic extracts of tissues were prepared for analysis of CRBP-I and CRBP-III expression by Western blot. For this purpose, tissues were excised and quickly flash frozen in liquid nitrogen. At the time of analysis, tissues were homogenized in buffer (10 mm Hepes, 10 mm KCl, 250 mm sucrose, 5 mm EDTA, 5 mm EGTA, 1.5 mm MgCl2, and protease inhibitor mixture (complete EDTA-free, Roche Diagnostics) using a Dounce homogenizer. The homogenate was centrifuged at 10,000 × g for 5 min. The resulting postnuclear supernatant was taken and centrifuged at 100,000 × g for 1 h to obtain a cytosolic fraction that was used in our analyses. Protein concentrations of the resulting supernatant were determined using the Bradford assay (Bio-Rad). Equal protein concentrations were separated on a 4–15% SDS/polyacrylamide gel (Bio-Rad) and transferred to nitrocellulose (Bio-Rad). Monoclonal antibody directed against β-actin (Sigma) was used as a control to verify protein load for all tissues except heart tissue, for which monoclonal antibody directed against α-actin (Sigma) was used. Immunoreactive proteins were visualized using a secondary antibody conjugated to horseradish peroxidase followed by chemiluminescence (Pierce).

**Reverse Transcription-PCR and Quantitative Real-time PCR Analysis of LRAT Expression in Mammary Tissue**—Tissue RNA at different stages of mammary development (virgin, pregnancy, and lactation) was extracted using RNA-Bee (Tel-Test) as described above. Random primers were used to generate cDNA (Invitrogen) followed by PCR using primers specific for the LRAT cDNA. Samples were processed with and without using reverse transcriptase. Liver total RNA was included as a positive control. For quantitative PCR comparing LRAT expression in wild type and CRBP-III−/− mice, LRAT levels were determined using SYBR Green (Applied Biosystems) real-time PCR. Primers for mouse LRAT were designed to minimize primer dimerization (Invitrogen), and β-actin was used as the internal control.

**LRAT Activity Assays**—LRAT assays were essentially performed as previously described (9, 10). In brief, the hamster ovaries/fetuses were dissected from pregnant females 180–190 days of age and homogenized in 100 μl of phosphatidylethanolamine (PE) emulsion (Invitrogen). The homogenates were centrifuged at 100,000 × g for 1 h. The PE extract was used to determine the LRAT activity of the estrous cycle. After incubation at 37 °C, the retinyl esters were extracted and measured in each sample using a kit according to the manufacturer’s instructions (Wako). Retinol and retinyl esters were separated on a Ultrasphere ODS C18 column (Beckman). A phospho diode array detector (Waters 996) was used to detect retinoids at 325 nm. All-trans-retinoic acid levels were measured in heart and adipose tissue from CRBP-III−/− and CRBP-III−/− mice as previously described (21).

**Plasma Clearance and Uptake of 3H-Retinoid into Tissues**—Postprandial clearance of retinoids by tissues was conducted as previously described (22). A gavage dose was prepared containing (11,12-3H)retinol (49.3 Ci/mmol; PerkinElmer Life Sciences) in peanut oil (106 cm2/100 μl). After an overnight fast, mice received an oral dose consisting of 100 μl of the oil containing the labeled retinol. One, 2, and 7 h after receiving the dose, the mice were sacrificed, and blood and tissues were taken for analysis. The tissues were quickly weighed and flash frozen in liquid nitrogen for analysis. Plasma was obtained after centrifugation of the whole blood at 4 °C. For each time point, four mice for each genotype were used. The experiments were repeated twice for mice of each gender at two different ages (11 and 18 weeks). 3H-Retinoid levels in plasma and tissues were determined as previously described (22).

**Collection of Dam Milk and Determination of Triglyceride Concentration**—At birth, litter size was culled to 6 pups/dam. Milk samples were collected at mid-lactation (day 13 postpartum). Milk was collected essentially as described previously (23). Dams were separated for 60 min from their pups prior to milk collection. Ten min after a subcutaneous injection of 0.2 unit of oxytocin (Sigma), milk was collected by gentle sucking of each nipple using a vacuum pump. The collected milk was directly transferred to an Eppendorf tube, diluted with saline and rinsed with 5 mm EDTA, and flash frozen at −80 °C. This method allowed us to collect 200–400 μl of milk per dam. Milk collection was performed under yellow light. Milk samples were analyzed for retinoids by reverse phase HPLC as described above using a 10 μl aliquot of milk for each extraction. Triglyceride concentrations were measured in each sample using a kit according to the manufacturer’s instructions (Wako). Retinoid levels are expressed relative to milk triglyceride content.

**RESULTS**

**Disruption of the CRBP-III Gene and Generation of CRBP-III−/− Mice**—To investigate the physiologic role of CRBP-III in vivo, a conventional, whole body knock-out mouse model lacking CRBP-III has been generated. The wild type allele, targeting vector, and targeted allele used for this purpose are depicted in Fig. 1A. A region spanning from intron 1 through part of intron 3 including exons 2 and 3 was replaced by the neomycin resistance cassette. The targeting vector was electroporated into 129/SvJ embryonic stem cells and put under selection of G418. Genomic DNA from embryonic stem cell clones was extracted and analyzed by Southern blot for homologous recombination. Based on Southern blotting of the DNA, 1 of 125 clones was identified that exhibited the Southern blot banding pattern predicted for homologous recombination (Fig. 1B). After injection of the positive embryonic stem cell clone into C57BL/6J blastocysts to generate chimeras, four male chimeras transmitted the mutation to their offspring. A representative Southern blot used for genotyping is depicted in Fig. 1C. A PCR method for routine
physiologic role of CRBP-III

geno-

type of the animals is shown in Fig. 1D. To confirm the successful disruption of the CRBP-III gene, RNA levels were analyzed in wild type (CRBP-III/−/−), heterozygous (CRBP-III+/−), and null mutant (CRBP-III−/−) heart extracts (heart tissue from CRBP-III−/− and CRBP-III+/− mice. F, Western blot analysis of heart tissue from CRBP-III+/− and CRBP-III−/− mice.

Retinol and Retinyl Ester Levels in the Milk of CRBP-III−/− Dams—Because CRBP-III is expressed at high levels during lactation, we analyzed the retinol and retinyl ester contents of milk from lactating females at mid-lactation. Total retinyl ester levels were significantly reduced in milk from dams that lack CRBP-III in comparison with levels in milk collected from CRBP-III+/− dams (Fig. 3). Several retinyl esters are present in the milk including retinyl palmitate, linoleate, oleate, and stearate (24) (Fig. 3). Interestingly, upon evaluation of levels of the different retinyl esters present in milk, it was apparent that the greatest reduction in retinyl ester levels occurred for retinyl palmitate (Fig. 3). These data indicate that CRBP-III has an important role in the incorporation of retinyl ester into the milk.

Retinol Bound to CRBP-III (Holo-CRBP-III) Is a Good Substrate for LRAT—The mammary gland can obtain retinol either postprandially from retinyl ester present on chylomicrons or from the circulation as retinol bound to serum retinol-binding protein (22, 25, 26). After its uptake into mammary tissue, retinol can either remain unesterified and bound to CRBP-III or be re-esterified to retinyl ester for storage or secretion of retinyl ester into the milk (27). Formation of retinyl ester can be catalyzed by one of two enzymes, acyl-CoA:retinol acyltransferase or LRAT (28). The latter has been cloned (19), and mice lacking LRAT reportedly have no retinyl esters in plasma, liver, lung, or eye (other tissues were not described) (29). As shown in Fig. 4A, LRAT is expressed at all stages of mammary development, suggesting that it may be important for catalyzing retinol esterification during the different stages of mammary gland development. Using real-time PCR, we found no quantitative differences in LRAT expression between CRBP-III+/− and CRBP-III−/− animals (Fig. 4B), inferring that changes in levels of LRAT do not contribute to decreased retinyl ester levels observed in the milk and mam-
The expression of CRBP-I is increased in adipose tissue of CRBP-III−/− mice. It has been well documented that retinol bound to CRBP-I and retinol bound to CRBP-II are good substrates for LRAT (5, 30). The lack of CRBP-I in the liver leads to decreased hepatic retinyl ester levels, supporting a role for holo-CRBP-I as a substrate for LRAT (12). Because retinyl esters are reduced in the milk from CRBP-III−/− dams, a question arises as to whether retinol-CRBP-III might also serve as a substrate for LRAT. If this were the case, the lack of CRBP-III might lead to decreased retinyl ester levels in these tissues and milk. We compared holo-CRBP-I and holo-CRBP-III as substrates for in vitro esterification by recombinant LRAT. As shown in Fig. 4C, holo-CRBP-III is a good substrate for LRAT, as good as holo-CRBP-I. It has been previously shown that excess apo-CRBP-I can inhibit LRAT-catalyzed retinyl ester formation. We conducted in vitro assays to assess whether this is also the case for apo-CRBP-III. Confirming the earlier findings, increasing apo-CRBP-I lead to a decline in LRAT activity reflected in a lessering in the rate of retinyl ester formation (Fig. 4D). In contrast, increasing apo-CRBP-III concentrations did not affect the LRAT activity. These data indicate that both holo-CRBP-I and holo-CRBP-III are able to serve as substrates for LRAT but that only apo-CRBP-I diminishes LRAT activity. This suggests that CRBP-I and CRBP-III may have overlapping but distinct roles in facilitating retinol metabolism in the mammary gland.

Expression of CRBP-I in CRBP-III−/− Mice—CRBP-I and CRBP-III are 56% identical; both proteins bind retinol, and both are substrates for LRAT. Considering that the two proteins have an overlapping tissue expression pattern, it seemed possible that CRBP-I and/or CRBP-III might be able to compensate functionally for the absence of the other. To assess this, we determined CRBP-I and CRBP-III expression levels in tissues from wild type mice. As seen in Fig. 5A, CRBP-I is ubiquitously expressed in the mouse, with the highest expression levels in liver, lung, testes, and eye. CRBP-I is also expressed at lower levels in heart, mammary, and adipose tissue; thus, its expression overlaps with CRBP-III expression (Fig. 5A). Moreover, the expression of CRBP-I is increased in adipose tissue of CRBP-III−/− mice as compared with CRBP-III+/+ mice (Fig. 5B). This parallels the mammary gland, in which CRBP-I has low expression in the virgin state and during lactation. However, when mice lack CRBP-III, CRBP-I expression is substantially increased in both the virgin and the lactating state (Fig. 5, C and D). This up-regulation is tissue-specific because CRBP-I expression remained unchanged in tissues such as testis that do not express CRBP-III (Fig. 5B).

CRBP-III Expression in CRBP-I−/− Mice—Mice lacking CRBP-I−/− kept on a control retinoid-sufficient diet exhibit a significant reduction of retinyl ester stores in the liver (12). Besides the liver, substantial amounts of retinyl ester are also present in the mammary gland and adipose tissue, both sites of CRBP-I expression (Fig. 5). However, in contrast to the liver, retinyl ester levels in mammary gland and adipose tissue of the CRBP-I−/− mutants are not different from those of wild type mice (data not shown). Unlike liver, both adipose tissue and

**Fig. 3.** Total retinyl ester and retinyl palmitate, stearate, oleate, and linoleate levels in milk collected from CRBP-III+/+(light bars) and CRBP-III−/− (dark bars) dams at mid-lactation (day 13). n = 7 dams for each genotype. Means ± S.D. are shown. *, p < 0.05.

**Fig. 4.** A, expression of LRAT in the mammary gland at different stages of mammary gland development and in the liver of wild type mice measured by reverse transcription-PCR. RT, reverse transcriptase. B, real-time PCR assessing expression of LRAT in mammary gland of female CRBP-III+/− (light bars) and CRBP-III−/− (dark bars) mice. Three different samples from CRBP-III+/− and CRBP-III−/− mice were used. Values are the means ± S.D. C, in vitro assay evaluating free retinol (black bars), CRBP-I-retinol (gray bars), and CRBP-III-retinol (white bars) as substrates for LRAT. In vitro, LRAT utilizes free retinol very efficiently, and the percentage of retinyl ester formed is therefore higher compared with that formed when retinol is bound to CRBPs. The LRAT activity was determined in three independent experiments. Values are the means ± S.D. D, the effect of increasing apo-CRBP-I (white bars) or apo-CRBP-III (gray bars) compared with holo-CRBP-III on the esterification of retinol by LRAT. The assay was repeated twice with similar results.
earliest total retinol determinations for these tissues were carried out 8 weeks after weaning. In the mammary gland, both retinol and retinyl ester levels were reduced in mice lacking CRBP-III at 11 weeks of age (Fig. 7, B and D). Interestingly, not all retinyl esters were decreased in these mice, but, specifically, retinyl palmitate levels were 30% reduced in the mammary gland from female CRBP-III−/− mice as compared with levels seen in CRBP-III+/+ mice (Fig. 7C). At 17 weeks of age, retinol levels remain reduced (Fig. 7D) in the mammary gland, whereas retinyl ester levels were no longer different between the two mouse strains (Fig. 7, B and C). No differences between strains were observed at any age for the total retinol concentration of white adipose tissue (Fig. 7E) or muscle (data not shown). In addition, levels of all-trans-retinoic acid were not different in heart (21.7 ± 4.2 ng/g for CRBP-III−/− mice and 21.3 ± 6.2 ng/g for CRBP-III+/+ mice) and adipose tissue (11.2 ± 5.7 ng/g for CRBP-III+/+ mice and 10.7 ± 8.6 ng/g for CRBP-III−/− mice) for mice lacking CRBP-III compared with wild type mice.

Postprandial Retinoid Clearance—Postprandially, chylomicrons containing intestinally derived retinyl esters are present in the circulation. The action of lipoprotein lipase, chylomicron remnants are generated from the chylomicrons. It has previously been shown that in vivo, lipoprotein lipase can facilitate the uptake of postprandial retinoids specifically into tissues in which it is expressed, namely, heart, muscle, and adipose tissue (31). These tissues also express CRBP-III. Therefore, we asked whether postprandial retinoid turnover was altered in mice lacking CRBP-III. Mice were given an oral bolus of peanut oil containing [3H]retinol, and its plasma clearance and tissue sites of uptake were monitored. As depicted in Fig. 8, there were no differences in 3H-retinoid uptake in heart, adipose tissue, and mammary gland between CRBP-III−/− and CRBP-III+/+ mice. Similar results were obtained in younger animals (10 weeks of age) and in male mice (data not shown). Based on these data, decreased uptake of postprandial retinol is not likely to be responsible for the lower retinoid levels observed in heart and mammary gland of young CRBP-III−/− mice.

DISCUSSION

The present study provides the first characterization of the phenotype of a new mouse strain that lacks CRBP-III. In the adult animal, CRBP-III is important for facilitating retinyl ester incorporation into milk. Retinyl ester levels are markedly reduced in milk of CRBP-III−/− mice compared with levels determined for milk from wild type dams. One possible explanation for this could be diminished postprandial retinol delivery to the mammary gland of CRBP-III−/− dams. Chylomicron delivery of retinoids is an important delivery pathway to the mammary gland, especially during lactation (22, 26, 32). The retinoid content of milk strongly correlates with the retinoid content of the diet (25, 33–39). The importance of postprandial retinol for retinyl ester incorporation into milk is further evidenced by our finding that the complete absence of retinol-binding protein, the sole plasma protein responsible for the delivery of retinol to tissues, does not diminish milk retinyl ester levels (22). However, because postprandial retinoid uptake and clearance was not different in mammary gland or other tissues of CRBP-III−/− mice as compared with wild type mice, this cannot account for the diminished retinyl ester content in milk of CRBP-III−/− dams. We have demonstrated that, like holo-CRBP-I and holo-CRBP-II, LRAT can utilize holo-CRBP-III as a substrate for retinyl ester formation. This observation, we believe, accounts for the reduced retinyl ester content of milk from CRBP-III−/− dams. Because high levels of CRBP-III are expressed during lactation, the lack of holo-
CRBP-III would lead to reduced substrate availability in the CRBP-III/H11002/H11002 mouse during lactation and reduced retinyl ester synthesis. This finding parallels the observation in mice lacking CRBP-I/H11002/H11002 that hepatic retinyl ester levels are reduced compared with wild type mice (12).

To better understand retinol metabolism in mammary gland and retinyl ester incorporation into milk, in the present work, we report the expression pattern of CRBP-I, CRBP-III, and LRAT during different developmental stages of the mammary gland. We confirmed earlier data showing high expression of CRBP-I during pregnancy but only relatively low levels of expression during the non-pregnant state and lactation (40, 41). CRBP-III, on the other hand, is expressed at high levels during lactation (see Fig. 5). LRAT is present at nearly con-
stent levels during all developmental stages. This suggests that retinoid incorporation into the mammary gland is modulated by changes in CRBP-I and/or CRBP-III activities rather than through modulation of LRAT.

Because we had previously demonstrated that changes in lipoprotein lipase activity influence the amount of postprandial retinoid that is taken up by heart, muscle, and adipose tissue (31), we predicted a priori that both the uptake of postprandial retinoid and tissue total retinol levels would be different for these tissues in CRBP-III-null mice. However, we were unable to detect differences in the uptake of postprandial retinoid for heart, muscle, or adipose tissue between CRBP-III−/− and CRBP-III+/− mice. Moreover, we only observed differences in tissue total retinol levels for some but not all of the tissues that normally express CRBP-III. At weaning, low levels of retinol and retinyl ester are detected in hearts from mice lacking CRBP-III. This, however, might be accounted for by the reduced levels of retinyl ester in the milk of CRBP-III−/− dams because milk is the sole source of retinoid for the first 21 days postpartum. After weaning, the chow diet that is provided to the mice provides substantial amounts of dietary retinoid, resulting in the normalization of retinol levels in the hearts of older CRBP-III−/− mice. We now believe that we did not observe differences in these parameters because of a functional compensation on the part of CRBP-I for CRBP-III in the null mice. This is supported by the observation that tissues for which expression of CRBP-III is high (namely, heart, adipose tissue, and mammary gland) is not clear. The elevation of CRBP-III protein in tissues in CRBP-III-null mice. However, we were unable to detect differences in the uptake of postprandial retinoid for heart, muscle, or adipose tissue between CRBP-III+/− and CRBP-III−/− mice. Moreover, we only observed differences in tissue total retinol levels for some but not all of the tissues that normally express CRBP-III. At weaning, low levels of retinol and retinyl ester are detected in hearts from mice lacking CRBP-III. This, however, might be accounted for by the reduced levels of retinyl ester in the milk of CRBP-III−/− dams because milk is the sole source of retinoid for the first 21 days postpartum. After weaning, the chow diet that is provided to the mice provides substantial amounts of dietary retinoid, resulting in the normalization of retinol levels in the hearts of older CRBP-III−/− mice. We now believe that we did not observe differences in these parameters because of a functional compensation on the part of CRBP-I for CRBP-III in the null mice. This is supported by the observation that tissues for which expression of CRBP-III is high (namely, heart, adipose tissue, and mammary gland) is not clear. The elevation of CRBP-III protein in tissues in CRBP-III-null mice. However, we were unable to detect differences in the uptake of postprandial retinoid for heart, muscle, or adipose tissue between CRBP-III+/− and CRBP-III−/− mice. Moreover, we only observed differences in tissue total retinol levels for some but not all of the tissues that normally express CRBP-III. At weaning, low levels of retinol and retinyl ester are detected in hearts from mice lacking CRBP-III. This, however, might be accounted for by the reduced levels of retinyl ester in the milk of CRBP-III−/− dams because milk is the sole source of retinoid for the first 21 days postpartum. After weaning, the chow diet that is provided to the mice provides substantial amounts of dietary retinoid, resulting in the normalization of retinol levels in the hearts of older CRBP-III−/− mice. We now believe that we did not observe differences in these parameters because of a functional compensation on the part of CRBP-I for CRBP-III in the null mice. This is supported by the observation that tissues for which expression of CRBP-III is high (namely, heart, adipose tissue, and mammary gland) is not clear. The elevation of CRBP-III protein in tissues in CRBP-III-null mice. However, we were unable to detect differences in the uptake of postprandial retinoid for heart, muscle, or adipose tissue between CRBP-III+/− and CRBP-III−/− mice. Moreover, we only observed differences in tissue total retinol levels for some but not all of the tissues that normally express CRBP-III. At weaning, low levels of retinol and retinyl ester are detected in hearts from mice lacking CRBP-III. This, however, might be accounted for by the reduced levels of retinyl ester in the milk of CRBP-III−/− dams because milk is the sole source of retinoid for the first 21 days postpartum. After weaning, the chow diet that is provided to the mice provides substantial amounts of dietary retinoid, resulting in the normalization of retinol levels in the hearts of older CRBP-III−/− mice.