Effects of dietary retinoid and triglyceride on the lipid composition of rat liver stellate cells and stellate cell lipid droplets

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Abstract Hepatic stellate cells store the majority of the liver's retinoid (vitamin A) reserves as retinyl esters in stellate cell lipid droplets. A study was conducted to explore the effects of differences in dietary retinoid and triglyceride intake on the composition of the stellate cell lipid droplets. Weanling rats were placed on one of five diets that differed in retinoid or triglyceride contents. The dietary groups were: *1)* control (2.4 mg retinol (as retinyl acetate)/kg diet and 20.5% of the calories supplied by triglyceride (as peanut oil)); *2)* low retinol (0.6 mg retinol/kg diet and control triglyceride levels); *3)* high retinol (24 mg retinol/kg diet and control triglyceride levels); *4)* low triglyceride (2.4 mg retinol/kg diet and 5% of the calories supplied by triglyceride); and *5)* high triglyceride (2.4 mg retinol/kg diet and 45% of the calories supplied by triglyceride). Stellate cells were isolated using the pronase–collagenase method and stellate cell lipid droplets were isolated by differential centrifugation. The levels of retinoids and other lipids were measured by high performance liquid chromatography. The stellate cells from control rats contained 113 μg total lipid/10^6 cells. Control stellate cell lipid droplets had the following mean percent lipid composition: 39.5% retinyl ester; 31.7% triglyceride; 15.4% cholesteryl ester; 4.7% cholesterol; 6.3% phospholipids; and 2.4% free fatty acids. Both the concentration of stellate cell lipids and the composition of stellate cell lipid droplets were markedly altered by changes in dietary retinoid. The low and high retinol groups contained, respectively, 82 and 566 μg total lipid/10^6 cells, with retinyl ester representing, respectively, 13.6% and 65.4% of the lipid present in the stellate cell lipid droplets. Low and high triglyceride groups were similar to controls in both stellate cell lipid content and the composition of the stellate cell lipid droplets. These findings indicate that the composition of stellate cell lipid droplets is strongly regulated by dietary retinoid status but not by dietary triglyceride intake.—**Moriwaki, H., W. S. Blaner, R. Piantedosi, and D. S. Goodman.** Effects of dietary retinoid and triglyceride on the lipid composition of rat liver stellate cells and stellate cell lipid droplets. *J. Lipid Res.* 1988. **29:** 1523–1534.

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It is well established that the liver plays the central role in retinoid (vitamin A) storage and metabolism in the body. The liver takes up dietary retinoid in the form of chylomicron retinyl ester (1, 2) and stores the majority of the body's retinoid reserves as retinyl esters in lipid droplets (3–5). To meet the requirements for retinoids in other organs, retinol is secreted from the liver, in a highly regulated manner, bound to its specific plasma transport protein, retinol-binding protein (RBP) (6, 7). During the period between chylomicron retinyl ester uptake and the secretion of the retinol-RBP complex, retinoids undergo a series of metabolic transformations that include hydrolysis of the dietary retinyl ester, reesterification of the retinol for storage, and hydrolysis of stored retinyl ester to provide retinol for secretion from the liver (see (3) for a recent review).

Recent studies have clearly shown that two types of hepatic cells, the parenchymal cells and the nonparenchymal stellate cells (also called fat-storing cells, lipocytes, and Ito cells) are importantly involved in hepatic retinoid metabolism and storage. The parenchymal cells are responsible for the uptake of newly absorbed chylomicron retinyl esters and for the synthesis and secretion of RBP (3, 7–9). Once taken up by the parenchymal cells, dietary retinoids are transferred to the stellate cells for storage (8, 10, 11). Studies from our laboratory suggest that chylomicron retinyl ester is not transferred directly from parenchymal to stellate cells without first undergoing hydrolysis to

Abbreviations: CRABP, cellular retinoic acid-binding protein; CRBP, cellular retinol-binding protein; HPLC, high performance liquid chromatography; RBP. retinol-binding protein; PBS, phosphate-buffered saline.

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retinol (11). The stellate cells are known to be the main storage site of retinoids in the liver (12–15); stellate cells are also highly enriched in both cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP), and in the enzymes that synthesize and hydrolyze retinyl esters (acyl-CoA:retinol acyltransferase and retinyl palmitate hydrolase) (13, 14, 16).

Stellate cells are characterized by their abundant lipid droplets, which normally represent the site of storage of the majority of the liver's retinoid reserves (5, 12–14). Recent studies have provided information about the chemical composition of the stellate cell lipid droplets obtained from normal rats (17, 18). Thus, it has been reported that these lipid droplets contain retinyl esters, triglycerides, cholesteryl esters, cholesterol, phospholipids, and free fatty acids, with approximately 70–85% of the total lipid mass consisting of retinyl ester and triglyceride (17, 18). The information available about the composition of the lipid droplets is, however, quite limited, and no detailed quantitative information is available about whether the composition of the lipid droplets is constant or variable, and about the factors that might regulate the composition of the stellate cell lipid droplets. It is known that the amount of dietary intake of retinol affects the number and size of the stellate cell lipid droplets (3, 5). However, nothing is known about the factors, other than dietary retinol status, that might control the stellate cell content of lipids and lipid droplets. Clearly, information is needed about the factors that influence, and the mechanisms that regulate, the content and composition of the lipid droplets in stellate cells.

The studies reported here were designed to address these issues, by exploring the effects of dietary variables on the content and composition of stellate cell lipid droplets. Specifically, these studies aimed to determine the effects of major changes in dietary intake of retinoids and of triglycerides on the content and composition of the stellate cell lipid droplets. The studies reported here demonstrate that the content and composition of these lipid droplets is strongly regulated by dietary retinoid status, and not at all influenced by dietary triglyceride intake. In contrast, dietary triglyceride intake strongly influences the lipid content and composition of the hepatic parenchymal cells.

EXPERIMENTAL PROCEDURES

Animals and diets

Weanling female Sprague-Dawley rats (weight range 32–59 g) were obtained from Camm Research Institute (Wayne, NJ). Each rat was housed in an individual cage and kept on one of five diets for 35–37 days. The rats were randomly assigned to a diet group and were allowed free access to food and water. The body weight and food intake of each rat were measured three times a week throughout the study. The rats were fasted the night before they were used for hepatic cell isolations.

The rats used for this study were each maintained on one of five diets that differed with regard to their contents of retinoid (vitamin A) and triglyceride. The five diets will be referred to as: control diet, high retinol diet, low retinol diet, high triglyceride diet, and low triglyceride diet. The nutritionally complete, purified control diet was prepared as described previously (19); it contained 2.4 mg of retinol (as retinyl acetate) per kg diet, and provided 52% of the total energy in the diet as dextrose, 27.5% as protein, and 20.5% as triglyceride (peanut oil). The other diets were identical to the control diet with the following modifications: i) low retinol diet, 0.6 mg of retinol (as retinyl acetate) per kg diet; ii) high retinol diet, 24 mg retinol (as retinyl acetate) per kg diet; iii) high triglyceride diet, 27.5% of the energy was provided by dextrose and 45% by triglyceride (peanut oil); and iv) low triglyceride diet, 67.5% of the energy was provided by dextrose and 5% by triglyceride (peanut oil). All diets were prepared fresh weekly.

Isolation of nonparenchymal hepatic cells

Perisinsuoidal stellate cells and a fraction consisting of Kupffer and endothelial cells were isolated using a modification (17) of the procedure of Hendriks et al. (12). This procedure, which uses pronase E to digest the liver (and destroy parenchymal cells) and a two-layer Nycodenz gradient, to separate stellate cells from Kupffer and endothelial cells, provides stellate cell preparations that are of high yield and purity. The two-layer Nycodenz gradient also separates a cell fraction that contains a mixture of Kupffer and endothelial cells (henceforth called Kupffer-endothelial cell fraction) in which very few cells of other cell types are present (12). Both the stellate cell and the Kupffer-endothelial cell fractions were collected for analysis from the two-layer Nycodenz gradient.

The compositions and purities of the isolated liver cell preparations were determined by light microscopic examination of stained cell preparations. Stellate cells were identified by Oil Red 0 Staining (5); Kupffer cells were identified by peroxidase staining (15); and endothelial cells were differentiated from parenchymal cells by hematoxylin–eosin staining.

Isolation of stellate cell lipid droplets

The procedure used for the isolation of lipid droplets from stellate cells is described in detail elsewhere (17). Briefly, stellate cells (3–5 $\times$ 10^6 cells) were suspended in 5 ml of phosphate-buffered saline (PBS).

The cell suspension was frozen (in acetone–dry ice) and thawed (in a water bath of 37° C) five times, and then gently homogenized with five strokes of a Teflon-on-glass homogenizer. The homogenate was mixed with a stock solution of 28.8% Nycodenz to yield a final concentration of 6% (w/v) Nycodenz, and then overlayered with 4 ml of PBS–H_2O 1:10 (v/v) and centrifuged at 3500 *g* for 20 min at 4° C. After centrifugation, the top layer that contained the lipid droplets was collected and the lower layer and pellet were again homogenized and centrifuged as described above. The top layers obtained from the two centrifugations were combined and used for further analyses. The lipid droplet preparations were judged, by electron microscopy, to be both highly pure and representative of the lipid droplets present in stellate cells.

Quantitation of neutral lipids and phospholipids in isolated hepatic cells and lipid droplets

Total lipids were extracted from the total liver digests, the isolated hepatic cell preparations, and the lipid droplets using a modification of the method of Folch, Lees, and Sloane Stanley (20). The extractions were carried out under argon in amber glass tubes in a darkened room. The neutral lipids and phospholipids in the total lipid extract were assayed quantitatively utilizing a modification of the high performance liquid chromatography (HPLC) procedure described by Schlager and Jordi (21). Some aspects of these methods will be summarized here in detail.

Total lipids were extracted with six volumes of chloroform–methanol 2:1 from 0.1 ml of either a total liver digest, a cell suspension (containing 0.1–0.5 × 10^6 cells), or an isolated lipid droplet preparation (derived from 3.0–5.0 × 10^6 cells). The lipid-containing chloroform phase was separated from the aqueous-methanol phase in a desk top centrifuge, and then evaporated to dryness under N_2 at room temperature. The resulting total lipid extract was immediately redissolved in 0.1 ml of hexane, and 40 μl of this solution was injected directly onto the HPLC column for neutral lipid or phospholipid analysis. The upper aqueous-methanol layer, including the protein interface, was also collected and evaporated to dryness; the dried protein extract so obtained was resuspended in 0.1 ml of deionized H_2O for assay of protein.

The HPLC determinations of neutral lipids and phospholipids were carried out on a 3.9 × 30 cm μPorasil column (Waters Association, Milford, MA). Two different solvent systems were used for the lipid analyses. For neutral lipid analysis, 100% hexane (solvent A) and hexane–2-propanol–water 6:8:0.75 (solvent B) were prepared and multiple linear gradients were generated as follows: 0 min, 100% solvent A, 0% solvent B; 3 min, 90% solvent A, 10% solvent B; 12 min, 70% solvent A, 30% solvent B; 30 min, 0% solvent A, 100% solvent B (the flow rates were constant at 2.0 ml/min). For the phospholipid separations, a linear gradient, over 30 min, was generated from 100% solvent B to 100% hexane–2-propanol–water 6:8:1.4 (the flow rate was constant at 2.0 ml/min). Neutral lipids and phospholipids were detected at 206 nm. The average recoveries of neutral lipids and phospholipids, using this procedure, were 95.1% and 92.7%, respectively.

Typical HPLC chromatograms of the neutral lipids and phospholipids extracted from the stellate cell derived lipid droplets are given in **Fig. 1.** The elution positions assigned for triglycerides in Fig. 1A are those obtained for pure single fatty acid triglycerides (tripalmitin, triolein, etc.). Since biological samples almost never consist of single fatty acid triglycerides, it is unlikely that the triglycerides extracted from the cellular or lipid droplet preparations and chromatographing with these retention times were single fatty acid triglycerides. In this study, we did not attempt to specifically identify the fatty acid composition of each triglyceride peak in the biological samples. The goal of our assays was, instead, to determine the mass of total triglyceride present in each sample. To validate the use of HPLC assays to meet this goal, the identity of each chromatographic peak, as a triglyceride was verified in a small number of selected samples by automated enzymatic analysis (ABA 100, Abbott Laboratories, Chicago IL) of peak fractions collected from the effluent stream of the HPLC. Hence, the total triglyceride determined in each experimental sample was measured as the sum of several peaks representing different species of triglycerides. Similarly, the cholesteryl esters and free fatty acids were measured as the sums of several peaks corresponding to individual cholesteryl esters or free fatty acids.

The quantities of neutral lipids and phospholipids present in the cell or lipid droplet preparations were determined from linear standard curves relating measured peak area with injected lipid mass. Standard curves were constructed from authentic samples of cholesterol, cholesteryl myristate, cholesteryl palmitate, cholesteryl stearate, cholesteryl oleate, cholesteryl linoleate, myristic acid, palmitic acid, arachidonic acid, tripalmitin, tristearin, triolein, trilinolein, trilinolenin, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin. The standard curves obtained for each individual cholesteryl ester were essentially identical, as were the standard curves for each individual triglyceride and free fatty acid. Hence, a single standard curve could be used for all cholesteryl ester peaks, a single standard curve for all triglyceride peaks, and a single standard curve for all free fatty acids. The lower detection limits for these

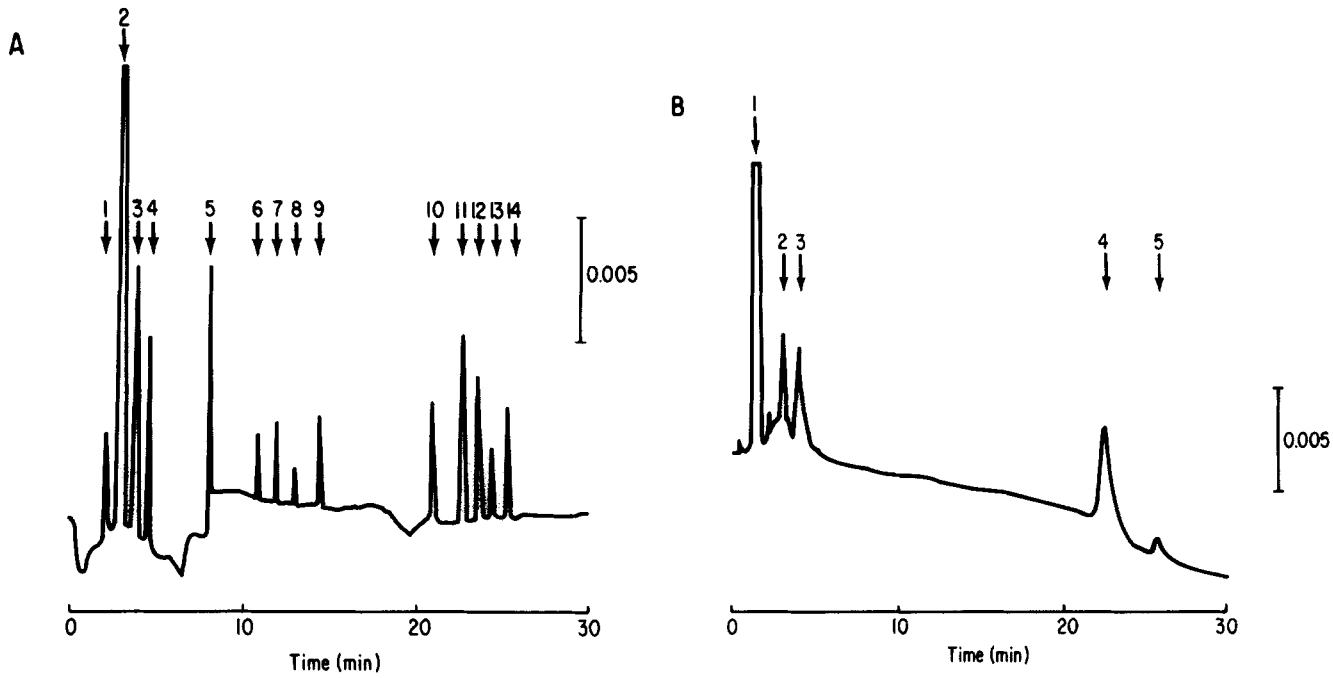

Fig. 1. High-performance liquid chromatograms of a lipid extract prepared from stellate cell lipid droplets isolated from a control rat liver. Panel A gives the chromatogram of the neutral lipids present in the stellate cell lipid droplets. Chromatography conditions were as described in Experimental Procedures. The eluting lipids were identified by comparison of the observed retention times with those of pure authentic neutral lipid standards. This comparison identified the HPLC peaks shown as follows: peak 1, cholesterol; peak 2, retinyl esters; peak 3, a mixture of cholesteryl myristate, palmitate, and stearate; peak 4, a mixture of cholesteryl oleate and linoleate; peak 5, solvent; peaks 6 through 9, free fatty acids (authentic standards eluted as follows: 6, myristic acid; 7, palmitic acid and oleic acid; 8, linoleic acid; 9, arachidonic acid); and peaks 10 through 14, triglycerides (authentic standards eluted as follows: 10, tripalmitin; 11, tristearin; 12, triolein; 13, trilinolein; 14, trilinolenin). Each of the chromatographic peaks eluting with retention times similar to the single fatty acid triglycerides was collected and confirmed by enzymatic analysis to be a species of triglyceride (see Experimental Procedures). The vertical bar at the left margin indicates 0.005 absorbance units. Panel B shows a chromatogram of the phospholipid present in the stellate cell lipid droplets. Chromatography conditions were as described in Experimental Procedures. The eluting phospholipids consisted of: peak 1, combined neutral lipid; peak 2, lysophosphatidylcholine; peak 3, phosphatidylethanolamine; peak 4, phosphatidylcholine; and peak 5, sphingomyelin. The vertical bar at the left margin indicates 0.005 absorbance units.

standard curves (and hence each individual assay) were 0.2 μg cholesterol, 0.6 μg cholesteryl ester, 0.1 μg free fatty acid, 0.4 μg triglyceride, and 0.2 μg phospholipid.

The total lipid masses of the isolated stellate cell lipid droplet preparations were calculated from measurements of the dry weight of the lipid droplet preparations. For this purpose, aliquots of each lipid droplet preparation were dried in a desiccator and weighed on a Cahn Gram Electobalance (Ventron Corp., Paramount CA). Each sample was repeatedly desiccated and weighed a total of six times and the dry weight of the lipid droplet preparation was taken as the mean of these determinations. The levels of the retinoid and nonretinoid lipids determined by HPLC are presented in the Results section as μg lipid per mg dry weight of lipid droplet.

Quantitation of retinoids in isolated cell fractions

The retinoid contents of the total liver digests, the isolated cell fractions, and the isolated lipid droplet preparations were determined by an HPLC procedure similar to that described by Amedee-Manesme, Furr, and Olson (22). Briefly, samples were ground with anhydrous Na_2SO_4 and retinoids were extracted with methylene chloride. Levels of retinol and individual retinyl esters (palmitate + oleate, stearate, linoleate, and myristate) were determined employing authentic standards of each ester and retinyl acetate as the internal standard.

Radioimmunoassays of RBP, CRBP, and CRABP

RBP was determined by radioimmunoassay as described previously (23). CRBP and CRABP contents in the isolated cell fractions were also measured by sensitive and specific radioimmunoassays, as has been described in detail elsewhere (24). CRBP and CRABP show no cross-reactivity for each other in these specific radioimmunoassays.

Other assays

Retinyl palmitate hydrolase activity was assayed according to the procedure of Prystowsky, Smith, and

Goodman (25). Protein was estimated using the method of Lowry et al. (26). Serum cholesterol and triglyceride concentrations were determined by automated enzymatic methods (ABA 100, Abbott Laboratories, Chicago, IL). Serum retinol concentration was determined by HPLC according to the procedure of Bieri, Tolliver, and Catignani (27).

Statistical methods

To test for the existence of diet-dependent differences in measured lipid levels in the total liver digest, in the isolated cell fractions, or in the isolated lipid droplet preparations, a one-way analysis of variance was performed for the level of each lipid determined to be present in each diet group for each of these types of preparations (28). Thus, for each of the tissue, cellular, or subcellular fractions examined, the measured levels of each different lipid in each of the five experimental groups were compared by analysis of variance. An overall diet effect was declared significant when P was found to be less than 0.01 (1% level of significance). When a statistically significant effect was found for a given lipid and preparation, then the mean level of that particular lipid of each diet group was compared by Dunnett's t-test with the mean lipid level measured in preparations from rats fed the control diet.

All lipid concentrations are given as the mean ± SD.

RESULTS

Nutritional status of experimental animals

The five experimental diets used in this study differed considerably with regard to the amount of retinoid or triglyceride that they provided to the rats in the different diet groups. The nutritional status of these rats was assessed by monitoring their growth rates and by measuring their serum and liver levels of retinol, cholesterol, and triglycerides at the time the rats were killed. The control diet, containing 2.4 mg retinol/kg diet and supplying 20.5% of the total energy as triglyceride, was sufficient to maintain the rats in normal nutritional status. **Table 1** and **Table 2** show, respectively, the serum and liver lipid levels of the rats in each of the five diet groups. Rats maintained on the low retinol diet had significantly lower hepatic total retinol levels than animals maintained on the control diet (Table 2). The high retinol diet group, which was provided 10-fold the amount of retinol necessary to maintain normal vitamin A status, was found to have significantly elevated hepatic total retinol levels over the control diet group (Table 2). These rats showed no clinical signs of vitamin A toxicity (i.e., weight loss, depilation, lethargy, decline of self-care) and had normal serum retinol levels (Table 1) without retinyl esters being observed in the serum. The low and high triglyceride diets markedly and directly affected the hepatic levels of neutral lipids, particularly triglycerides, in the animals receiving these diets (Table 2).

Fig. 2 shows the mean growth curves for the rats in each of the dietary groups. No significant differences were found between the body weights of the rats maintained on the control diet and those maintained on any of the other four diets. Over the entire duration of the experiment, the control rats consumed 520 ± 26 g diet/rat, the low retinol diet group 520 ± 26 g diet/rat, the high retinol diet group 525 ± 23 g diet/rat, the low triglyceride group 512 ± 22, and the high triglyceride group 525 ± 28 g diet/rat.

Characteristics of the isolated cell fractions

Nonparenchymal cells were isolated using a modification (17) of the procedure developed by Hendriks et al. (12) (see Experimental Procedures). The yields and purities of the isolated stellate cell preparations are given in **Table 3.** The stellate cell preparations usually contained some contaminating Kupffer and endothelial cells; however, parenchymal cell contamination of the stellate cell preparations was always less than 1%. For the control rats, the estimated recovery of stellate cells from the whole liver was 10.2%, assuming that the liver comprised 4% of the total body weight of the animal (29) and contained 16 × 10^6 stellate

TABLE 1. Serum levels of retinol, triglyceride, total cholesterol, and protein

Diet Group	(n)	Retinol	Triglyceride	Total Cholesterol	Protein
		μg/dl	*mg/dl*		*g/dl*
Control	6	57.2 ± 7.1	53.0 ± 5.6	87.5 ± 10.9	6.25 ± 0.45
Low retinol	14	52.6 ± 9.6	59.1 ± 9.5	84.9 ± 19.1	6.58 ± 0.55
High retinol	6	57.9 ± 11.3	58.0 ± 8.5	95.8 ± 16.4	6.21 ± 0.48
Low triglyceride	6	60.1 ± 16.3	43.2 ± 6.6	84.4 ± 23.3	6.45 ± 0.50
High triglyceride	5	60.3 ± 14.5	68.7 ± 8.8	88.4 ± 15.1	6.39 ± 0.47

TABLE 2. Lipid composition of the total liver digest

Diet Group	(n)	Retinoid[a]	Triglyceride	Cholesterol Ester	Cholesterol	Free Fatty Acid	Phospholipids	Total Lipid
					mg/g liver			
Control	6	0.7 ± 0.1	13.2 ± 2.4	0.6 ± 0.1	1.9 ± 0.2	0.4 ± 0.1	25.5 ± 3.9	42.2 ± 4.8
Low retinol	14	0.2 ± 0.1[b]	13.3 ± 2.0	0.6 ± 0.1	1.8 ± 0.3	0.4 ± 0.1	24.0 ± 3.9	40.1 ± 5.4
High retinol	6	6.1 ± 0.4[b]	14.1 ± 2.6	0.8 ± 0.2	1.9 ± 0.4	0.4 ± 0.1	24.2 ± 4.5	47.8 ± 4.7
Low triglyceride	6	0.6 ± 0.1	6.3 ± 1.8[b]	0.2 ± 0.1[b]	1.5 ± 0.4	0.3 ± 0.1	23.8 ± 4.9	32.8 ± 4.2[b]
High triglyceride	5	0.6 ± 0.1	28.6 ± 2.7[b]	3.0 ± 1.0[b]	1.7 ± 0.6	0.8 ± 0.3[b]	24.4 ± 3.9	59.1 ± 7.3[b]

[a]Retinol + retinyl esters.

[b]Statistically different from the control values ($P < 0.01$). The measured lipid levels in each of the experimental groups were compared by analysis of variance, and an overall diet effect was declared significant when P was found to be less than 0.01. When a statistically significant diet effect was found, the mean lipid level of each diet group was contrasted by Dunnett's t-test with the mean control lipid level.

cells/g liver (12, 14). The number of stellate cells isolated from rats fed the low retinol, high retinol, low triglyceride, and high triglyceride diets were not significantly different at the $P < 0.01$ level from the number of cells obtained from the rats fed the control diet. However, the number of stellate cells isolated from rats fed the low retinol diet was significantly lower ($P < 0.01$) than the number of stellate cells obtained from the livers of rats fed the high retinol diet. It should be noted that the procedure used to isolate stellate cells is in part dependent on the lipid content of these cells. As demonstrated below, the lipid content of the stellate cells is strongly determined by the retinol content of the diet. Hence, it is not surprising that more cells were isolated from livers of rats receiving 40-fold higher levels of retinol in the diet.

A population of cells enriched in Kupffer and endothelial cells (called Kupffer-endothelial cell fraction) was also collected from the two-layer Nycodenz gradient used for stellate cell isolation. The characteristics of this cell fraction are summarized in **Table 4;** this fraction was found to contain a small number of stellate cells but was not observed to contain parenchymal cells. No statistically significant diet-dependent differences were observed for the yields of Kupffer and endothelial cells or for the relative cellular composition of this cell fraction.

Stellate cells isolated from rats maintained on the control diet were found to contain 155 ± 13 μg protein/10^6 cells and the Kupffer-endothelial cell fraction contained 107 ± 20 μg protein/10^6 cells. Stellate cell fractions and the Kupffer-endothelial cell fractions isolated from rats maintained on the other four experimental diets showed no significant differences in protein content from those observed for the control diet.

Lipid concentrations in the total liver digest and in isolated nonparenchymal hepatic cells

As shown in Table 2, significantly decreased hepatic retinoid levels were observed in the rats fed the low retinol diet and significantly elevated levels were observed in the rats fed the high retinol diet. However, neither the low nor the high triglyceride diets had any effect on the retinoid concentrations measured in the liver digest. Triglyceride concentrations in the liver digest were significantly elevated in the rats maintained on the high triglyceride diet and significantly reduced in the rats maintained on the low triglyceride diet. Similar effects of these latter two diets were also observed on the cholesteryl ester concentrations in the whole liver. Thus, cholesteryl ester levels were significantly elevated in the livers of animals fed the high triglyceride diet and significantly decreased in the livers of animals fed the low triglyceride diet. In addition, significantly elevated levels of free fatty acids were found in the whole liver of the rats fed the high fat diet. Total lipid levels in the whole liver and the levels of neutral lipids (other than retinoids) were not altered by either the high or low retinol diets.

Table 5 shows the lipid composition of isolated stellate cells in terms of μg lipid per 10^6 cells and **Table 6** gives the relative distribution of the total lipids recovered from the isolated stellate cells. Stellate cell retinoid levels were markedly influenced by dietary retinoid intake. Stellate cells isolated from animals fed the

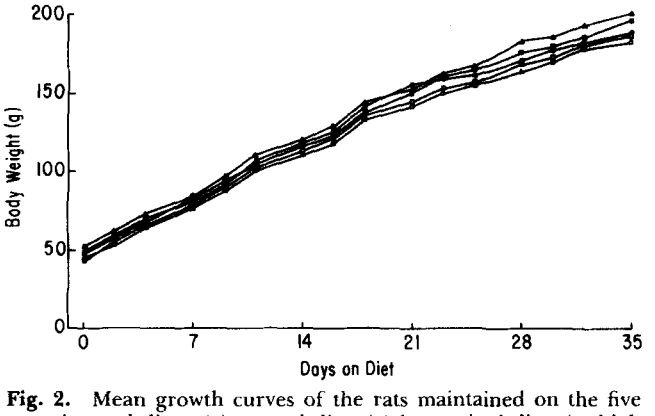

Fig. 2. Mean growth curves of the rats maintained on the five experimental diets: (○) control diet; (□) low retinol diet; (■) high retinol diet; (△) low triglyceride diet; and (▲) high triglyceride diet.

TABLE 3. Yield and purity of the isolated stellate cell preparations

Diet Group	(n)	Yield[a]	Purity
		no. cells × 10^{-6}	%
Control	6	13.4 ± 2.9	90.0 ± 3.8
Low retinol	14	8.1 ± 3.7	85.5 ± 8.0
High retinol	6	17.0 ± 3.1	85.6 ± 3.7
Low triglyceride	6	12.5 ± 4.0	93.1 ± 6.1
High triglyceride	5	14.8 ± 2.5	88.0 ± 4.9

[a]The yield represents the actual number of stellate cells isolated from each of the livers.

low retinoid diet had significantly lower retinoid levels than those observed for the control group. For the rats fed the low retinol diet, a significant reduction in stellate cell free fatty acid concentration also was observed, but other lipid levels were not significantly changed from control levels. In these rats, the stellate cell retinoid content represented a significantly smaller percentage of the total stellate cell lipid than was observed in the control rats. Stellate cells isolated from rats fed the high retinol diet had significantly higher retinoid levels than those isolated from animals fed the control diet. In addition, in the rats maintained on the high retinol diet, the isolated stellate cells contained concentrations of triglyceride, cholesteryl ester, and free fatty acid that were significantly higher than those observed for control animals. The mean retinoid level in stellate cells isolated from rats fed the high retinol diet was 10.8-fold greater than the mean retinoid level of the control group. Similarly the mean triglyceride, cholesteryl ester, and free fatty acid levels for the high retinol stellate cells were elevated 3.7-, 3.3-, and 3.8-fold, respectively, over the mean control levels. Since cellular retinoid levels were increased more extensively in this group than were the levels of the other lipids, retinoids comprised 63% of the total lipid of the stellate cells and the relative contributions of the other lipids to the stellate cell total lipid mass were decreased.

Like the liver digest, stellate cell retinoid concentrations were not affected by changes in triglyceride intake. However, unlike the diet effects observed in the total liver digest, neither high nor low triglyceride diets had any effect on the neutral lipid concentrations present in stellate cells.

The lipid composition of the Kupffer-endothelial cell fraction obtained from rats fed the control diet (**Table 7**) was very different from that determined for stellate cells isolated from rats fed the control diet. The total lipid mass present in the Kupffer-endothelial cell fraction was substantially less than that measured in the isolated stellate cells. In addition, the Kupffer-endothelial cell fraction retinoid levels were quite low compared to stellate cell levels (and it is very probable that the retinoid present in this fraction arose mainly from stellate cells present in the Kupffer-endothelial cell fraction (see Table 4) (14)). A significant effect of dietary retinoid was observed on the total retinoid levels of the Kupffer-endothelial cell fraction isolated from rats maintained on the high retinol diet but this effect can be explained by the stellate cell contamination of this fraction. In contrast to the stellate cells, dietary triglyceride significantly affected the lipid composition of the Kupffer-endothelial cell fraction. The triglyceride levels in the Kupffer-endothelial cell fractions were significantly reduced in rats maintained on the low triglyceride diet and significantly elevated in the rats maintained on the high triglyceride diet. Additionally, the Kupffer-endothelial cell fractions obtained from rats fed the high triglyceride diet also were found to contain sig-

TABLE 4. Characteristics of the isolated Kupffer-endothelial cell fractions

Diet Group	(n)	Yield[a]	Cellular Composition			Estimated Recovery	
			Kupffer Cells	Endothelial Cells	Stellate Cells	Kupffer Cells	Endothelial Cells
		no. cells × 10^{-6}	%			%	
Control	6	31.1 ± 4.1	30.9 ± 2.1	64.3 ± 1.5	3.9 ± 0.7	15.1 ± 2.4	14.8 ± 1.6
Low retinol	14	30.7 ± 14.3	32.1 ± 3.2	62.7 ± 1.9	2.6 ± 1.3	14.4 ± 6.7	13.5 ± 6.8
High retinol	6	31.5 ± 4.0	31.3 ± 3.4	62.8 ± 2.2	3.7 ± 1.9	15.5 ± 3.3	14.6 ± 2.2
Low triglyceride	6	34.5 ± 2.7	33.2 ± 1.3	62.9 ± 1.6	2.2 ± 1.0	18.0 ± 2.6	16.1 ± 2.6
High triglyceride	5	36.4 ± 9.0	32.1 ± 2.3	61.7 ± 7.9	1.9 ± 0.6	16.9 ± 4.9	15.5 ± 4.7

[a]The yield represents the total number of cells (Kupffer + endothelial + stellate) isolated from each of the livers in this cell fraction.

TABLE 5. Lipid composition of isolated stellate cells

Diet Group	(n)	Retinoid[a]	Triglyceride	Cholesteryl Ester	Cholesterol	Free Fatty Acid	Phospholipid	Total Lipid
				μg/10⁶ cells				
Control	6	33.1 ± 6.3	27.5 ± 5.6	16.6 ± 2.7	14.7 ± 1.5	2.5 ± 0.2	19.7 ± 3.1	114.1 ± 9.0
Low retinol	14	6.6 ± 3.7[b]	24.6 ± 3.8	15.2 ± 4.4	15.0 ± 2.6	1.6 ± 0.7[b]	20.1 ± 2.2	83.1 ± 7.5[b]
High retinol	6	358.6 ± 26.7[b]	102.7 ± 19.3[b]	54.6 ± 22.2[b]	18.8 ± 3.8	9.6 ± 4.5[b]	24.0 ± 3.5	568.3 ± 52.4[b]
Low triglyceride	6	31.0 ± 3.0	25.1 ± 4.7	18.3 ± 4.0	14.6 ± 2.0	2.3 ± 0.4	21.6 ± 4.2	112.9 ± 6.2
High triglyceride	5	30.1 ± 9.0	29.3 ± 4.8	17.5 ± 5.0	14.1 ± 1.5	2.6 ± 0.5	19.2 ± 3.1	112.8 ± 7.6

[a]Retinol + retinyl ester.
[b]See footnote *b* of Table 2.

nificantly elevated levels of free fatty acid and of total lipid.

None of the experimental diets showed any significant effect on the concentration of phospholipids for either the whole liver or isolated cell preparations.

Lipid composition of lipid droplets derived from stellate cells

Table 8 gives the lipid composition of lipid droplets that were derived from the isolated stellate cell preparations. The lipid mass is expressed as μg lipid per mg dry weight of lipid droplets (see Experimental Procedures for details). Since proteins and other nonlipid materials are present in the lipid droplet preparations, the lipid mass of the lipid droplets was always less than 100% of the lipid droplet dry weight. In the rats fed the control diet, the lipid composition of the lipid droplets consisted of 60.5% nonretinoid lipid and 39.5% retinoid. The nonretinoid lipid consisted of 58.6% triglycerides, 28.4% cholesteryl ester, 8.7% cholesterol, and 4.4% free fatty acid. The phospholipids consisted of 50% phosphatidyl choline, 22% phosphatidylethanolamine, 20% lysophosphatidylcholine, and 8% sphingomyelin.

The effects of dietary retinoid on the lipid composition of the isolated lipid droplets were marked. For the lipid droplets derived from stellate cells isolated from animals maintained on the low retinol diet, a significant reduction in absolute and relative retinoid content and a relative elevation of neutral lipids were observed. Lipid droplets isolated from the low retinol diet group showed a significant relative increase (in terms of percent of total lipid represented by each lipid) in triglyceride, cholesteryl ester, cholesterol, and free fatty acid levels over control levels. The high retinol diet had the opposite effects; this diet resulted in a significant absolute and relative elevation in retinoid content and a relative reduction of neutral lipid and phospholipid content in the lipid droplets. The effects of dietary retinoid intake on stellate cell lipid droplet retinoid levels directly paralleled the effects on whole stellate cell retinoid levels.

The high triglyceride and low triglyceride diets did not alter the lipid composition of the stellate cell lipid droplets.

The phospholipid distributions determined for the isolated stellate cell lipid droplets were identical for each of the five diet groups.

Stellate cell levels of RBP, CRBP, CRABP, and retinyl palmitate hydrolase activity

The levels of RBP, CRBP, CRABP, and of retinyl palmitate hydrolase activity measured in the different stellate cell preparations are given in **Table 9.** No significant differences for any of these parameters were observed in any of the five different dietary groups. The levels of these three retinoid-binding proteins and of this retinoid-metabolizing enzyme were in excellent agreement with levels previously reported to

TABLE 6. Relative distribution of total recovered lipids in isolated stellate cells

		Lipid Mass		Mean Relative Distribution					
Diet Group	(n)	Retinoid[a]	Nonretinoid	Retinoid[a]	Triglyceride	Cholesteryl Ester	Cholesterol	Free Fatty Acid	Phospholipid
		μg/10⁶ cells				%			
Control	6	33.1 ± 6.3	81.0 ± 5.5	29.0	24.1	14.5	12.9	2.2	17.3
Low retinol	14	6.6 ± 3.7[b]	76.5 ± 5.4	7.9[b]	29.6	18.3	18.1	1.9	24.2
High retinol	6	358.6 ± 26.7[b]	209.7 ± 37.8[b]	63.1[b]	18.1	9.6	3.3[b]	1.7	4.2[b]
Low triglyceride	6	31.0 ± 3.0	81.9 ± 5.1	27.4	22.2	16.2	13.1	2.0	19.1
High triglyceride	5	30.1 ± 9.0	82.7 ± 6.1	26.7	26.0	15.5	12.5	2.3	17.0

[a]Retinol + retinyl esters.
[b]See footnote *b* of Table 2.

TABLE 7. Lipid composition of Kupffer-endothelial cell fraction

Diet Group	(n)	Retinoid[a]	Triglyceride	Cholesteryl Ester	Cholesterol	Free Fatty Acid	Phospholipids	Total Lipid
				μg/10⁶ cells				
Control	6	1.2 ± 0.5	5.2 ± 2.2	1.9 ± 0.9	6.0 ± 1.2	0.2 ± 0.2	18.8 ± 4.4	33.2 ± 9.1
Low retinol	14	0.8 ± 0.4	5.0 ± 1.9	2.1 ± 1.0	6.2 ± 1.2	0.2 ± 0.1	19.4 ± 2.3	33.6 ± 8.0
High retinol	6	2.2 ± 0.6[b]	5.0 ± 2.0	2.1 ± 0.9	6.3 ± 1.3	0.2 ± 0.2	20.5 ± 4.1	36.2 ± 10.3
Low triglyceride	6	1.1 ± 0.3	3.4 ± 1.2[b]	1.4 ± 0.8	5.3 ± 1.7	0.2 ± 0.2	17.9 ± 2.6	29.3 ± 5.9
High triglyceride	5	1.0 ± 0.4	17.3 ± 7.2[b]	2.3 ± 1.4	6.5 ± 1.6	1.6 ± 1.2[b]	20.2 ± 4.6	48.9 ± 7.7[b]

[a]Retinol + retinyl esters.
[b]See footnote *b* of Table 2.

be present in stellate cells isolated from control animals (13, 14, 17).

DISCUSSION

The studies reported here provide detailed and quantitative information about the effects of selected dietary perturbations on the content and composition of the lipids and lipid droplets of rat liver stellate cells. This work greatly extends the limited information recently reported, from our laboratory (17) and by others (18), about the chemical composition of stellate cell lipid droplets from normal rats. The results reported here demonstrate and define the dramatic changes in the content and composition of the stellate cell lipids and lipid droplets that occur in response to changes in dietary retinol intake. In contrast, major changes in dietary triglyceride intake had no effect on stellate cell lipid droplet content and composition. Quite opposite effects, from each of these two kinds of dietary perturbations, were seen on the lipid content and composition of parenchymal cells, as reflected in the analyses of total liver digests. It is clear that very different mechanisms regulate the lipid content and composition of stellate as compared to parenchymal cells.

The present study was carried out using highly purified stellate cells which were isolated from the livers of rats kept on one of five different diets. The purities and recoveries of stellate cells from rats maintained on each of the dietary regimens were very good, and compared favorably with the purities and recoveries of stellate cells reported in other studies (12, 13, 17, 30, 31). As judged by their levels of RBP, CRBP, CRABP, and retinyl palmitate hydrolase activity, the stellate cells used in these studies had characteristics similar to those of stellate cells isolated in other laboratories (13, 14). It is of interest that the stellate cell levels of the three retinoid-binding proteins and of retinyl palmitate hydrolase activity were similar in rats from each of the five different dietary treatments.

For rats maintained on the control diet, the lipid compositions found in the isolated stellate cells and in stellate cell lipid droplets were, in general, in agreement with the recently reported lipid compositions of stellate cells (18) and their lipid droplets (17, 18). Thus, in the control rats, retinoids (mainly retinyl esters) and triglycerides were the two major stellate cell lipids, and together comprised approximately 70% of the lipids of the isolated lipid droplets. For rats fed the high retinol diet, a marked increase in retinyl ester content was found in the isolated stellate cells and in their lipid droplets. Thus, in these rats, stellate cell retinoid content increased more than 10-fold (compared to controls), and the retinoid content of the lipid droplets increased from 39.5% to 65.4%. In addition, the increase in retinyl ester (retinoid) content in the high

TABLE 8. Relative distribution of total recovered lipid in stellate cell lipid droplets

Diet Group	(n)	Lipid Mass		Mean Relative Distribution					
		Retinoid[a]	Nonretinoid	Retinoid[a]	Triglyceride	Cholesteryl Ester	Cholesterol	Free Fatty Acid	Phospholipid
		μg/mg dry weight				%			
Control	6	318 ± 40	486 ± 40	39.5	31.7	15.4	4.7	2.4	6.3
Low retinol	14	101 ± 35[b]	679 ± 60[b]	12.9[b]	39.9[b]	27.0[b]	9.6[b]	4.3[b]	6.3
High retinol	6	527 ± 60[b]	345 ± 32[b]	65.4[b]	23.5	6.6[b]	1.0[b]	2.3	1.2[b]
Low triglyceride	6	284 ± 59	498 ± 38	36.3	33.1	17.8	4.9	2.4	5.4
High triglyceride	5	274 ± 50	515 ± 48	34.7	33.5	17.8	5.2	3.2	5.7

[a]Retinol + retinyl ester.
[b]See footnote *b* of Table 2.

TABLE 9. Stellate cell levels of RBP, CRBP, CRABP and retinyl palmitate hydrolase activity

Diet Group	(n)	RBP	CRBP	CRABP	Retinyl Palmitate Hydrolase Activity
		ng/10⁶ cells			*pmol FFA/hr per 10⁶ cells*
Control	6	27.9 ± 8.4	191 ± 40	18 ± 4	1672 ± 602
Low retinol	14	23.8 ± 10.0	192 ± 44	19 ± 9	1588 ± 449
High retinol	6	28.1 ± 7.6	190 ± 72	16 ± 5	1591 ± 917
Low triglyceride	6	24.2 ± 5.4	172 ± 21	16 ± 5	1455 ± 361
High triglyceride	5	28.4 ± 12.7	192 ± 13	19 ± 4	1651 ± 459

retinol diet group was accompanied by a substantial elevation in stellate cell triglyceride, cholesteryl ester, and free fatty acid levels. Overall, the total lipid content of the stellate cells was increased 5-fold in rats on the high retinol diet compared to the control diet. Opposite changes were seen in stellate cell lipids and lipid droplets in rats fed the low retinol diet. Thus, in these rats, statistically significantly lower levels of both retinoid and total lipid were observed, as compared to control rats. These quantitative changes in stellate cell retinoid and total lipid levels, resulting from changes in dietary retinol intake, are consistent with previous qualitative observations that the number and size of stellate cell lipid droplets are strongly influenced by retinol intake (5).

Significant changes in total lipid content or in lipid composition (except for retinoid levels), resulting from changes in dietary retinol intake, were not observed in either the total liver digests or in the Kupffer-endothelial cell fractions. It should be noted that these analyses of the total liver digest mainly reflect the composition of the parenchymal cells, since parenchymal cells comprise approximately 66% of the total cells present (12) and account for an even much larger majority of the cellular volume (32) of the liver. Taken together, our results demonstrate that changes in dietary retinol intake markedly affect stellate cell lipid levels but have very little or no effect on lipid levels in the other hepatic cell types. With regard to stellate cells, however, dietary retinol intake not only influences the level of retinoids in the stellate cells, but markedly influences the levels of several other kinds of lipids in these cells, and also the lipid composition of the stellate lipid droplets.

The high and low triglyceride diets strongly affected the concentrations of triglyceride, cholesteryl ester, and total lipid present in the total liver digest, presumably mainly reflecting the lipid content and composition of the parenchymal cells. These two diets also affected the triglyceride levels in the Kupffer-endothelial cell fractions. However, the high and low triglyceride diets had no effects on the lipid levels or composition of the stellate cells. Changes in dietary triglyceride intake had no effect on the retinoid levels in the total liver or in any of the different liver cell types. Thus, although the lipid levels of the total liver, and of parenchymal cells (by inference) and of Kupffer-endothelial cells, were strikingly influenced by dietary triglyceride intake, stellate cell lipid levels were not altered by large changes in dietary triglyceride intake.

It is now well established that chylomicron retinol (present mainly as retinyl esters) is taken up by the liver parenchymal cells and then transferred to the stellate cells for storage (8, 10, 11). The mechanisms responsible for this transfer process are still undefined. Studies from our laboratory indicate that chylomicron retinyl esters are not transferred intact from parenchymal to stellate cells without first undergoing hydrolysis (11). It has been suggested that RBP may be involved in the exchange of retinol from parenchymal to stellate cells (33, 34). This suggestion is not supported by the finding that RBP is synthesized in parenchymal and not in stellate cells (17) nor by the fact that the secretion of RBP by parenchymal cells is inhibited when the cells are depleted of retinol (35–37). This later finding suggests that (at least during periods of low vitamin A intake) retinol must be transferred from stellate to parenchymal cells in order to maintain the normal rates of RBP secretion by parenchymal cells and, hence, of retinol mobilization from the liver. Various possible mechanisms involved in the intercellular transfer of retinol between parenchymal and stellate cells have been discussed recently (11, 38). More research is clearly needed to address this important issue. Information is also needed about the question of whether the mechanism(s) responsible for the intercellular transfer of retinol between parenchymal and stellate cells are in some way linked to the mechanisms that regulate the content and the composition of the stellate cell lipid droplets.

What is the physiological significance of the present finding that dietary retinol intake markedly and specifically influences the content and composition of the lipid droplets in the stellate cells? It is tempting to speculate that these observations might reflect the mechanisms whereby retinyl esters are deposited (and stored) in, or mobilized from, the stellate cell lipid droplets. Thus, retinyl ester incorporation into or

mobilization from the lipid droplets might occur not as a movement of retinoid alone, but rather through the movement of a mixture of lipids, as a lipoprotein particle of some sort. The previously described retinyl ester-rich lipid-protein aggregate (39) might represent such a lipoprotein particle, but other kinds of lipoprotein particles are also feasible. It is possible that the entire mixture of core lipids present in such a lipoprotein particle might be deposited in, or removed from, the stellate cell lipid droplets. From the results reported here, one might anticipate that the chemical composition of such a lipoprotein particle could vary with retinoid intake, and that the metabolism and movement of such a lipoprotein particle in the stellate cell would specifically be regulated by retinol nutritional status. Regardless of whether or not this hypothesis is correct, further research on the factors and mechanisms that regulate the content and composition of the stellate cell lipid droplets should help to provide insights into the mechanisms that control retinyl ester storage in and mobilization from the stellate cells and the liver.

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REFERENCES

1. Goodman, D. S., H. S. Huang, and T. Shiratori. 1965. Tissue distribution and metabolism of newly absorbed vitamin A in the rat. *J. Lipid Res.* **6:** 390–396.
2. Lawrence, C. W., F. D. Crain, F. J. Lotspeich, and R. F. Krause. 1966. Absorption, transport, and storage of retinyl-15-^{14}C palmitate-9,10-^{3}H in the rat. *J. Lipid Res.* **7:** 226–229.
3. Goodman, D. S., and W. S. Blaner. 1984. Biosynthesis, absorption, and hepatic metabolism of retinol. *In* The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, Inc., Orlando, FL. 1–39.
4. Zachman, R. D., and J. A. Olson. 1965. Uptake and metabolism of retinol (vitamin A) in the isolated perfused rat liver. *J. Lipid Res.* **6:** 27–32.
5. Wake, K. 1980. Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their relative structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs. *Int. Rev. Cytol.* **66:** 303–353.
6. Kanai, M., A. Raz, and D. S. Goodman. 1968. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J. Clin. Invest.* **47:** 2025–2044.
7. Goodman, D. S. 1984. Plasma retinol-binding protein. *In* The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, Inc., Orlando, FL. 41–88.
8. Blomhoff, R., P. Helgerud, M. Rassmussen, T. Berg, and K. R. Norum. 1982. In vivo uptake of chylomicron [^{3}H]retinyl ester by rat liver: evidence for retinol transfer from parenchymal to nonparenchymal cells. *Proc. Natl. Acad. Sci. USA.* **79:** 7326–7330.
9. Dixon, J. L., and D. S. Goodman. 1987. Effects of nutritional and hormonal factors on the metabolism of retinol-binding protein by primary cultures of rat hepatocytes. *J. Cell. Physiol.* **130:** 6–13.
10. Blomhoff, R., K. Holte, L. Naess, and T. Berg. 1984. Newly administered [^{3}H]retinol is transferred from hepatocytes to stellate cells in liver for storage. *Exp. Cell Res.* **150:** 186–193.
11. Blaner, W. S., J. L. Dixon, H. Moriwaki, R. A. Martino, O. Stein, Y. Stein, and D. S. Goodman. 1987. Studies on the in vivo transfer of retinoids from parenchymal to stellate cells in rat liver. *Eur. J. Biochem.* **164:** 301–307.
12. Hendriks, H. F. J., W. A. M. M. Verhoofstad, A. Brouwer, A. M. de Leeuw, and D. L. Knook. 1985. Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver. *Exp. Cell Res.* **160:** 138–149.
13. Blomhoff, R., M. Rasmussen, A. Nilsson, K. R. Norum, T. Berg, W. S. Blaner, M. Kato, J. R. Mertz, D. S. Goodman, U. Eriksson, and P. A. Peterson. 1985. Hepatic retinoid metabolism: distribution of retinoids, enzymes and binding proteins in isolated rat liver cells. *J. Biol. Chem.* **260:** 13560–13565.
14. Blaner, W. S., H. F. J. Hendriks, A. Brouwer, A. M. de Leeuw, D. L. Knook, and D. S. Goodman. 1985. Retinoids, retinoid-binding proteins, and retinyl palmitate hydrolase distributions in different types of rat liver cells. *J. Lipid Res.* **26:** 1241–1251.
15. Batres, R. O., and J. A. Olson. 1987. Relative amount and ester composition of vitamin A in rat hepatocytes as a function of the method of cell preparation and of total liver stores. *J. Nutr.* **117:** 77–82.
16. Kato, M., K. Kato, and D. S. Goodman. 1984. Immunocytochemical studies on the localization of plasma and of cellular retinol-binding proteins and of transthyretin (prealbumin) in the rat liver and kidney. *J. Cell. Biol.* **98:** 1698–1704.
17. Yamada, M., W. S. Blaner, D. R. Soprano, J. L. Dixon, H. M. Kjeldbye, and D. S. Goodman. 1987. Biochemical characteristics of isolated rat liver stellate cells. *Hepatology.* **7:** 1224–1229.
18. Hendriks, H. F. J., P. J. A. M. Brekelmans, R. Buytenhek, A. Brouwer, A. M. de Leeuw, and D. L. Knook. 1987. Liver parenchymal cells differ from the fat-storing cells in their lipid composition. *Lipids.* **22:** 266–273.
19. Blaner, W. S., J. E. Smith, R. B. Dell, and D. S. Goodman. 1985. Spatial distribution of retinol-binding protein and retinyl palmitate hydrolase activity in normal and vitamin A-deficient rat liver. *J. Nutr.* **115:** 856–864.
20. Folch, J., M. Lees, and G. H. Sloane Stanley. 1956. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
21. Schlager, S. I., and H. Jordi. 1981. Separation of cellular phospholipid, neutral lipid and cholesterol by high-pressure liquid chromatography. *Biochim. Biophys. Acta.* **665:** 355–358.
22. Amedee-Manesme, O., H. C. Furr, and J. A. Olson. 1984. The correlation between liver vitamin A concentrations in micro- (needle biopsy) and macrosamples of human liver specimens obtained at autopsy. *Am. J. Clin Nutr.* **39:** 315–319.
23. Smith, J. E., D. D. Deen, D. Sklan, and D. S. Goodman.

1980. Colchicine inhibition of retinol-binding protein secretion by rat liver. *J. Lipid Res.* **21:** 229–237.
24. Kato, M., W. S. Blaner, J. R. Mertz, K. Das, K. Kato, and D. S. Goodman. 1985. Influence of retinoid nutritional status on cellular retinol- and cellular retinoic acid-binding protein concentrations in various rat tissues. *J. Biol. Chem.* **260:** 4832–4838.
25. Prystowsky, J. H., J. E. Smith, and D. S. Goodman. 1981. Retinyl palmitate hydrolase activity in normal rat liver. *J. Biol. Chem.* **256:** 4498–4503.
26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.* **193:** 265–275.
27. Bieri, J. C., T. J. Tolliver, and G. L. Catignani. 1979. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin Nutr.* **32:** 2500–2507.
28. Steel, R. G. D., and J. H. Torrie. 1960. Principals and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York, NY. 144–160.
29. Caster, W. O., J. Poncelet, A. B. Simon, and W. D. Armstrong. 1956. Tissue weights of the rat. I. Normal values: determined by dissection and chemical methods. *Proc. Soc. Exp. Biol. Med.* **91:** 122–126.
30. Friedman, S. L., J. F. Roll, J. Boyles, and M. D. Bissell. 1985. Hepatic lipocytes: the principal collagen-producing cells in normal rat liver. *Proc. Natl. Acad. Sci. USA.* **82:** 8681–8685.
31. Andus, T., G. Ramadori, P. C. Heinrich, T. Knittel, and K-H. Meyer Zum Buschenfelde. 1987. Cultured Ito cells of rat liver express the α_2-macroglobulin gene. *Eur. J. Biochem.* **168:** 641–646.
32. Blouin, A., R. P. Bolender, and E. R. Weibel. 1977. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. *J. Cell Biol.* **72:** 441–455.
33. Blomhoff, R., K. R. Norum, and T. Berg. 1985. Hepatic uptake of [^{3}H]retinol bound to the serum retinol binding protein involves both parenchymal and perisinusoidal stellate cells. *J. Biol. Chem.* **260:** 13571–13575.
34. Gjøen, T., T. Bjerkelund, H. K. Blomhoff, K. R. Norum, T. Berg, and R. Blomhoff. 1987. Liver takes up retinol-binding protein from plasma. *J. Biol. Chem.* **262:** 10926–10930.
35. Smith, J. E., Y. Muto, P. O. Milch, and D. S. Goodman. 1973. The effects of chylomicron vitamin A on the metabolism of retinol-binding protein in the rat. *J. Biol. Chem.* **248:** 1544–1549.
36. Smith, J. E., C. Borek, and D. S. Goodman. 1978. Regulation of retinol-binding protein metabolism in cultured rat liver cell lines. *Cell.* **15:** 865–873.
37. Dixon, J. L., and D. S. Goodman. 1987. Studies on the metabolism of retinol-binding protein by primary hepatocytes from retinol-deficient rats. *J. Cell. Physiol.* **130:** 14–20.
38. Hendriks, H. F. J., A. Brouwer, and D. L. Knook. 1987. The role of hepatic fat-storing (stellate) cells in retinoid metabolism. *Hepatology.* **7:** 1368–1371.
39. Sklan, D., W. S. Blaner, N. Adachi, and D. S. Goodman. 1982. Association of cellular retinol-binding protein and several lipid hydrolase activities with a vitamin A-containing high-molecular-weight lipid-protein aggregate from rat liver cytosol. *Arch. Biochem. Biophys.* **214:** 35–46.