Dietary Restriction Alters Retinol and Retinol-Binding Protein Metabolism in Aging Rats

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Recent studies reported that retinoid metabolism was influenced by long-term dietary restriction (DR) in rats. Because plasma retinol was decreased in rats subjected to DR, it was thought that this dietary manipulation may have an effect on retinol-binding protein (RBP) metabolism. Thus, the aim of this study was to assess retinoids, RBP, and transthyretin (TTR) levels in plasma and liver of young (3 months), adult (12 months), and old (22 months) female Sprague-Dawley rats fed ad libitum (AL) or subjected to a 40% DR, enriched (DR+), or not (DR), with vitamins and minerals. Results indicate that hepatic total retinoid concentrations and content increased with age in all the groups. DR rats showed higher hepatic retinoid concentrations than age-matched AL and DR rats. Adult and old DR and DR+ rats exhibited significantly lower plasma RBP-retinol and higher total retinoic acid levels than corresponding controls, although these parameters were not influenced by aging. Liver RBP levels were also decreased in DR and DR+ rats when compared to respective AL controls. There was a slight age-related decline in plasma TTR levels in DR and DR+ rats which was not associated with modifications in liver TTR levels. Hepatic gene expression of RBP and TTR, as evaluated by Northern blot hybridization, did not change with age or diet, suggesting that the lower levels of plasma RBP-retinol and liver RBP in vitamin A–sufficient rats subjected to DR may reflect post-transcriptional alterations and/or accelerated degradation of hepatic RBP. The elevated plasma levels of retinoic acid may represent an adaptive mechanism developed by DR rats to maintain retinoid-dependent functions.

METHODS

Animals and Diets

This protocol was revised and approved by the Animal Care Committee of the University of Montreal in compliance with guidelines of the Canadian Council on Animal Care. Details regarding the aging rat colony have been previously published (3). Briefly, weanling 4-week-old female Sprague-Dawley rats (Charles River Canada Inc., St.-Constant, Québec, Canada) were either fed ad libitum (AL), a semisynthetic diet prepared according to the Teklad control diet #TD89248 (Teklad Test Diets, Madison, WI) or subjected to a 40% DR of the same diet. One additional group, DR+, was fed this diet enriched with vitamins and minerals so that daily intakes of micronutrients were similar to AL rats. Rats were raised until the age of 3 (young), 12 (mature), and 22 months.

After an overnight fast, rats were sacrificed between 8 and 12 AM. Under ether anesthesia, blood was drawn from rats by abdominal aorta puncture. Heparinized blood was centrifuged at 4°C, 500 g, to obtain plasma. Liver, kidneys, small intestine (postpylorus to caecum), lungs, intra-abdominal adipose tissue, and brain were quickly excised, washed in a 0.9% NaCl solution, weighed, and frozen in liquid nitrogen. Plasma and tissues were kept at -80°C and protected from light until further analysis. Rats with overt renal disease at autopsies (observed exclusively in the old AL rats) were excluded from further analysis, because these pathologies are known to interfere with retinol metabolism.

HPLC Analysis of Retinoids

All the extraction procedures were carried out under yellow light to protect retinoids from isomerization. Retinol and retinyl
esters were extracted from plasma and tissue homogenates in phosphate buffer, pH 7.4, by petroleum ether following ethanol precipitation of the proteins. These retinoids were separated and quantified by an isocratic reverse-phase HPLC system, using retinyl decanoate as the internal standard, as described elsewhere (4). Retinoids were eluted from a LC-18 column (Supelcosil #5-8298, Supelco Park, Bellefonte, PA) with a mobile phase consisting of acetonitrile:methylene chloride:methanol (70:20:10) at a flow rate of 1.7 mL/min and detected by UV absorbance at 326 nm. Retinol and different retinyl esters were identified by coelution with authentic standards, either obtained as a generous gift from Hoffman-LaRoche, Nutley, NJ (retinol), purchased from Sigma, St. Louis, MO (retinyl palmitate), or synthesized in our laboratory (retinyl oleate, stearate, linoleate, laurate, myristate) according to known procedures (5).

Retinoic acid was extracted from individual plasma samples by chloroform:methanol (2:1), separated from other lipids by solid phase extraction on aminopropyl columns (Supelco), and analysed by normal-phase HPLC (Waters Associates). All-trans, 13-cis, and 9-cis retinoic acid isomers were eluted with hexane:acetone:acetic acid (99.5:0.4:0.1) from two silica columns linked in series and quantified by in-line detection (350 nm) of tracer [3H]-retinoic acid added to the extracts. Using this system, the limit of detection was about 0.5 ng/mL of plasma (1.83 nmol/mL). It should be mentioned that the plasma samples used for retinoic acid measurements were stored at ~80°C for approximately one year and a half, and stability of retinoic acid has not been assessed from plasma stored for that period of time.

**Radioimmunoassays of RBP and TTR**

Levels of RBP and TTR were determined in plasma and liver homogenates by radioimmunoassay techniques previously described (6). RBP and TTR were detected and quantified using antirat plasma RBP raised in sheep and antirat TTR raised in rabbits. Known amounts of purified rat RBP and TTR were used as standards.

**RNA Isolation and Northern Analysis**

The procedures used for RNA isolation and Northern analysis have been described in detail in a recent publication (7). Total RNA was isolated from liver homogenates using phenol:chloroform:isoamyl alcohol extractions according to the method of Tushinski and colleagues (8). Twenty micrograms of total RNA was subjected to electrophoresis on 1% agarose, X% agarose, and transferred to nylon membranes. The membranes were prehybridized for 1 hour at 65°C and then hybridized overnight with 32P-labeled probes (2.5 - 3.5 x 10^6 cpm/mL) specific for rat RBP and CRBP mRNAs and murine TTR and $\beta$-actin mRNAs. The probes were obtained by nick translation of respective cDNA clones, as previously described (9-11). Specific mRNA levels were determined on washed membranes using a PhosphorImager. RBP, TTR, and CRBP mRNA levels were normalized for $\beta$-actin expression, which was not influenced by DR (12,13).

**Acid Retinyl Ester Hydrolase Activity**

We measured the activity of a newly described acid retinyl ester hydrolase (aREH) (14), enriched in the lysosomal fraction, and able to hydrolyse in vitro the retinyl esters contained in the lipid droplets of the rat stellate cells (15). Acid REH activity was assayed in liver homogenates as previously described (14), except that the liposomes which contained the substrate were prepared according to Grolier and colleagues (16). Briefly, an ethanolic solution of retinyl palmitate, phosphatidylcholine, cholesterol, and dicetyl phosphate was dispersed in a 30 mM phosphate buffer, containing 1 µM 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Fluka Chemie AG, Buchs, Switzerland), a tocopherol analogue. After evaporation and filtration, the liposomes obtained were used as the substrate for aREH activity. They were incubated at 37°C in a citrate buffer (pH 4.1) together with the liver homogenate, which contained the enzyme to be assayed. Retinol formation was monitored after 90 minutes and corrected for endogenous retinol in the liver homogenates and for nonenzymatic retinol formation, using blank incubations. Every assay was carried out in duplicate.

**Neutral Retinyl Ester Hydrolase Activity**

Neutral bile salt-dependent retinyl ester hydrolase (nREH) activity was measured in whole liver homogenate according to the method described by Cooper (17). Retinol formed during incubation was quantified by HPLC procedures detailed above, and corrected for endogenous retinol and nonenzymatic retinol formation. Liver protein concentrations were evaluated by the method of Lowry and colleagues (18) using bovine albumin as the standard (Sigma).

**Statistical Analysis**

Results are presented as means ± SEM for groups of 4-8 samples. Data were analysed with two-factor analysis of variance (ANOVA) models by using the generalized linear model procedure. The main effect of age, diet, and the interaction effect of the two factors are presented. Analyses were performed with the SPSS 8.0 (SPSS Inc., Chicago) software program.

**RESULTS**

**Body and Organ Weights**

All groups started with similar initial body weights, but DR and DR+ rats had a much slower, though constant, body weight gain, as evidenced by a significant Age by Diet interaction effect. DR and DR+ rats demonstrated a significant difference in body weight from AL rats from 8 weeks of life (4 weeks after the onset of DR) until the end of the study (results not shown). Maximum body weight was reached at 12 months of age in all the groups. Enrichment of the DR diet with vitamins and minerals did not result in any difference in body weight gain or final body weight compared to the DR group (Table 1).

In general, there was an increase in weight for all the tissues examined between 3 and 12 months of age, but no further major increase thereafter. Although the liver, lungs, and kidney weights were significantly reduced in DR and DR+ rats, their intestine and brain weights were not different from those of AL rats. Thus, the influence of energy restriction on growth was tissue-specific. As observed for body weight, there was no difference between the two DR-diet groups with regard to the weights of the tissues examined.

**Plasma, Liver, and Tissue Retinoids**

Plasma retinol levels were not significantly modified by age (Figure 1). In contrast, they were decreased in DR and DR+ rats...
eral target tissues such as the small intestine, the lungs, the kidney, and the brain (Table 2). In control AL rats, retinoid levels significantly with advancing age. The lower plasma retinol levels in DR and DR+ rats were not associated with low levels observed for the three diets studied. Considering similar daily liver retinoid content than respective AL rats. DR rats, who showed retinoid concentrations (per gram of liver) to age-matched AL hepatic retinoids. In fact, mature and old DR+ rats had similar retinoid levels compared to respective controls eating ad libitum. Although the intake of vitamin A, mature and old DR+ rats had higher total retinol levels were not significantly influenced by diet (Figure 3). The opposite effect on all-trans retinoic acid, whereas 13-cis and 9-cis retinoic acid were markedly elevated in these animals when compared to AL rats. Age had a significant increasing effect on 9-cis retinoic acid, but the opposite effect on all-trans retinoic acid. Therefore, plasma total retinoic acid levels were not significantly influenced by age under our conditions.

**Liver Acid and Neutral REH Activity**

As can be seen in Figure 2a, both young DR and DR+ groups showed a lower acid REH activity as compared to their age-matched AL group. However, this effect of DR was not observed later in life, as demonstrated by higher enzyme activity for 12-month-old DR+ rats compared to respective controls and similar activity in 22-month-old rats (significant Age by Diet interaction effect).

Liver neutral REH activity showed large intragroup variability and was not significantly influenced by diet (Figure 2b). There was a tendency towards decreased nREH activity in old rats fed ad libitum.

Despite some differences in acid and neutral REH activities between the groups, the amount of free retinol present in the liver tended to decrease with aging but was not significantly altered by diet. As a percentage of total hepatic retinoid, free

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![Figure 1](https://example.com/figure1.png)  
*Figure 1. Effect of age and dietary restriction on plasma retinol levels in young, mature, and old rats. Results are presented as means ± SEM for 4–8 animals per group. Abbreviations are AL: ad libitum-fed rats; DR: dietary-restricted (40%) rats; DR+: dietary-restricted (40%) rats ingesting a diet enriched with micronutrients. Age effect: p < .135; diet effect: p < .001; Age by Diet interaction: p = .339. A significant (p < .05) trend was found in the difference of plasma retinol between AL and DR groups with increasing age, as measured by contrast tests following one way ANOVA. Values of plasma retinol concentrations were previously published (3).*

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**Table 1. Body and Organ Weights of Young, Mature, and Old Rats Fed Ad Libitum (AL) or Dietary-Restricted (DR)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>AL</th>
<th>DR</th>
<th>DR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>Body weight (g)</td>
<td>Liver weight (g)</td>
<td>Intestine weight (g)</td>
</tr>
<tr>
<td>3</td>
<td>231.3 ± 9.6</td>
<td>191.3 ± 4.5</td>
<td>224.8 ± 12.3</td>
</tr>
<tr>
<td>12</td>
<td>486.0 ± 28.1</td>
<td>263.6 ± 11.8</td>
<td>236.6 ± 12.3</td>
</tr>
<tr>
<td>22</td>
<td>468.5 ± 73.5</td>
<td>252.8 ± 8.0</td>
<td>236.0 ± 12.3</td>
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</tbody>
</table>

Note. Data are mean ± SEM (n = 4–8); DR+ = dietary restricted rats ingesting a diet enriched with micronutrients.

Body weight: age effect, p < .001; diet effect, p < .001; Age by Diet interaction, p = .001.
Liver weight: age effect, p < .001; diet effect, p < .001; Age by Diet interaction, p = .001.
Intestine weight: age effect, p < .001; diet effect, p = .940; Age by Diet interaction, p = .926.
Lungs weight: age effect, p < .001; diet effect, p = .049; Age by Diet interaction, p = .098.
Kidney weight: age effect, p < .001; diet effect, p = .014; Age by Diet interaction, p = .445.

as compared to respective controls eating ad libitum. Although the Age by Diet interaction effect was not significant, the difference between plasma retinol of AL and DR groups increased significantly with advancing age. The lower plasma retinol levels in DR and DR+ rats were not associated with low levels of hepatic retinoids. In fact, mature and old DR rats had similar retinoid concentrations (per gram of liver) to age-matched AL rats, whereas DR+ rats had almost twice that amount (Table 2). An increased accumulation of hepatic retinoids with age was observed for the three diets studied. Considering similar daily intake of vitamin A, mature and old DR+ rats had higher total liver retinoid content than respective AL rats. DR rats, who ingested 40% less vitamin A, showed lower hepatic content than AL and DR+ rats (results not shown).

Besides liver, retinoid concentrations were measured in several target tissues such as the small intestine, the lungs, the kidney, and the brain (Table 2). In control AL rats, retinoid levels tended to increase with age in intestine and kidney, but not in lungs and brain. DR and DR+ rats did not show decreased levels of retinoids in the peripheral tissues studied when compared to AL controls, and DR+ rats even exhibited higher levels in intestine and lungs. Thus, although plasma retinol levels were decreased in DR and DR+ rats, retinol was transported efficiently to the peripheral tissues of these animals.

In contrast to the reduced plasma retinol levels, total retinoic acid levels were higher in adult and old DR and DR+ than in respective controls (Table 3). Of the isomers evaluated, all-trans retinoic acid levels were slightly increased in DR and DR+ rats, whereas 13-cis and 9-cis retinoic acid were markedly elevated in these animals when compared to AL rats. Age had a significant increasing effect on 9-cis retinoic acid, but the opposite effect on all-trans retinoic acid. Therefore, plasma total retinoic acid levels were not significantly influenced by age under our conditions.
Liver RBp, TTR, CRBp, and $\beta$-Actin mRNA Expression

To assess whether the low levels of liver and plasma RBp in DR rats resulted from alterations in the RBP gene expression, levels of mRNA were quantified in liver. Given the involvement of TTR and CRBP in retinoid metabolism, the mRNA levels of those proteins were also measured in hepatic tissue. RBp, TTR, and CRBP mRNA levels were normalized for $\beta$-actin expression to correct for differences in sample loading. Data presented in Figure 4 indicate that neither age nor diet had a significant effect on liver RBp and TTR mRNA levels. In contrast, CRBP mRNA levels were 50% higher than control levels in DR and DR+ rats, but only at the age of 22 months.

### DISCUSSION

The present study reports a paradoxical retinoid status in rats subjected to prolonged DR: their plasma retinol levels are significantly reduced, with values usually indicative of vitamin A deficiency (19), and are paralleled by a decrease in plasma and hepatic RBp, but not TTR levels, whereas their retinoid tissue stores are adequate. Data also revealed that changes in plasma retinol homeostasis resulted from global energy restriction rather than reduction in the intake of specific nutrients involved in retinoid metabolism, such as vitamin A and zinc, as evidenced by a similar effect of DR and DR+. The protein intake of DR rats, even if reduced by 40%, still meets the recent recommendations set by the National Research Council (20) and others (21).

Some aspects of the retinol transport were explored. Two distinct proteins with different functions are responsible for retinol transport: one is intracellular (CRBP) and the other circulating (RBP). Within the cell of most tissues, retinol is translocated by sequestering retinol and directing it to different enzymes responsible for its esterification, hydrolysis, and oxidation (22). Here, liver CRBP mRNA levels were main-

### Table 2. Tissue Total Retinoid Concentrations of Young, Mature, and Old Rats Fed Ad Libitum (AL) or Dietary-Restricted (DR)

<table>
<thead>
<tr>
<th>Diet</th>
<th>AL</th>
<th>DR</th>
<th>DR+</th>
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</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>Liver (nmol/g)</td>
<td>Intestine (nmol/g)</td>
<td>Lungs (nmol/g)</td>
</tr>
<tr>
<td>3</td>
<td>3.49 ± 0.54</td>
<td>2.50 ± 0.44</td>
<td>4.68 ± 0.69</td>
</tr>
<tr>
<td>12</td>
<td>7.49 ± 1.49</td>
<td>7.58 ± 1.06</td>
<td>14.25 ± 2.89</td>
</tr>
<tr>
<td>22</td>
<td>10.59 ± 1.51</td>
<td>10.92 ± 1.53</td>
<td>21.10 ± 4.40</td>
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</table>

Note. Data are mean ± SEM (n = 6–8). Total retinoids = retinol + retinyl esters. Values of liver retinoid concentrations were previously published (3).

Liver: age effect, p < .001; diet effect, p < .001; Age by Diet interaction, p < .001.

Intestine: age effect, p = .058; diet effect, p = .001; Age by Diet interaction, p = .380.

Lungs: age effect, p = .339; diet effect, p < .001; Age by Diet interaction, p = .070.

Kidney: age effect, p = .002; diet effect, p = .485; Age by Diet interaction, p = .582.

Brain: age effect, p = .332; diet effect, p = .195; Age by Diet interaction, p = .629.

retinol represented 1.35 ± 0.55, 1.72 ± 0.99, 1.34 ± 0.75, 0.73 ± 0.45, 0.70 ± 0.30, 0.45 ± 0.23, 0.54 ± 0.43, 1.27 ± 0.88, and 0.48 ± 0.28 in young AL, DR, DR+; mature AL, DR, DR+; and old AL, DR, and DR+ rats, respectively.

### Plasma and Liver RBp and TTR Levels

As illustrated in Figure 3a, plasma RBp levels were significantly lower in DR and DR+ rats than in corresponding controls, and were therefore highly correlated (r = .70, p < .001) to plasma retinol levels. The same diet effect was observed in liver RBp levels (Figure 3b), but in contrast to plasma RBp, which was not significantly affected by age, there was a gradual decline in liver RBP with advancing age.

Plasma TTR was not affected by DR to the same extent as plasma RBP (Figure 3c). However, both young DR groups showed higher plasma TTR levels than respective AL rats, and the opposite for older ages, as demonstrated by a significant interaction of age and diet factors. In contrast to RBP, hepatic TTR concentrations did not vary significantly with age or diet (Figure 3d).

### Table 3. Plasma Retinoic Acid Levels of Young, Mature, and Old Rats Fed Ad Libitum (AL) or Dietary-Restricted (DR)

<table>
<thead>
<tr>
<th>n</th>
<th>13-cis RA (nmol/L)</th>
<th>9-cis RA (nmol/L)</th>
<th>all-trans RA (nmol/L)</th>
<th>Total RA (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>AL 4 1.90 ± 1.11</td>
<td>ND</td>
<td>4.39 ± 1.14</td>
<td>6.30 ± 2.03</td>
</tr>
<tr>
<td></td>
<td>DR + DR+ 7 1.42 ± 0.94</td>
<td>0.26 ± 0.26</td>
<td>5.02 ± 1.55</td>
<td>6.94 ± 2.63</td>
</tr>
<tr>
<td>12 months</td>
<td>AL 5 0.81 ± 0.81</td>
<td>0.58 ± 0.58</td>
<td>2.11 ± 0.80</td>
<td>3.50 ± 1.33</td>
</tr>
<tr>
<td></td>
<td>DR + DR+ 4 4.59 ± 1.47</td>
<td>4.81 ± 0.89</td>
<td>3.61 ± 1.21</td>
<td>12.29 ± 2.30</td>
</tr>
<tr>
<td>22 months</td>
<td>AL 4 ND</td>
<td>ND</td>
<td>2.52 ± 2.06</td>
<td>2.52 ± 2.06</td>
</tr>
<tr>
<td></td>
<td>DR + DR+ 4 3.95 ± 1.62</td>
<td>5.87 ± 0.74</td>
<td>2.62 ± 0.57</td>
<td>12.44 ± 1.71</td>
</tr>
</tbody>
</table>

Note. Data are mean ± SEM (because there was no significant difference in RA levels between DR and DR+ (dietary restricted rats ingesting a diet enriched with micronutrients)) groups, these data were pooled. ND, below detection limit (see Materials and Methods). For statistical analysis, ND, as a mean, was considered as zero. Most AL groups included detectable values and ND values; the latter were not considered for calculations of the mean.

13-cis RA: age effect, p = .508; diet effect, p = .010; Age by Diet interaction, p = .105.

9-cis RA: age effect, p < .001; diet effect, p < .001; Age by Diet interaction, p < .001.

All-trans RA: age effect, p = .007; diet effect, p = .042; Age by Diet interaction, p = .287.

Total RA: age effect, p = .843; diet effect, p < .001; Age by Diet interaction, p = .027.
Figure 2. Effect of age and dietary restriction on liver acid (a) and neutral (b) retinyl ester hydrolase (REH) activity in young, mature, and old rats. Results are presented as means ± SEM (n = 4–8). Abbreviations are as in Figure 1. For acid REH, age effect: \( p = .038 \); diet effect: \( p = .103 \); Age by Diet interaction: \( p < .001 \). For neutral REH, age effect: \( p = .015 \); diet effect: \( p = .456 \); Age by Diet interaction: \( p = .520 \).

Low levels of plasma RBP were found in DR and DR\(^+\) rats of mature and advanced age, suggesting alterations in RBP synthesis. However, expression of hepatic RBP mRNA was not significantly modified by either DR diets, when compared to respective controls. Similarly, liver TTR mRNA expression as well as CRBP mRNA levels were not modified by age or diet. Recent studies showed that DR can regulate the expression of certain hepatic genes such as insulin receptor (24), cytochromes P\(_{450}\) and P\(_{2}-450\), superoxide dismutase (25), and glucose-regulated protein 78 (26), whereas the expression of other genes, namely that of basal heat shock protein 70, ubiquitin (27), catalase, and glutathione peroxidase (28), is unaffected by the DR regimen. Thus, it appears that RBP, TTR, and CRBP genes are part of the second category and do not respond to a prolonged DR.

Hepatic RBP levels were decreased with aging and to a greater extent with DR, whereas RBP gene expression was unaffected by these parameters. Interestingly, Imamine and colleagues (28) reported similar changes in a model of cholestasis.
Figure 3. Effect of age and dietary restriction on plasma RBP (a), liver RBP (b), plasma TTR (c), and liver TTR (d) levels in young, mature, and old rats. Results are presented as means ± SEM (n = 4–8). Abbreviations are as in Figure 1. For plasma RBP (a), age effect: p = .166; diet effect: p = .002; Age by Diet interaction: p = .245. For liver RBP (b), age effect: p = .001; diet effect: p < .001; Age by Diet interaction: p = .943. For plasma TTR (c), age effect: p = .013; diet effect: p = .200; Age by Diet interaction: p = .001. For liver TTR (d), age effect: p = .619; diet effect: p = .564; Age by Diet interaction: p = .695.
Figure 4. Effect of age and dietary restriction on liver RBP, TTR, and CRBP expression in young, mature, and old rats. Figure 4a illustrates a representative Northern blot per group for each protein. Mean level of RBP (b), TTR (c), and CRBP (d) mRNA levels are presented as a percentage of the expression of the 3-month-old AL group (controls) after correction for the mean expression of β-actin expression. Each bar represents the mean ± SEM from 3-7 rats per group. For RBP mRNA (b), age effect: \( p = .598 \); diet effect: \( p = .626 \); Age by Diet interaction: \( p = .163 \). For TTR mRNA (c), age effect: \( p = .906 \); diet effect: \( p = .528 \); Age by Diet interaction: \( p = .224 \). For CRBP mRNA (d), age effect: \( p = .043 \); diet effect: \( p = .022 \); Age by Diet interaction: \( p = .113 \).
induced by bile duct obstruction. They explained the low synthetic rate of RBP by modifications in the subcellular distribution of the functioning mRNA (known as the polysome membrane-bound mRNA); only 36% of the mRNA was membrane-bound in the bile-obstructed rats compared to 80% in the control rats. Although their hypothesis is attractive, it could hardly explain our findings, because the effects of age and DR were specific to RBP and did not affect TTR. The observation that the RBP gene and its protein expression were not parallel may suggest that RBP synthesis might be impaired at the post-transcriptional level. The exact mechanism explaining this effect of DR still needs to be assessed.

It is generally thought that a decline in the general turnover of hepatic and whole body proteins occurs with advancing age and that this decline is counteracted by DR, resulting from both increased protein synthesis and breakdown (29). It may be possible that RBP degradation is enhanced in DR rats. In fact, there is some evidence of increased degradation of total protein, as demonstrated in the perfused livers of male Fisher F344 DR rats (30) and of horseradish peroxidase and ovalbumin in hepatocytes of aged mice subjected to DR for 70 days (31). Because of its shorter half-life, RBP could be affected to a greater extent than TTR, leading to reduced protein levels despite maintained synthesis.

Because retinol appears to be efficiently transported to peripheral tissues despite a 50% lower level of the main transport protein, alternative transport pathways of retinol might be considered. It was demonstrated that dietary retinyl esters contained in chylomicrons could be taken up by cultured adipocytes and that lipoprotein lipase was able to hydrolyse retinyl esters to form retinol in vitro (32). The postabsorptive pathway could contribute in part to the retinol pool of extrahepatic tissues. Interestingly, we have recently shown that adult and old DR rats absorbed significantly more retinol via intestinal lymphatic transport than control rats eating ad libitum (3). Thus, it is possible that the increased lymphatic retinol absorption not only contributes to the accumulation of retinoids in hepatic tissues but also to the maintenance of adequate stores in peripheral tissues. This, in turn, would reduce the need for export of retinol-RBP from the liver, hence the lower plasma retinol levels.

An unexpected finding was the higher plasma retinoic acid levels seen in DR rats, which suggest a stimulation of retinol oxidation in these animals. It may be hypothesized that the higher biotransformation of retinol into its active form represents a compensatory mechanism resulting from reduced delivery of retinol-RBP. Another study, in TTR deficient mice, reported a similar situation where very low plasma levels of retinol-RBP were associated with elevated retinoid acid levels (7). In the TTR model, as in DR, the animals did not show any apparent sign of retinoid deficiency, supporting the view that normal retinoid functions are maintained despite perturbation of plasma retinol homeostasis. Furthermore, in view of previous studies in which lower retinol levels were reported as a response to retinoic acid supplementation (33–35), it seems that plasma retinol and retinoic acid levels may be under reciprocal regulation.

In conclusion, hepatic retinoid content increased with age in AL and DR rats, but was markedly higher in DR groups. By contrast, these rats exhibited lower plasma retinol-RBP levels than AL controls, and this was associated with decreased hepatic RBP but unchanged RBP mRNA levels. Extrahepatic tissues retinoid content was not reduced by DR, suggesting alternative pathways of retinol delivery other than the one involving RBP.

Results indicate that DR enhanced retinoid acid synthesis, which could compensate for the decreased retinol delivery to tissues. Finally, one may speculate that the higher levels of retinoic acid, and especially of the 9-cis isomer, the exclusive ligand of the retinoid acid receptors, may contribute to certain beneficial effects of DR through regulation of gene expression.

ACKNOWLEDGMENTS

This research was funded by the Natural Science and Engineering Research Council of Canada. S.C. was supported by a scholarship from the Fonds pour la Formation de Chercheurs et l’Aide à la Recherche. The authors are grateful to Roseanne Panfalone, Aline Perea, Maurice Audet, and Anne Partier for valuable technical support.

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Received September 18, 1998
Accepted April 7, 1999