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Effect of Dietary Avocado Oils on Hepatic Collagen Metabolism

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Abstract. The effect of various avocado and soybean oils on collagen metabolism in the liver was studied in growing female rats for 8 weeks and in day-old chicks for 1 week. In comparison with rats fed either refined avocado oil, refined or unrefined soybean oils, rats fed unrefined avocado oil showed a significant decrease in total collagen solubility in the liver, while there were no changes in total collagen, protein and moisture content. Chicks fed unrefined avocado oil as compared to those fed refined avocado oil also showed a decrease in hepatic total soluble collagen while hepatic total collagen remained unaffected. Electron micrographs and light-microscope examinations of rats' liver revealed collagen accumulation in the periportal location. This is suggestive of the early stages of fibrosis.

Introduction

The avocado is one of the few cultivated fruits in which the oil is the main component. The oil, which makes up 15–30% of the fresh weight, depending on the variety, is unsaturated [1]. The two major methods of production of the oil are organic solvent extraction and centrifugal force separation [2]. Feeding rats with unrefined avocado oil produced by solvent extraction from intact fruits was found to cause some metabolic alterations as compared to refined avocado oil which is produced by centrifugal separation from cored fruits [3]. In this study, growing rats fed unrefined avocado oil showed liver enlargement, an increased amount of hepatic lipids which was a consequence of enhanced lipid biosynthesis and impaired lipid transport. Lipid accumulation, identified by histological examinations, appeared as macrovesicular and microvesicular steatosis. These phenomena were accompanied by a subsequent reduction in se-

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rum triglyceride content. It was suggested that these effects were due to some hepatotoxic factors originating from the avocado seed [4]. Robert et al. [5, 6] and Lamaud et al. [7] showed that feeding or topical application of unsaponifiable material obtained from avocado and soybean lipidic extracts caused an increase in collagen solubility in rat's skin. Collagen is the major structural protein component in vertebrates and in normal mammalian liver it accounts for about 1% of the total dry weight [8]. Extensive deposition of collagen, as produced by various hepatotoxins, is a prominent feature of liver injury [9]. This component of the hepatic extracellular biomatrix has received increasing interest due to its role in the pathogenesis of liver cirrhosis [10] in which fibrosis is generalized and is responsible for distorting normal histoarchitecture. A fibrotic response may be induced by any noxious agent of a physical (temperature, radiation, incision), chemical (CCl_4, bleomycin) or biological (infection, immune reaction) nature [11]. In addition, it is known that liver collagen metabolism may alter as a result of changes in lipid metabolism [12]. Since feeding unrefined avocado oil was shown to affect liver lipid metabolism in rats, the purpose of this study was to evaluate the effect of this oil on liver collagen metabolism.

Material and Methods

Animals and Diets

Female Charles River CD rats, weighing 80–100 g, were obtained from the animal colony of the Department of Food Engineering and Biotechnology, Technion Israel. They were randomly divided into four groups, each of 7 rats, and housed individually in a temperature-controlled room (25 °C) having a 12-hour light/dark cycle. Food and water were supplied ad libitum. Food consumption of all groups was recorded daily. Each group was fed a basic ground commercial rat chow, obtained from Asia Maabarot, Israel, which meets the recommended dietary allowances of growing rats [13]. The ground chow was supplemented at a level of 10% (w/w) with avocado or soybean oil as follows.

Group A: Refined avocado oil (RAO) derived from cored avocado fruit, Hass variety, produced by centrifugal force separation, obtained from Avochem, Santa Paula, Calif., USA.

Group B: Unrefined avocado oil (URAO) extracted with hexane from the intact fruit, Fuerte variety, obtained from Miluot, Haifa, Israel.

Group C: Refined soybean oil (RSO), a commercial product of Shemen, Haifa, Israel.

Group D: Unrefined soybean oil (URSO) obtained from Shemen, Haifa, Israel.

The refined oils (RAO and RSO) employed in this study passed all the commercial standard refining procedures. The composition of the unsaponifiables of the oils employed in this study was previously given by Werman et al. [14] and Gutfinger and Letan [15].

Day-old Leghorn male chicks obtained from a local hatchary fed commercial chicken ration were given a daily oral dose of 1 ml RAO or URAO for 1 week.

Experiment

At the end of the 8-week feeding period, the rats were fasted overnight and then sacrificed by carbon dioxide asphyxiation. The abdominal and thoracic cavities were opened and the liver, spleen, lungs and heart were removed, blotted, and weighed. One lobe of the liver was then cut into slices of 1 × 1 mm each, with a surgical blade and used for transmission electron microscopy (TEM) and light-microscope examinations. The remaining liver tissues were used for the determination of moisture, protein, fat and collagen content, and collagen solubility profile. The chicks were sacrificed by decapitation and the liver was removed and weighed. Total and soluble collagen were immediately determined.

TEM Examinations

Liver slices were placed in 0.1 *M* phosphate buffer containing 2% glutaraldehyde and 7.5% sucrose for

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fixation. The slices were postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon. Sections were counterstained with lead-citrated uranyl acetate and examined with a Philips 300 electron microscope.

Light-Microscope Examinations

Liver slices were placed in 4% formaldehyde for fixation. The formaldehyde-fixed liver slices were embedded in paraffin, yielding blocks which were sectioned on a rotatory microtome at 7 µm and stained for reticulin.

Moisture Content

The remaining liver tissues, approximately 2.5 g each, were cut to small pieces and freeze-dried for 24 h to constant weight.

Protein Content

Total nitrogen content of the freeze-dried liver samples, 0.3–0.5 g each, was determined by the Kjeldahl procedure as described by the AOAC official method [16]. Protein content was expressed as nitrogen content × 6.25.

Lipid Content

Lipids were extracted from liver samples, 2.5–3.0 g each, as described by Folch et al. [17]

Collagen Content

Total collagen content of the freezed-dried liver samples was determined according to the method suggested by Rompala and Jones [18] with some modification. Samples, 0.05–0.1 g each, were hydrolyzed with 3 ml of 6 *N* HCl at 115 °C for 24 h. The acid was vaporized at 60 °C in the presence of solid KOH. Hydroxyproline (Hy-Pro) content, as an index of collagen in the hydrolyzed sample, was determined as described by Grant [19].

Collagen Solubility Profile

Liver samples, 0.5 g each, cut into small pieces were extracted with 20 ml of 0.5 *N* NaCl in 0.02 *M* Tris-HCl buffer pH 7.4, for 24 h in a shaker at a rate of 100 strokes/min. Following the extraction, the samples were centrifuged at 10,000 *g* for 20 min. The supernatant was dialyzed overnight against 0.5 *N* acetic acid, pH 3.4. All these operations were performed at 4 °C. Aliquots after acid hydrolysis (6 *N* HCl, 115 °C, 24 h) were analyzed for Hy-Pro [20], as an index of collagen, and neutral soluble collagen (NSC) was expressed as Hy-Pro content in the hydrolysate. The precipitates following NaCl extraction were reextracted with 0.5 *N* acetic acid pH 3.4, 4 °C, and centrifuged under the same conditions. Acid soluble collagen (ASC) was expressed as Hy-Pro content in the supernatant. Total soluble collagen (TSC) was determined by extraction of fresh liver samples, 0.5 g each, directly with 0.5 *N* acetic acid, pH 3.4, as described above, and the Hy-Pro measured in the supernatant.

Statistical Analysis

All data were expressed as the mean ± SD and analyzed by Student's t test or by one-way analysis of variance (ANOVA). If the ANOVA showed a significant value ($p < 0.01$), Fisher's least significant difference test for multiple comparison was applied [21].

Results

The effect of various dietary oils on rats' body weight during the 8-week feeding period is presented in figure 1. In agreement with our previous results [3], feeding weanling rats with URAO caused a significant growth inhibition of 23% as compared to rats fed RAO. Rats fed diets containing RSO and URSO exhibited the same body weight as that of rats fed RAO. Rats fed URAO showed a marked decrease of approximately 20% in food consumption as compared to the other tested oil. No significant differences were found between the groups in the relative weights of spleen, lungs and heart. However, a significant liver enlargement (4.3% of body weight) was found in the animals fed URAO, compared to those fed the other diets in which the relative liver weight was only 3%. Liver content of moisture, protein and collagen (Hy-Pro) did not differ sig-

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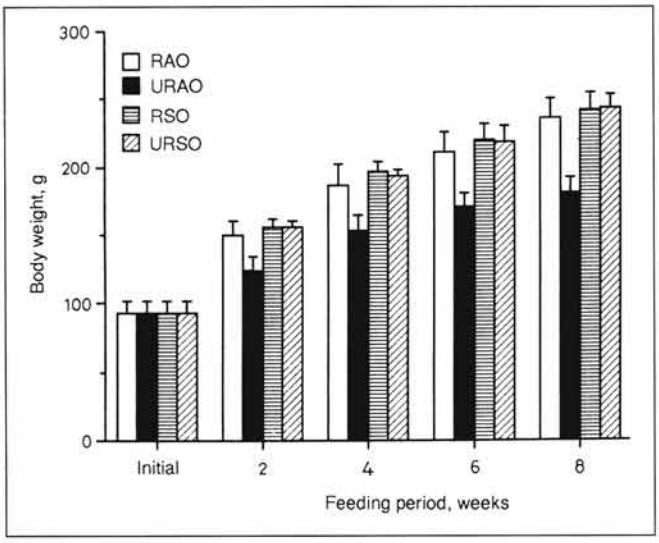

Fig. 1. Body weight of rats fed URAO, URSO, RAO or RSO during 8 weeks. Values are means ± SD for 7 rats in each group.

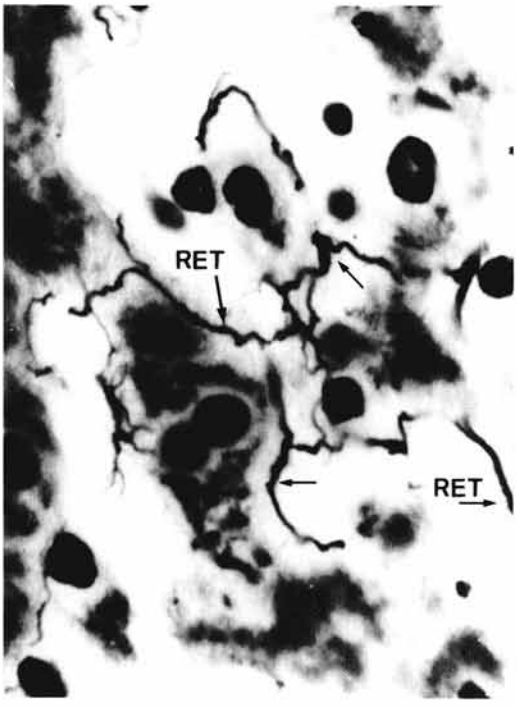

Fig. 2. Rat liver. Deposition of reticulin (RET) in the periportal location following a feeding period of 8 weeks with URAO. × 400.

nificantly between the dietary groups, and was found to be approximately 70%, 20% and 172 μg/g liver, respectively (table 1). Feeding rats with URAO caused a significant increase in liver fat content to 5.6%, while rats fed RAO, RSO, and URSO showed lower levels of 4.0, 4.3 and 4.6%, respectively (table 1). As far as food consumption data are concerned, we have previously shown in a pair-fed study [4] that low food consumption of RAO did not cause liver enlargement and fatty liver as compared to rats fed URAO. Differences in liver collagen solubility following the 8-week administration of refined or unrefined avocado oils are shown in table 2. The amount of TSC decreased significantly, by 16.5%, in the liver of rats fed URAO in comparison with rats fed RAO. Examination of the collagen solubility profile in the liver of the rats fed URAO revealed a significant decrease in NSC, while ASC remained unaffected. A similar trend was observed in chicks given URAO for 1 week. In these

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Table 1. Effect of avocado oils in comparison to soybean oils on moisture, protein, collagen and soluble collagen content in the liver of growing rats

Liver	RAO	URAO	RSO	URSO	p
Relative weight, %	3.04 ± 0.11^{a}	4.30 ± 0.26^{b}	3.02 ± 0.13^{a}	2.88 ± 0.27^{a}	< 0.01
Moisture, %	71.44 ± 1.80	70.05 ± 1.24	71.56 ± 1.76	71.16 ± 0.77	
Protein ($N_2 \times 6.25$), %	20.53 ± 1.85	20.39 ± 0.75	20.24 ± 1.73	20.35 ± 0.52	
Fat, %	4.00 ± 0.29^{a}	5.60 ± 0.48^{b}	4.29 ± 0.34^{a}	4.60 ± 0.57^{a}	< 0.05
Collagen, μg Hy-Pro/g WT	171 ± 15	186 ± 12	162 ± 18	170 ± 13	
TSC, μg Hy-Pro/g WT	$41.2 \pm 3.1^{b,c}$	36.1 ± 1.9^{a}	$42.4 \pm 4.3^{b,c}$	43.4 ± 3.8^{c}	< 0.01

Values are means ± SD for 7 rats in each group. Values with different superscripts differ significantly (least significant difference multiple comparison test, as indicated). WT = Wet tissue.

Table 2. Total collagen content and collagen solubility profile in the liver of growing rats fed URAO produced by hexane extraction and RAO

	Collagen, μg Hy-Pro/g WT	
	RAO	URAO
Total content	196 ± 21	203 ± 24
TSC	44.8 ± 2.9	37.4 ± 2.4*
NSC	33.9 ± 2.8	28.4 ± 2.3*
ASC	9.4 ± 1.6	9.1 ± 0.9

Values are means ± SD for 8 rats in each group. * $p < 0.01$, significantly difference (t test). WT = Wet tissue.

Table 3. Effect of URAO on total collagen content and collagen solubility in the liver of growing chicks

Parameters	RAO	URAO
Number of chicks	25	21
Initial body weight, g	41.2 ± 4.8	40.9 ± 3.6
Final body weight, g	104.9 ± 12.5	62.3 ± 18.6*
Liver		
Relative weight, %	3.07 ± 0.24	4.88 ± 0.80*
Collagen (Hy-Pro) content[1] μg/g WT	245.1 ± 34.4	264.7 ± 54.3
TSC (Hy-Pro) content[1] μg/g WT	26.7 ± 3.3	18.4 ± 2.9*

Values are means ± SD for the number of chicks indicated. * $p < 0.01$, significant difference (t test). WT = Wet tissue.

[1] The results are for 6 chicks only.

chicks a 65% decrease in body weight, a significant increase in relative liver weight and in a decrease TSC content with no change in total collagen content were found (table 3).

Light-microscope examinations of livers from rats fed URAO (fig. 2) showed accumulation of fat droplets and reticulin which was randomly distributed in the periportal and midzonal locations. The distribution of reticulin around the central vein, bile canaliculi and liver arteries was normal, as in the rats fed RAO. TEM examinations (fig. 3) verified the light-microscope observations.

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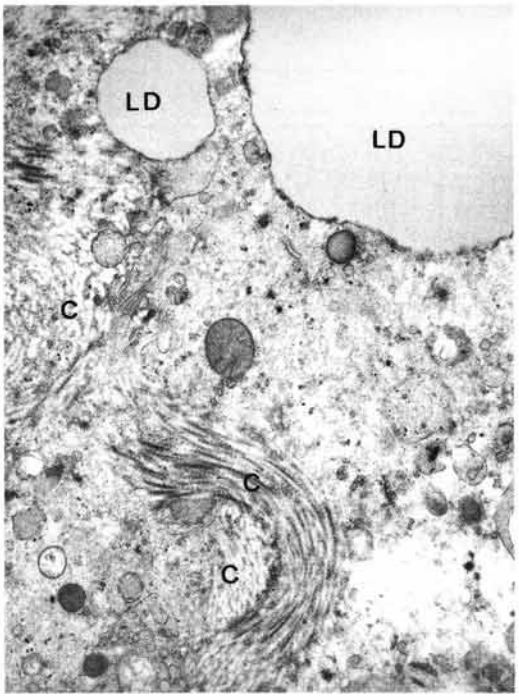

Fig. 3. Electron micrograph of a rat hepatocyte, following a feeding period of 8 weeks of URAO. Note the accumulation of collagen clusters (C) located within the zone of lipid droplet (LD) accumulation. ×24,750.

Discussion

Our results indicate the occurrence of alterations in collagen metabolism in the liver of rats and chicks fed diets containing URAO as compared to those fed RAO. No differences in total collagen and TSC content were observed between rats fed RAO-, RSO- and URSO-supplemented diets. While TSC was significantly decreased both in the livers of rats and chicks fed URAO, total collagen content did not change. It seems that this phenomenon (tables 1, 2) could be caused by an imbalance between synthesis and degradation of collagen [20]. Although hepatic collagen content was not altered, the accumulation of collagen clusters near the lipid droplet location in the fatty liver (fig. 3) supports the possibility of early stages of focal fibrosis formation in the rats fed URAO [9]. Hepatic collagen deposition during fat infiltration, caused by CCl_4 intoxication [22] or by ethanol administration [23], is a well-documented finding. In the early stages of hepatic fibrosis, collagen synthesis is accelerated but the content of collagen is not increased, as was also observed in this study. This is presumably due to a concomitant rise in its rate of degradation. Increase in collagen degradation by collagenolytic enzymes is a self-protective mechanism which serves to limit fibrosis [24]. Following the development of fibrosis an increase in collagen levels might not be accompanied by a proportionate increase in activity of these enzymes, supporting the concept that hepatic fibrosis may be caused, in part, by insufficient collagen degradation [5]. Another characteristic feature of the early stages of liver fibrosis is the elevated lysyl oxidase activities [25], which reached a maximum when hepatic collagen became apparent histologically [26]. Lysyl oxidase is the enzyme which catalyzes the oxidative deamination of the ε-amino groups of certain lysyl and hydroxylysyl residues in collagen molecules. Lysyl oxidase initiates intra- and intermolecular cross-linking in the extracellular space [27], producing stable, mature and insoluble collagen fibrils [28]. Elevated activities of hepatic lysyl oxidase might explain the decrease in hepatic collagen solubility observed in rats fed URAO. Although we observed no differences in hepatic collagen content, it is possible that if our study had been more prolonged, an increase in total collagen content

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would have occurred and might have been accompanied by additional hepatotoxic effects, such as liver necrosis and liver malfunction in the rats fed URAO. The decreased hepatic collagen solubility found in this study is in contrast with our previous findings showing that feeding URAO caused increased skin collagen solubility due to the inhibition of lysyl oxidase activity in the skin [29]. It seems that the increase in lysyl oxidase activity as a result of fibrosis formation in the liver might overcome the inhibitory effect of the URAO.

In conclusion, the unrefined avocado oil produced by organic solvent extraction from intact fruits contains some hepatotoxic agents which cause alterations in lipid and collagen metabolism. Although the precise nature of these toxic factors and the mechanisms by which their effects are exerted are unknown, it seems that they originate from the lipid fraction of the avocado seed and more specifically from the unsaponifiable components of this fraction [3, 4].

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