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ORIGINAL CONTRIBUTIONS

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Fat infiltration in liver of rats induced by different dietary plant oils: high oleic-, medium oleic- and high petroselinic acid-oils

Fettinfiltration in die Leber von Ratten durch Fütterung unterschiedlicher Pflanzenöle

Summary Beneficial effects on health parameters by ingestion of plant oils having high levels of monounsaturated fatty acids such as oleic (*cis*-9-octadecenoic) acid have been described in several studies. Recently, we have shown that feeding of coriander oil containing high proportions of a positional isomer of oleic acid, i.e. petroselinic (*cis*-6-octadecenoic) acid, led to significantly decreasing proportions of arachidonic (all *cis*-5,8,11,14-eicosatetraenoic) acid in the cellular lipids. Here, we report histopathological findings in male Wistar rats fed isocaloric diets containing 2 % corn oil and 12 % (w/w), each, of different plant oils (high-oleic and conventional sunflower oil, olive oil, rapeseed oil as well as coriander oil) containing varying levels of monounsaturated fatty acids, i.e. oleic or petroselinic acids. One group of animals was fed a standard diet containing 4 % (w/w) of fat as control. Liver, heart, aorta, stomach, and spleen were anatomically and histologically examined. Livers of animals from all the groups that had received high-fat diets – except the coriander oil group – showed moderate non-degenerative fat infiltrations of the hepatocytes beginning in the periportal fields. Marked to severe fat infiltration was observed in hepatocytes of animals fed coriander oil. In this experimental group the livers were found to contain fatty cysts in addition to hepatocytes with mixed-size lipid vesicles. Moreover, enlarged nuclei were observed in numerous hepatocytes without fat infiltration. Fat infiltration was not observed in livers of animals fed a rat standard diet. In the cells of all other organs and tissues studied, in particular heart and aorta, neither non-degenerative fat infiltrations nor other degenerative changes were observed for any group. Our results show that feeding of fat-rich diets containing varying proportions of C_{18} *cis*-monoenoic fatty acids induced different histopathological alterations in the livers of rats, depending on position of the double bond. Plant oils containing varying proportions of oleic acid lead exclusively to moderate fat infiltration in the hepatocytes, whereas in the animals fed high-petroselinic coriander oil degenerative alterations (fatty cysts) as well as – in several cases – enlarged nuclei were found that are likely due to a non-specific stimulation of the hepatocytes resulting from the cell degeneration observed.

Zusammenfassung In verschiedenen Studien wird eine günstige Wirkung von Pflanzenölen mit hohem Anteil an einfach ungesättigten Fettsäuren, z.B. Ölsäure (*cis*-9-Octadecensäure), auf die Gesundheit beschrieben. Kürzlich haben wir gezeigt, daß die Fütterung von Ratten mit Korianderöl, das hohe Anteile eines Positionsisomers der Ölsäure enthält, nämlich Petroselinsäure (*cis*-6-Octadecensäure), zu einer signifikanten Erniedrigung der Arachidonsäure (all *cis*-5,8,11,14-Eicosatetraensäure)-Anteile in den zellulären Lipiden führte. Hier berichten wir über histopathologische Befunde an männlichen Wistar-Ratten, denen isokalorische Diäten mit 2 % Maiskeimöl und je-

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weils 12 % (w/w) Pflanzenöl (ölsäure-reiches oder konventionelles Sonnenblumenöl, Olivenöl, Rapsöl, Korianderöl) und unterschiedlichen Anteilen an isomeren Monoenfettsäuren, d.h. Öl- und Petroselinsäure, verabreicht wurden. Die Tiere der Kontrollgruppe erhielten eine Standarddiät mit 4 % (w/w) Fett. Leber, Herz, Aorta, Magen und Milz wurden anatomisch und histologisch untersucht. Die Lebern der Tiere aller Gruppen, die eine fettreiche Diät erhalten hatten – mit Ausnahme der Korianderöl-Gruppe –, entwickelten eine mäßige, nicht-degenerative Fettinfiltration der Hepatocyten, die in den Periportalfeldern einsetzte. Eine ausgeprägte bis schwere Fettinfiltration wurde dagegen in den Hepatocyten von Tieren gefunden, die mit petroselinsäurereichem Korianderöl gefüttert worden waren. In dieser experimentellen Gruppe enthielten die Lebern Fettzysten und Lipidvesikel unterschiedlicher Größe. Außerdem wurden vergrößerte Kerne in zahlreichen Hepatocyten beobachtet, die keine Fettinfiltration aufwiesen. In Ratten, die mit einer Standarddiät gefüttert worden waren, wurde dagegen keine Fettinfiltration in den Leberzellen gefunden. Die Zellen aller anderen untersuchten Organe und Gewebe, insbesondere Herz und Aorta, zeigten weder nicht-degenerative Fettinfiltrationen noch irgendwelche anderen degenerativen Veränderungen. Unsere Ergebnisse zeigen, daß die Fütterung fettreicher Diäten mit unterschiedlichen C_{18} *cis*-Monoenfettsäuren in Abhängigkeit von der Position der Doppelbindung unterschiedliche histopathologische Veränderungen in der Leber von Ratten hervorruft. Während Pflanzenöle mit variierenden Ölsäureanteilen ausschließlich zu mäßigen Fettinfiltrationen in den Hepatocyten führten, wurden in den Lebern der mit petroselinsäurereichem Korianderöl gefütterten Tiere degenerative Veränderungen (Fettzysten) sowie – in einigen Fällen – vergrößerte Zellkerne beobachtet, die auf eine unspezifische Stimulierung der Hepatocyten hindeuten.

Key words Fatty liver – isomeric octadecenoic acids – oleic acid – petroselinic acid – coriander oil

Schlüsselwörter Fettleber – Isomere Octadecensäuren – Ölsäure – Petroselinsäure – Korianderöl

Introduction

Beneficial effects on health parameters have been reported by ingestion of dietary fats having high levels of the monounsaturated fatty acid, oleic acid (*cis*-9-octadecenoic), in the constituent fatty acids of triacylglycerols (6, 7, 11, 14–16, 25). Only a few studies are known on the biological effects of dietary fats containing naturally occurring positional isomers of oleic acid, such as *cis*-vaccenic (*cis*-11-octadecenoic) acid (22) or petroselinic (*cis*-6-octadecenoic) acid (9, 18).

cis-Vaccenic acid is a minor constituent of many fats and oils of plant origin (19). Seeds of Umbelliferae, such as parsley (*Petroselinum rubrum*), fennel (*Foeniculum vulgare*) and coriander (*Coriandrum sativum*) are known to contain high levels of petroselinic acid as the constituents of the triacylglycerols (12). Agronomically suitable varieties of coriander are being developed as renewable resources for petroselinic acid (4) in view of potential use of this fatty acid as raw material for the production of specific oleochemicals (21).

Experiments *in vitro* have revealed that – as compared to other triacylglycerols – those containing petroselinoyl moieties are hydrolyzed by pancreatic lipase at much lower rates (8, 24). We have found recently that petroselinic acid from dietary triacylglycerols, such as coriander oil is absorbed by rats as readily as oleic acid from high-oleic olive and sunflowerseed oils, medium-oleic rapeseed oil and high-linoleic conventional sunflowerseed oil; however, dietary petroselinic acid was found to decrease the level of arachidonic (all *cis*-5,8,11,14-eicosatetraenoic) acid in the cellular lipids (26) most probably by inhibition of Δ^6-desaturase (Weber et al., in preparation). Here, we report the histopathological findings observed upon feeding high proportions of the above oils.

Materials and methods

Materials

Refined, bleached and deodorized coriander oil was a generous gift of Dr. Westfechtel, Henkel Cie & KG, Düsseldorf, Germany. Refined, bleached and deodorized oils of high-oleic sunflowerseed, conventional sunflowerseed and double-zero ("Canola type") rapeseed were kindly provided by Dr. Holtmeier, Noblée & Thörl, Hamburg, Germany. Corn oil and virgin grade olive oil were obtained from local supermarkets. Rat standard diet (Altromin Kontrolldiät C 1000, Altromin International, Lage, Germany) containing 4 % fat was used as control.

Isocaloric pelleted feeds containing (per kg diet): crude protein 170.8 g, disaccharides 111.4 g, polysaccharides 151.3 g, crude fiber 29.9 g, crude ash 75.8 g, moisture 53.8 g, vitamin A 15 000 IU, vitamin D_3 500 IU, vitamin E 193.6 mg, vitamin K_3 10.0 mg, vitamin B_1 20.0 mg, vitamin B_2 20.3 mg, vitamin B_6 15.0 mg, vitamin B_{12} 0.04 mg, nicotinic acid 50.2 mg, pantothenic acid 50.1 mg, folic acid 10.0 mg, biotin 0.20 mg, choline chloride 1.0 mg, *p*-amino benzoic acid 100.0 mg, inositol 111.0 mg, vitamin C 20.0 mg, calcium 9.26 g, available phosphorus 7.16 g, magnesium 0.67 g, sodium 2.44 g, potassium 7.01 g, sulfur 2.0 g, chlorine 3.63 g, iron 0.18 g, manganese 0.10 g, zinc 28.8 mg, copper 5.5 mg, iodine 0.45 mg, molybdenum 0.20 mg, fluorine 4.17 mg, selenium 0.32 mg, cobalt 0.13 mg (3) and one of the above experimental oils (120 g/kg diet) plus corn oil (20 g/kg diet) were prepared by Altromin International. Composition of the oils (almost exclusively triacylglycerols) in the final diets are given in Table 1.

Table 1 Acyl composition of the total oil (triacylglycerols) in the diets[1)]

Group	g Acyl moieties/100 g oil				
	16:0 (Palmitoyl–)	18:1 (n-9) (Oleoyl–)	18:1 (n-12) (Petroselinoyl–)	18:2 (n-6) (Linoleoyl–)	18:3 (n-3) (Linolenoyl–)
Coriander oil	5	3	72	19	< 1
High-oleic sunflower oil	6	75	0	15	< 1
Olive oil	14	66	0	17	< 1
Rapeseed oil	7	54	0	28	7
Sunflower oil	9	25	0	61	< 1

[1)] Including 2 g/100 g of corn oil supplement

Methods

Animals and maintenance

Weaned male Wistar rats (Lippische Versuchstierzucht, Extertal, Germany) weighing 85–90 g were caged individually and divided into groups of ten animals, each. The rats were kept in rooms with adequate ventilation at a temperature of 22 °C and a relative humidity of 60 %. The animals had free access to feed and water at all times until 12 h before they were killed. Feeding was carried out for a total period of 10 weeks. For comparison one group (n = 5) of animals was fed Altromin standard diet. The experiments were approved by the official commission for animal experimentation (Der Regierungspräsident Münster, permission no. 26.0834 (48/90) of November 29, 1990).

Histological examination

At the end of the feeding period, the rats were killed by subjecting them to ether narcosis followed by section of the external jugular vein and common carotid artery. Liver, heart, aorta, stomach, and spleen were rapidly removed. For histological examination, parts of these organs were fixed in formalin. Frozen sections (8 μ) of liver were stained with oil red 0 - hematoxylin (1). Paraffin sections of heart, aorta, stomach, and spleen were stained with hematoxylin – eosin (H-E). Microscopic examination was performed by subjective visual assessment of morphological changes, as given in Table 2.

For the livers of animals fed coriander oil and control diet, the areas of cell nuclei in histological sections were quantitatively measured by an Image Analyzer Zeiss Axioskop 40 X/0.75 using a data acquisition system (Kontron Instruments, München, Germany). For each section, measurements were carried out on up to 10 nuclei of intact hepatocytes that did not undergo fat infiltration. The data were statistically evaluated using a Statgraphics two-sample analysis program (STSC, Rockville, MD, USA).

Results

Each of the high-fat diets administered to the rats induced fatty livers with total lipid contents of 7.1 to 9.7 % (26). Liver lipids of these experimental groups mainly consisted of triglycerides (data not shown).

Coriander oil

Figure 1A–B shows microphotographs of hepatic tissues of rats fed coriander oil with high petroselinic acid content. As is evident from this figure, the severe fat infiltration in the hepatocytes of this experimental group is characterized predominantly by hepatocytes with small, medium and large lipid droplets (mixed-size lipid vesicles). In particular, large macrovesicular lipid droplets (fatty cysts) were observed which were localized predominantly in periportal fields and, in part, their distribution was ubiquitary. A substantial number of the fatty

Table 2 Fat infiltration in liver of rats after feeding various plant oils for 10 weeks

Dietary oil/group[a)]	Percentage of monounsaturated fatty acids in the high-fat diets[b)]	Nature and localization of fat infiltration	Number (n) of animals with different degrees of simple fat infiltration — Degree[c)]: 0	0.5	1	1.5	2	2.5	3	Average degree per animal
Coriander oil	72	hepatocytes with mixed-size lipid vesicles and fatty cysts; mainly periportal, partly ubiquitary	–	–	–	2	2	2	3	2.3
High-oleic sunflower oil	75	hepatocytes with mixed-size lipid vesicles; predominantly periportal, partly ubiquitary	–	–	3	3	2	2	–	1.6
Olive oil	66		–	–	2	3	4	1	–	1.8
Rapeseed oil	54		–	–	–	3	6	1	–	1.9
Sunflower oil	25		–	–	2	3	3	1	1	1.8
Control (Standard diet 'Altromin')	–	hepatocytes with small lipid vesicles only; predominantly periportal, partly ubiquitary	4	1	–	–	–	–	–	0.1

a) Number of animals per group n = 10, except coriander oil group (n = 9); control group, n = 5.

b) Group fed coriander oil, petroselinic acid; all other groups, oleic acid.

c) Subjective visual assessment of the degree of fat infiltration (figures in parentheses show the approximate percentage of total hepatocytes with fat infiltration): 0 = none; 0.5 = minimal (< 10 %); 1.0 = slight (10–20 %); 1.5 = moderate (20–30 %); 2.0 = marked (30–40 %); 2.5 = severe (40–50 %); 3.0 = very severe (> 60 %).

cysts was found to be too large so that they slipped away during the staining procedure. The average degree of fat infiltration was estimated as marked to severe (Table 2).

Moreover, in the livers of various animals fed the coriander oil – but not in other groups – the size of the nuclei differed significantly within the various hepatocytes. In particular, cells without fat infiltration often showed large nuclei; the average area (± standard deviation = 43) of the nuclei of these hepatocytes of rats fed coriander oil (88.2 ± 15.0 μm^2) was significantly ($p < 0.01$) larger than the corresponding areas of the nuclei of the hepatocytes of rats fed the standard diet (62.7 ± 15.1 μm^2). Indications of inflammatory reactions, however, were not observed.

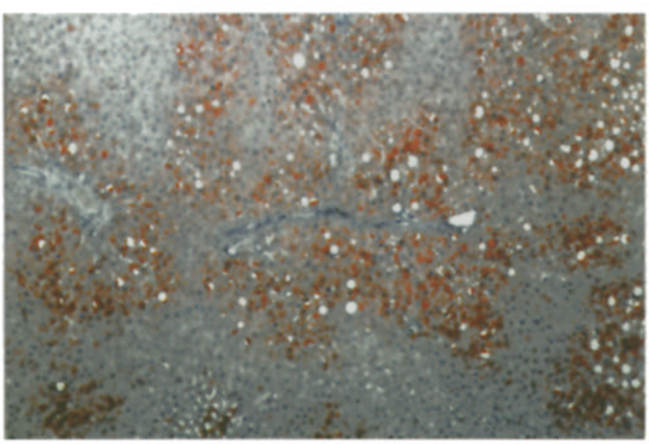

Fig. 1A Liver of rats fed high-petroselinic acid plant oil (coriander oil): Severe periportal and ubiquitary fat infiltration (stained with oil red O – hematoxylin; red, fat droplets; blue, cell nuclei. Extremely large lipid vesicles partly slipped away during the staining procedure and are, therefore, optically blank; magnification x 60).

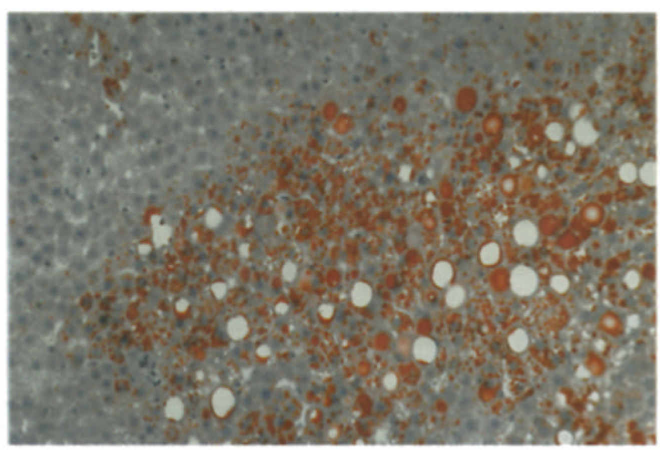

Fig. 1B Higher magnification (x 150) of Fig. 1A: Hepatocytes with mixed-size lipid vesicles and fatty cysts (red or optically blank, mostly without blue-stained nuclei); functional swelling of nuclei (blue) in hepatocytes that did not undergo fat infiltration.

Other plant oils

Figures 2A–B, 3A–B, and 4A–B show microphotographs of hepatic tissues of rats fed different plant oils containing varying proportions of oleic acid such as olive oil, rapeseed oil, and conventional sunflower oil. In contrast to coriander oil, feeding of these plant oils as well as high-oleic sunflower oil (microphotographs not shown) led to a moderate fat infiltration with mixed-size lipid vesicles which were localized predominantly in hepatocytes of periportal fields and, in part, their distribution was ubiquitary. In these experimental groups no indication of fatty cysts and nuclei enlargement was found as was observed in the coriander oil group.

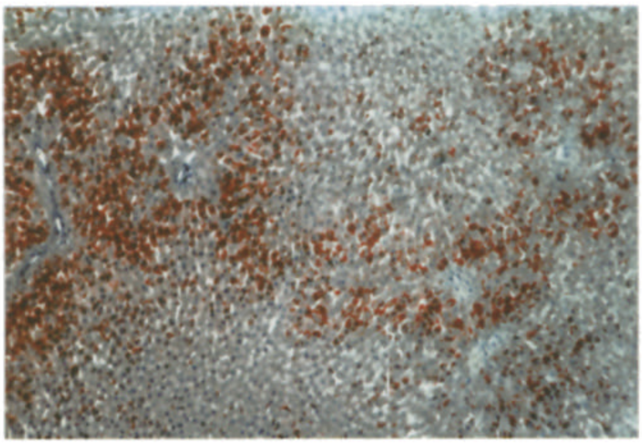

Fig. 2A Liver of rats fed a high-oleic acid plant oil (olive oil): Moderate predominantly periportal and partly ubiquitary fat infiltration (stained with oil red O – hematoxylin; red, fat droplets; blue, cell nuclei; magnification x 60).

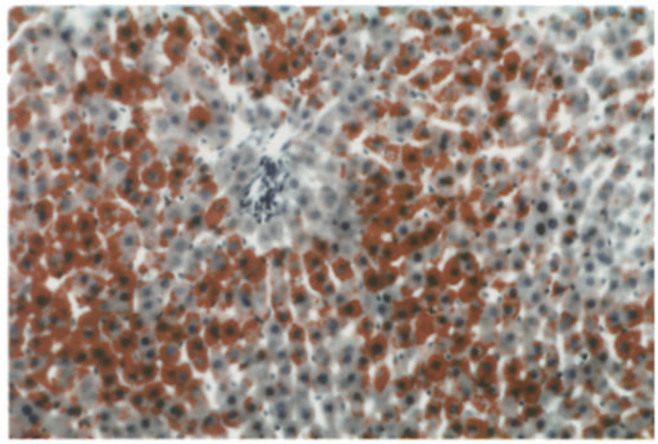

Fig. 2B Higher magnification (x 150) of Fig. 2A: Hepatocytes with mixed-size lipid vesicles (red).

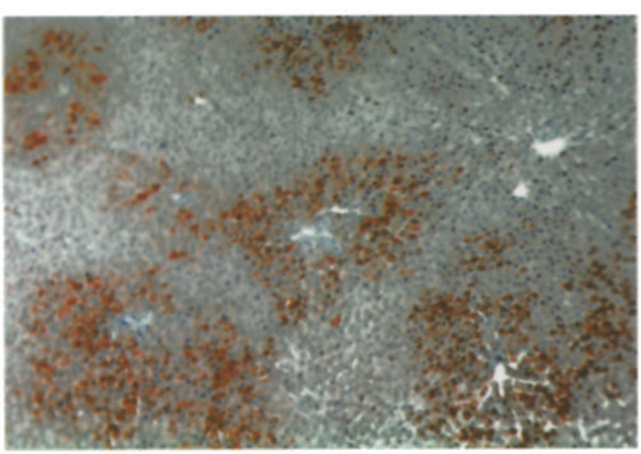

Fig. 3A Liver of rats fed a medium-oleic acid plant oil (rapeseed oil): Moderate predominantly periportal and partly ubiquitary fat infiltration (stained with oil red O – hematoxylin; red, fat droplets; blue, cell nuclei; magnification x 60).

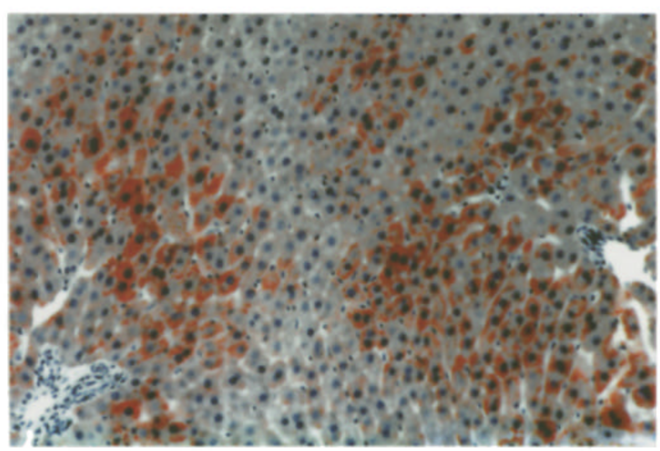

Fig. 3B Higher magnification (x 150) of Fig. 3A: Hepatocytes with mixed-size lipid vesicles (red).

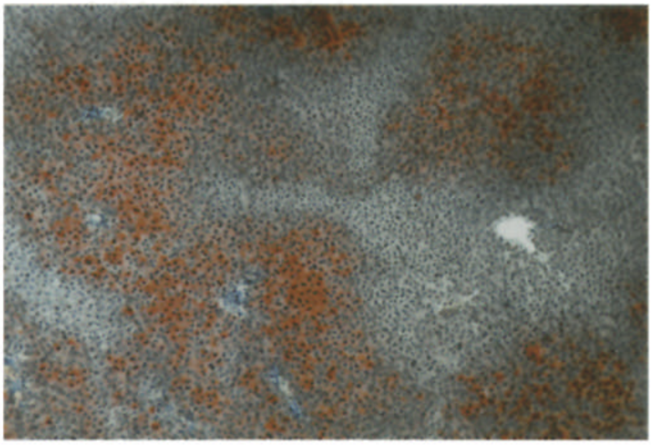

Fig. 4A Liver of rats fed a medium-oleic acid plant oil (common sunflower oil): Moderate predominantly periportal and partly ubiquitary fat infiltration (stained with oil red O – hematoxylin; red, fat droplets; blue, cell nuclei; magnification x 60).

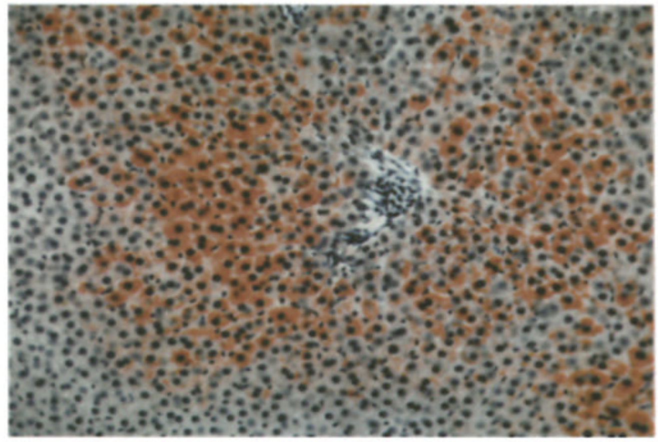

Fig. 4B Higher magnification (x 150) of Fig. 4A: Hepatocytes with mixed-size lipid vesicles (red).

Standard diet

Microphotographs of hepatic tissues of rats fed a standard Altromin diet are given in Fig. 5A–B. Obviously, feeding of the standard diet containing only 40 g fat/kg diet led to no fat infiltration in four out of five animals; only in one animal small lipid vesicles were found which were localized predominantly in periportal fields and, in part, their distribution was ubiquitary. In this context, it has to be kept in mind that lipid droplets are not detectable in histological preparations of "normal" liver (5).

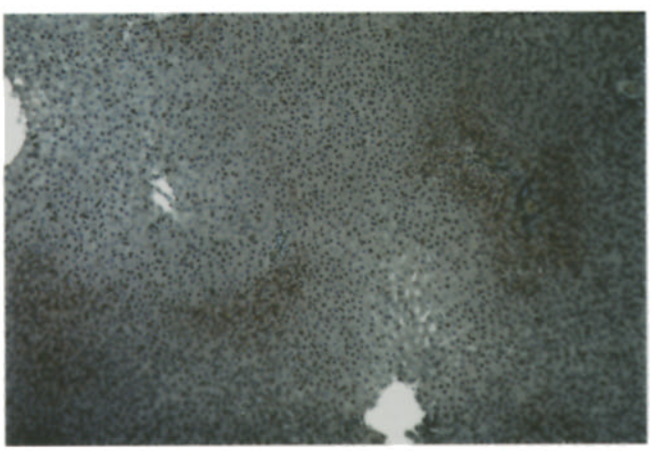

Fig. 5A Liver of rats fed a standard diet: Minimal predominantly periportal and partly ubiquitary fat infiltration (stained with oil red O – hematoxylin; red, fat droplets; blue, cell nuclei; magnification x 60).

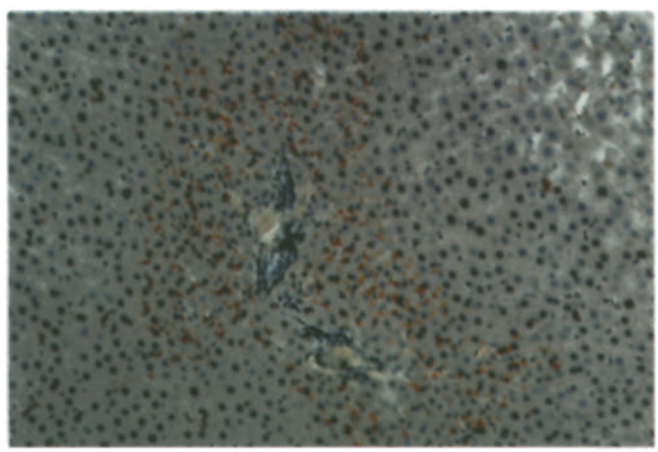

Fig. 5B Higher magnification (x 150) of Fig. 5A: Hepatocytes with single small lipid vesicles (red) only.

Comparison of the various diets

The results of histological examination of liver sections of the experimental animals are summarized in Table 2. They show for the coriander oil group hepatocytes with degenerative fat infiltrations which are characterized by mixed-size lipid vesicles and fatty cysts as well as – in some cases – high numbers of enlarged nuclei in hepatocytes that did not undergo fat infiltration. All the other plant oil groups including high-oleic sunflower group demonstrate, however, simple non-degenerative fat infiltrations in the liver tissues beginning in the periportal fields. These results demonstrate that the varying proportions of oleic and linoleic acids in the different plant oils have no significant influence on the degree of simple fat infiltration in the rat liver.

Discussion

Fatty livers

Various non-glyceridic constituents of plant oils may also induce pathological changes in liver tissues as was suggested recently (17, 20, 27). This assumption is supported by our earlier finding that liver weights of the group fed olive oil are relatively high as compared to the other experimental groups – except for the group fed coriander oil (26) – although histological examination of the livers of the group fed olive oil shows no significant differences as compared to the groups fed high-oleic and conventional sunflower oils or rapeseed oil (Table 2 and Figs. 1–5).

A moderate fat infiltration in the liver cells of the groups fed olive oil, high-oleic and conventional sunflower oil as well as rapeseed oil, as observed by histological investigations (Table 2), is attributed to a rather high level of dietary fat; similar effects have been described by others (10, 27). Highest degree of fat infiltration is observed in liver sections of rats fed coriander oil. In this group, the livers are found to contain hepatocytes with small, medium, and large lipid droplets as well as fatty cysts, the latter resulting obviously from ruptured hepatocytes. Moreover, in the group fed coriander oil extensive enlargement of the area of the nuclei (88.2 ± 15.0 μm^2) vs. 62.7 ± 15.1 μm^2) for the group fed standard diet is observed in hepatocytes that did not undergo fat infiltration. These results point at a non-specific stimulation of hepatocytes which is known to occur frequently as a compensatory response to cell degeneration or cell death, e.g. to the formation of fatty cysts (5, 23). In addition, it is of special interest that the levels of liver lipids of the group fed coriander oil are significantly higher than those of all the other experimental groups, except olive oil group (26). The extensive fat infiltration in the livers of rats fed coriander oil containing high levels of the unusual petroselinic acid contrasts recent histopathological findings reported after feeding less common plant oils, such as evening primrose oil or sal oil, which did not show any adverse physiological effects on liver as compared to oils such as cottonseed, peanut and linseed oils (13). It is possible that the coriander oil contained specific natural components, in addition to triacylglycerols containing petroselinic acid, that give rise to the infiltrations and cellular changes.

It is conceivable that severe fat infiltration (triacylglycerol accumulation) in the livers of rats fed coriander oil might have resulted from the relative inability of the liver lipases to hydrolyze triacylglycerols containing petroselinoyl moieties, as reported for pancreatic lipase in vitro (8, 24). A further reason for the accumulation of triacylglycerols containing petroselinoyl moieties in the liver could be due to the fact that, although petroselinic acid is incorporated into membrane phospholipids, its metabolism is limited, e.g., it is neither desaturated, nor chain-elongated substantially, but the major pathway of metabolism of petroselinic acid is catabolism by β-oxidation (Weber et al., in preparation).

Other organs and tissues

Histological findings of heart, aorta, stomach, and spleen show no histopathological lesions. It is of particular interest that in our studies neither fat infiltration nor necrotic lesions are observed in heart and aorta of rats fed novel "Canola type" rapeseed oil as opposed to earlier observations in hearts of rats fed high-erucic rapeseed oils (for review see 2). These results are in good agreement with our recent findings that no significant differences are observed in the heart weights and heart lipid contents of the animals of the groups fed rapeseed oil and those of the other experimental groups (26).

Conclusion

In conclusion, the results of our study on rats show for all the experimental groups fed high levels of various plant oils (140 g/kg diet) – except the coriander oil group – moderately developed simple non-degenerative fat infiltrations predominantly in the periportal hepatocytes. Marked to severe fat infiltration as well as fatty cysts are observed in livers of rats fed coriander oil which contains large proportions (72 %) of the unusual monoenoic fatty acid, petroselinic acid. Moreover, in the livers of various animals of this group enlarged nuclei are found in numerous hepatocytes that did not undergo fat infiltration which indicates a compensatory response to cell degeneration or cell death. Our data suggest that the severity of fat infiltration and degenerative changes of hepatocytes is qualitatively and quantitatively different after feeding of the coriander oil diet containing petroselinic acid as compared to diets containing plant oils with high proportions of oleic and linoleic (*cis,cis*-9,12-octadecadienoic) acids, e.g. olive oil, Canola-type rapeseed oil, high-oleic and conventional sunflower oil.

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