are lower in milk phospholipids of the SO (high linoleic in the diet) group in respect of the control values. It, thus, appears that, although, in milk phospholipids, the level of linoleic acid in the SO group is 7 times higher than in the SF group, the level of tetraenes (20:4 and 22:4 n-6) is almost half in the former group with respect to the second. The data suggest increased conversion of linoleic acid to polyunsaturated derivatives with low dietary levels of linoleate and inhibition in this conversion in the presence of high levels of linoleate in the diet. The observations of an increased formation of long chain polyunsaturated fatty acids of the (n-6) series with low dietary levels of the precursor linoleic acid, and vice versa, suggest a control of the supply of polyunsaturated fatty acids to the sucklings in conditions of reduced maternal intake of EFA.

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Myocardial Alterations Resulting from Feeding Partially Hydrogenated Marine Oils and Peanut Oil to Rats

ABSTRACT

Myocardial alterations were observed in 5 groups of rats fed diets containing 20% fat for 16 weeks. The incidence was comparable to that from other studies and uniform at 6/20 in hearts from rats fed: partially hydrogenated herring oil to give dietary levels of either 16.7% or 4.6% 22:1; partially hydrogenated redfish/flatfish oil to give 4.5% 22:1 in the dietary fat; and peanut oil (of unknown origin) containing 0.1% 22:1. The incidence was 9/20 in the hearts of rats fed an unrefined and unprocessed redfish oil at a dietary level of 16.0% 22:1 in fatty acids.

INTRODUCTION

Several of the reports of myocardial alteration in experimental animals fed marine oils in partially hydrogenated form have originated in one Canadian laboratory (1-5). The results from one earlier study are difficult to ascribe to marine oils due to the mixture of marine oil and oil from plants of the genus *Brassica* which were involved (6) and a more recent experiment involved only a study of short term lipidosis (7).

EXPERIMENTAL PROCEDURES

To broaden the data base on this subject, the Fisheries and Marine Service, Environment Canada, contracted for the execution of long term studies comparable to those carried out elsewhere, with three marine oil samples as part of 20% (w/w) fat in diets. The levels of docosenoic acids (shorthand notation 22:1), which are thought to be one of the causative agents of myocardial alterations (8,9), were adjusted by dilution with lard-corn oil mixture so that two different partially hydrogenated oils would provide 22:1≈5% of dietary fat. This level was recommended to industry by Health and Welfare Canada as a voluntary restriction pending further studies (10). The other two oils

were the same partially hydrogenated herring oil blended with corn oil (3:1) and an unrefined and unprocessed redfish oil also blended with corn oil (3:1). These oils were selected so that, after corn oil supplementation, the percentage of 22:1 was ca. the same percentage (ca. 16%) in both dietary fats primarily (see below) to compare the effects of the refining and partial hydrogenation. In addition to the control lard-corn oil mixture (3:1), a vegetable oil not hitherto tested in Canada (peanut oil, origin unknown) was included in the experiment. Pertinent details of fatty acid composition obtained in this laboratory are given in Table I. All marine oils fed were supplemented with 0.05% of commercial antioxidant mixture, and α-tocopherol was blended into the diet at 50 mg/100 g diet. Peroxide values at the beginning and end of the experiment were respectively: lard, 0.6 and 0.8; corn oil, 3.3 and 1.8; partially hydrogenated herring oil, 0.9 and 0.8; partially hydrogenated redfish/flatfish oil, 1.3 and 2.8; unprocessed redfish oil plus corn oil, 2.1 and 8.2; and peanut oil, 1.4 and 4.8.

The Sprague-Dawley male rats were Caesarian originated, barrier-sustained, from the Bio-Breeding Laboratories, Ottawa, Canada. At weaning, 20 rats were started on each diet contained 20% fat. The balance of the semisynthetic diet comprised 20% casein, 20% sucrose, 30% cornstarch, 1% vitamin mix (TD 71048, General Biochemicals, Shagrin Falls, Ohio), 4% Bernhart-Tomarelli salt mixture, and 5% Alphacel. After 16 weeks the rats were executed. Organs recovered for wt studies included the adrenals, hearts, and livers (Table II). The hearts were fixed in buffered 10% formalin, embedded in paraffin, and a single frontal section prepared and stained with hematoxylin and eosin. The sections were examined on a "blind" basis by a certified pathologist and scored for incidence and severity of myocardial alterations. Staining of ventricular sections with Sudan IV did not show any fatty deposits associated with the experiment.

RESULTS

The histopathological results, Table III, show no experiment induced myocardial alterations in the animals on the control fat of lard-corn oil mixture. The three diets containing partially hydrogenated fish oils showed a moderate incidence of lesions of low severity. The unrefined fish oil showed a higher incidence with a slightly higher severity score. The adrenals for the rats fed this oil showed slight enlargement. The hearts of the rats on the two oils high in 22:1 showed slight enlargement, respectively 0.307 and 0.292% of body wt for the partially hydrogenated herring oil and unrefined redfish oil, vs 0.276% for controls. Liver wt was increased for all four marine oil diets and depressed for peanut oil compared to the control rats. Basically, our results support the findings of Beare-Rogers, et al., (3) and can be interpolated numerically into their data. With 5% 22:1 from partially hydrogenated herring oils included in diets with 20% (w/w) fat, the balance being lard-corn oil, for 16 weeks, their histopathological score for rat hearts was 2/10. At 15% 22:1 the score was 6/10.

The slightly higher score for the unrefined redfish oil in our experiment may reflect the concentration of effectively all 22:1 into three particular isomers (normally 22:1ω11>22:1ω13>22:1ω9) in lieu of the greater isomer spread found in partially hydrogenated oils (4,11). Alternatively, the animals may have been more highly stressed metabolically because of the inclusion of volatile or thermolabile oxidation products (12) normally removed during hydrogenation and on refining and thus were less able to adapt to the inclusion of 22:1 in the diet.

Astorg and Rocquelin (7) tested peanut oil, partially hydrogenated herring oil, and partially hydrogenated herring oil plus corn oil in short term rat experiments. They found no difference in heart wt, i.e. no short term lipidosis, for the marine oils, but their fatty acid analyses showed more 22:1 deposited from both herring oil diets than from peanut oil containing 0.9% of this acid. Our analysis of the control oil supplied as pure peanut oil (Table I) shows virtually no erucic acid. It is always possible, but improbable, that some low erucic acid Brassica oil could have been mixed into any particular lot of peanut oil. In our study, the amount of such admixture would have been limited, as proportions of other acids, especially 18:3ω3, were not unusual. In an earlier experiment with peanut oil containing no erucic acid, 2.5% behenic acid (22:0), and probably some lignoceric acid (24:0), Rocquelin and Cluzan (13) reported "doubtful myocarditis" in 3 out of 20 rats. Other studies from this group present innocuous results for peanut oil (14,15). Partially hydrogenated marine oils contain 22:0 at 2-5% of fatty acids and 24:0 at 0.5% or less. In these oils, the digestibility of 22:0 and 24:0 is comparable to 22:1 (16,17), but no physiological effect has been attributed to the saturated acids from either animal or vegetable sources.

TABLE I

Details of Important Fatty Acids in Lard and Corn Oil Used for Control Diet in 3:1 Mixture of Dietary Fat Mixtures Used in Rat Feeding Experiments and of Peanut Oil[a]

Fatty acid	Lard	Corn oil	Partially hydrogenated herring oil + Corn oil	Partially hydrogenated herring oil + Lard-corn oil (3:1)	Partially hydrogenated redfish/flatfish oil + Lard-corn oil (3:1)	Unprocessed redfish oil + Corn oil	Peanut oil
14:0	1.7	---	5.2	1.7	3.7	4.1	---
16:0	25.6	10.8	12.8	20.4	18.5	10.2	13.6
18:0	16.4	2.2	2.7	10.1	7.1	1.0	2.8
20:0	0.3	0.4	0.6	0.1	0.9	0.1	1.0
22:0	---	---	1.0	0.2	0.7	---	2.7
24:0	---	---	---	---	---	---	0.4
16:1	2.8	0.1	10.2	4.3	6.8	11.7	0.1
18:1	42.1	24.9	17.1	33.2	27.3	17.2	41.7
20:1	0.8	0.2	12.9	3.9	7.2	14.0	0.7
22:1	---	0.4	16.7	4.6	4.5	16.0	0.1
24:1	---	---	0.6	0.1	0.1	0.4	0.01
18:2	9.1	60.2	18.8	20.8	20.5	16.3	36.5
18:3	0.6	0.8	---	---	---	---	0.1
20:4	---	---	---	---	---	0.2	---
20:5	---	---	---	---	---	5.0	---
22:6	---	---	---	---	---	2.3	---

[a]Further details on all samples, except peanut oil, available from author.

TABLE II

Relative Organ Wt (Percent of Body Wt) for 4 Organs from Rats on 6 Different Dietary Fat Mixtures at 20% (w/w) of Diet for 16 Weeks

	Lard-corn oil (3:1) control	Dietary Oil: Partially hydrogenated herring oil + Corn oil	Partially hydrogenated herring oil + Lard-corn oil (3:1)	Partially hydrogenated redfish/flatfish + Lard-corn oil (3:1)	Unprocessed redfish oil + Corn oil	Peanut oil
Percent 22:1	0.1	16.7	4.6	4.5	16.0	0.1
Adrenal R	0.005	0.005	0.005	0.005	0.006[a]	0.005
Adrenal L	0.005	0.005	0.005	0.005	0.006[a]	0.005
Heart	0.276	0.307[a]	0.274	0.284	0.292[a]	0.284
Liver	2.091	2.497[a]	2.251[a]	2.402[a]	2.463[a]	1.871[a]

[a]Statistically different from control.

TABLE III

Histopathological Scoring[a] of Hearts from Rats Kept on 6 Different Dietary Fat Mixtures at 20% (w/w) for 16 Weeks

Oil or mixture	Percent 22:1 in dietary fat	Histopathological observations of myocardial alterations	
		Severity	Incidence
Lard and corn oil (3:1 mixture)	0.1	0	0/20
Partially hydrogenated herring oil and corn oil (3:1 mixture)	16.7	0.30	6/20
Partially hydrogenated herring oil (same batch) in corn oil and lard	4.6	0.30	6/20
Partially hydrogenated redfish-flatfish oil in corn oil and lard	4.5	0.30	6/20
Unprocessed redfish oil and corn oil (3:1 mixture)	16.0	0.60	9/20
Peanut oil	0.1	0.35	6/20

[a]The following is the grading system that was employed in the assessment of the heart lesions: (A) ± this heart lesion was represented by occasion focal scars or groups of swollen intersitial fibroblasts representing a lesion or an effect of a low grade focal myocardial injury *not related* to experimental treatment and occurring in all groups in occasional animals; (B) + this lesion was regarded as treatment induced and consists of multiple small foci of collapse or replacement fibrosis composed of capillaries, fibroblasts, mononuclear cells, and Anitschkow's myocytes; (C) ++ this lesion represented multiple foci of confluent areas of cellular proliferation or scars surrounding and replacing the degenerating cardiac muscle fibres; and (D) +++ this lesion signified massive myocardial alteration in which remnants of necrotic or degenerating cardiac muscle cells were surrounded by reactive mononuclear cell infiltrates, hemosiderin-laden macrophages, or proliferating fibroblasts. It is emphasized that the histopathological examinations were carried out by the pathologist on a "blind" basis without prior knowledge of the various experimental treatments.

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