TABLE IV

The Effect of Heating on Grease Color

Bleaching treatment (100 g grease)	Color increase (red units) after various heating times at 100C					
	Solvent		Grease A		Grease B	
	4 hr	16 hr	4 hr	16 hr	4 hr	16 hr
None	0.3	1.1	0.1	0.6	0.1	0.1
5% H_2SO_4 (2 ml) + 35% H_2O_2 (4 ml)	0.3	1.0	0.3	0.9	0.2	0.2
1% H_3PO_4 (4 ml) + 35% H_2O_2 (4 ml)	0.1	0.4	0.2	0.3	0.1	0.1
5% Calgon (8 ml) + 35% H_2O_2 (4 ml)	0.3	0.7	0.2	0.4	0.1	0.2
5% H_3PO_4 (20 ml) + 10% $NaClO_2$ (40 ml)	0.2	0.7	0.3	0.7	0.4	0.6
5% H_3PO_4 (4 ml) + 10% $NaClO_2$ (20 ml)	0.2	0.5	0.3	0.6	0.1	0.2

bleaching with hydrogen peroxide or sodium chlorite (Table IV). With the higher concentrations of sodium chlorite, the darkening rate of Grease A was increased, but as this system is not recommended, the effect has little practical importance.

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A Comparison of the Effects of the Polyunsaturated Fatty Acids of Cuttlefish Liver Oil and Cottonseed Oil on Cholesterol Metabolism

T. KANEDA [1] and R. B. ALFIN-SLATER, Division of Nutritional Sciences, School of Public Health, University of California, Los Angeles, California

Abstract

Rats fed ad lib. for 12 weeks either a fat-free diet, a diet containing 15% cottonseed oil, or a diet containing 15% cuttlefish liver oil, with or without exogenous (1%) cholesterol, were studied to evaluate and compare the effect of polyunsaturated fatty acids of cuttlefish liver oil and cottonseed oil on cholesterol metabolism. The results indicate that the longer chain polyunsaturated fatty acids contained in the fish oil cannot substitute for the essential fatty acid, linoleic, either as far as effect on various aspects of cholesterol metabolism are concerned or in the ability to form arachidonic acid. The observed interference of cuttlefish liver oil with the absorption of exogenous cholesterol may be caused by the presence in this oil of the highly unsaturated long chain fatty acids.

Introduction

THE HYPOCHOLESTEROLEMIC effect of vegetable oil has been ascribed to its high polyunsaturated fatty acid content, which, in most vegetable oils consists primarily of the essential fatty acid linoleic acid. Recently investigators have reported hypocholesterolemic activity in fish oils which, although high in polyunsaturated fatty acid, are relatively low in essential fatty acids. Ahrens et al. (1) fed a diet containing 40% menhaden oil (4% essential fatty acids) to two patients, one with hyperlipemia and the other with hypercholesterolemia and found that there was a continued depression of the serum levels of cholesterol, phospholipids, and triglycerides. They concluded that the observed effects on serum lipid levels were unrelated to the essential fatty acid and sterol content of the dietary fats. Other investigators (2,3,4) obtained similar serum cholesterol-depressing effects with various fish oils, and concluded that there was no basis for the suggestion that elevated serum cholesterol levels resulted from an essential fatty acid deficiency.

The investigation was undertaken in order to evaluate these observations and to determine the effects of the polyunsaturated fatty acids in a fish oil, as compared with the linoleic acid contained in cottonseed oil, on some aspects of cholesterol metabolism.

Cuttlefish are common in the Japanese diet, and cuttlefish liver oil from the liver of *Ommastrephes sloani pacificus,* in addition to its commercial uses, is considered an edible oil (obtained from Nippon Kagaku Shiryo Co., Japan). The cuttlefish liver oil used in this investigation had an iodine value of 193 whereas the cottonseed oil had an iodine value of 115. The fatty acid composition of cuttlefish liver oil compared with that of cottonseed oil (determined by gas-liquid chromatography) is shown in Table I. The major differences between these two oils are in the linoleic acid content (cottonseed oil contains 44.7% linoleic acid compared with 1.4% linoleic acid in cuttlefish liver oil) and in the large quantities of longer chain length polyunsaturated fatty acids found in cuttlefish liver oil and not in cottonseed oil.

Experimental

Weanling albino male rats of the U.S.C. strain were divided into groups of 12 each and were fed the following diets: Group I, a fat-free diet (FF); Group II, a diet containing 15% cottonseed oil (CSO);

[1] Present address: Chief, Oils Section, Tokai Regional Fisheries Research Laboratory, Tokyo, Japan.

TABLE I

Principal Fatty Acids of Cuttlefish Liver Oil and Cottonseed Oil

Fatty acid	Cuttlefish oil	Cottonseed oil
	%	%
C14:0	5.1	1.7
14:1	trace	.....
14:2	0.5	.....
14:3[a]	0.6	.....
16:0	19.9	23.9
16:1	6.4	1.6
16:2	1.1	.....
18:0	3.1	4.5
18:1	20.9	23.6
18:2	1.4	44.7
20:1	13.0	.....
?	1.6	.....
20:4	1.1	.....
20:5[a]	7.3	.....
22:3	18.1[a]	.....
22:4		.....
22:5		.....
24:5[a]	trace	.....

[a] No standards available for comparison. These are calculated values.

Group III, a diet containing 15% cuttlefish liver oil CtLO); Group IV, a fat-free diet plus 1% cholesterol (FF+C); Group V, a diet containing 15% cottonseed oil plus 1% cholesterol (CSO+C); and Group VI, a diet containing 15% cuttlefish liver oil and 1% cholesterol (CtLO+C).

The complete diets are listed in Table II. Since the highly unsaturated fatty acids of cuttlefish liver oil are easily oxidized, 0.02% 2,5 di-tert-butylhydroquinone was added to prevent oxidation and the diets in Groups III and VI were changed daily.

The rats were fed the diets ad lib. for 12 weeks. Feces were collected during the last week of the experimental period and analyzed for total lipid, unsaponifiable matter, and cholesterol. At the end of the experimental period, the animals were killed by

TABLE II

Composition of Diets

Dietary constituents	I	II	III	IV	V	VI
Sucrose	67.53	52.53	52.53	66.28	51.28	51.38
Casein	22.23	22.23	22.23	22.23	22.23	22.23
Celluflour	4.0	4.0	4.0	4.0	4.0	4.0
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0
Choline chloride	0.24	0.24	0.24	0.24	0.24	0.24
Vitamin mixture[a]	2.0	2.0	2.0	2.0	2.0	2.0
Cholesterol	.....	.....	.....	1.0	1.0	0.9[b]
Cottonseed oil	.....	15.0	.....	.....	15.0	.....
Cuttlefish liver oil	.....	.....	15.0	.....	.....	15.0
Bile salts	.....	.....	.....	0.25	0.25	0.25

[a] The vitamin mixture had the following composition: Vitamin Test Casein 61.35 g; p-aminobenzoic acid 2.42 g; inositol 2.0 g; tocopherol acetate 1.3865 g; ascorbic acid 0.8 g; thiamine 0.288 g; Ca-pantothenate 0.24 g; niacin 0.24 g; vitamin B_{12} 0.24 g; riboflavin 0.11 g; pyridoxine 0.108 g; crystalets (500,000 U.S.P./g vitamin A, 50,000 U.S.P./g vitamin D) 0.052 g; folic acid 0.046 g; menadione 0.022 g; biotin 0.016 g.

[b] The amounts of cholesterol in cuttlefish liver oil corresponded to 0.14%; therefore group VI was administered 0.9% cholesterol.

the intraperitoneal injection of nembutal after which blood was removed by heart puncture. Livers, lungs, adrenals, and testes were removed, weighed, and frozen for later analyses.

Cholesterol analyses were performed on extracts of plasma and tissue by a modified Sperry-Schoenheimer method as described by Nieft and Deuel (5). Total liver lipids were determined gravimetrically. Hepatic cholesterol esters were isolated by silicic acid chromatography by a modification of the method of Mead and Gouze (6), and their fatty acid composition determined by gas-liquid chromatography using the Barber Coleman Gas Chromatography Apparatus, Model 20, with a 9.5 ft column, ¼ in. diameter, packed with 15.4% by weight of diethylene glycol succinate polyester (DEGS) on 80–100 mesh acid-washed Chromosorb W. Chromatographic peaks were identified by the use of retention times of standards and by log retention times.

TABLE III

Food Consumption and Gain in Weight

Group No.	Wt. gain after 12 weeks	Food consumption	Food efficiency (approx) wt. gain/food eaten
	g	*g/wk*	
I (FF)	180	108	0.14
II (CSO)	317	122	0.21
III (CtLO)	237	85	0.24
IV (FFC)	176	112	0.13
V (CSOC)	308	114	0.24
VI (CtLOC)	252	94	0.22

Results and Discussion

Weight gain, plasma, and liver cholesterol values, and liver total lipid levels of the animals fed the various diets are shown in Tables III and IV. Although the efficiency of the cottonseed oil and cuttlefish liver oil diets were equal and higher than that of the fat-free diet, the gain in weight and food consumption were decreased in the animals fed the cuttlefish liver oil as compared with those on the cottonseed oil diet; but the weight gain was increased over that observed for the animals fed the fat-free diet. A retardation in the growth of rats fed various fish oils, i.e., cod liver, sardine, tuna, and menhaden, has also been reported by Ershoff (7).

Table IV shows that of the groups receiving no cholesterol (Groups I, II, and III), the highest plasma cholesterol levels were found in rats fed the cottonseed oil diet (Group II). Plasma cholesterol values of animals given the fat-free (Group I) and fish oil diets (Group III) were almost identical and lower than the vegetable oil-fed animals. The cholesterol content of the livers of the fat-free and cuttlefish liver oil-fed groups were similarly identical and higher than the value observed in the livers of rats fed the cottonseed oil diet. Evaluations in hepatic cholesterol levels of essential fatty acid deficient rats have previously been reported by this laboratory (8).

The addition of 1% cholesterol to the diet caused a three-fold increase in plasma cholesterol values in the fat-free group (Group IV), whereas levels in the cottonseed oil and fish oil groups (Groups V and VI) were relatively unchanged. Liver cholesterol levels, however, were elevated in all three groups receiving the dietary cholesterol supplement, with the greatest increase occurring in the cottonseed oil group. The hepatic cholesterol content of the animals on the fat-free diet was less affected; the least increase occurred in the livers of rats on the fish liver oil diet.

A possible explanation for the differences in plasma and hepatic cholesterol values found in the various experimental groups is that, as has been previously suggested (9), essential fatty acids are concerned with

TABLE IV

Plasma and Liver Cholesterol Levels and Liver Total Lipids

Group No.	Plasma cholesterol			Liver cholesterol			Total lipid[a] liver
	Total[a]	Free[a]	% Free	Total[a]	Free[a]	% Free	
	mg%	*mg%*		*mg/g*	*mg/g*		*mg/g*
I	43.5±0.8	11.8±0.6	27.1	3.66±0.28	1.99±0.13	54.4	61.5±1.3
II	77.4±0.8	19.6±0.4	25.3	1.94±0.17	1.56±0.13	80.4	50.7±0.6
III	43.9±1.0	8.8±0.6	20.0	3.38±0.25	2.44±0.24	71.6	59.6±0.9
IV	122±3	25.8±1.1	21.0	11.4±0.6	2.56±0.22	22.5	84.6±1.0
V	80.8±1.1	23.2±0.7	28.7	16.4±0.5	2.12±0.12	13.0	105.0±1.0
VI	44.4±1.2	10.0±0.7	22.5	8.6±0.4	2.56±0.18	29.7	75.3±0.8

[a] Including standard error of the mean.

TABLE V

Fecal Lipid, Unsaponifiable Matter, and Sterol Concentration

Group No.	Total lipid in feces,[a]	Unsaponifiable matter		Sterols as cholesterol	
		In fecal fat,[a]	In feces,	In fecal fat, total	In feces, total
	%	%	%	(mg/g)	(mg/g)
I	4.15±0.18	70.7±0.9	2.94	205±1	8.5
II	4.62±0.24	57.6±0.7	2.66	83±1	3.8
III	4.16±0.21	47.6±1.1	1.98	108±1	4.5
IV	13.1±0.4	67.1±0.8	8.75	539±3	70.4
V	8.97±0.23	57.9±0.7	5.19	279±3	25.0
VI	8.09±0.33	70.2±1.1	5.68	346±3	28.0

[a] Including standard error of the mean.

cholesterol transport and that the cholesterol esters of essential fatty acids are more mobile than the esters of saturated or non-essential polyunsaturated long chain fatty acids. In Groups I through III, with no exogenous cholesterol added, cholesterol movement would be from the site of synthesis, the liver, to the plasma. The presence of essential fatty acids in cottonseed oil would therefore tend to elevate plasma cholesterol values while lowering hepatic cholesterol values. In those groups receiving dietary cholesterol supplements, cholesterol biosynthesis would be suppressed (10,11) and a reversal of cholesterol transport (from plasma to liver) would probably occur. In this situation, an increased supply of essential fatty acids would result in lower plasma cholesterol and elevated hepatic cholesterol levels.

The low plasma and hepatic cholesterol levels observed in rats fed the cuttlefish liver oil diet supplemented with cholesterol (Group VI) cannot be explained in this way. These values can only partially be attributed to the lower food consumption in this group (Table III). It is possible, however, that the long-chain polyunsaturated fatty acids in this oil interfere with the absorption of exogenous cholesterol, since the excretion of fecal cholesterol is higher in this group (Group VI) than in the animals fed the cottonseed oil plus cholesterol diet (Table V). Feces which were collected during the last week of the experimental period and analyzed for total sterols showed no significant differences between the cottonseed oil and cuttlefish liver oil groups.

The cholesterol values of testes, lungs, and adrenals are shown in Table VI. Although the cholesterol content of the testes is similar in all groups and that of the lungs similar in both groups fed the oils, the cholesterol content of lungs of animals fed the fat-free diet is elevated, indicating once again the requirement for polyunsaturated fatty acids for cholesterol transport. The elevated adrenal cholesterol level of the animals fed the cuttlefish liver oil may indicate a stress condition induced by this dietary constituent.

Table VII lists the fatty acid composition as determined by gas-liquid chromatography of hepatic cholesterol esters. The fatty acid pattern of the animals fed the cuttlefish liver oil (Group III) was quite similar to that found in the fat-free animals (Group I), the main difference being a higher percentage of polyunsaturated fatty acids (18:3 and higher) in Group III. The very low concentration of linoleic acid (18:2) is noticeable in both groups. The high percentage of oleic acid (18:1) found in all groups has also been observed in similar studies by Okey et al. (12). It is quite possible that the 2.7% of the 20:4 fatty acid in Group III and 2.6% in Group VI are not arachidonic acid but a non-essential eicosatetraenoic acid isomer derived from the more highly unsaturated fatty acids in the fish oil. The 20:3 fatty acid, 5,8,11 eicosatrienoic acid, observed in the fat-free group (Group I) is characteristic of essential fatty acid deficiency (13). When cholesterol was added to the fat-free diet and to the cottonseed oil diet (Groups IV and V, respectively), an increase in monoenoic fatty acids and a decrease in trienoic (Group IV) and tetraenoic fatty acids (Group V) esterified to cholesterol was observed. No similar change was noted in Group VI (cuttlefish liver oil and cholesterol) where the fatty acid pattern remained similar to that obtained by feeding cuttlefish liver oil without the cholesterol supplement (Group III).

It is concluded from these results that the effects of dietary cuttlefish liver oil in the rat are not similar to those observed when cottonseed oil is fed as the source of fat in the diet, and that the non-essential longer chain polyunsaturated fatty acids cannot substitute for linoleic acid in its role in growth and in cholesterol metabolism.

TABLE VI

Cholesterol Content of Various Organs of the Rat

Group No.	Cholesterol		
	Testes[a]	Lung[a]	Adrenal
	(mg/g)	(mg/g)	(mg/g)
I	1.54±0.10	5.09±0.32	37.5
II	1.53±0.10	4.46±0.21	29.2
III	1.48±0.10	4.82±0.85	55.6
IV	1.66±0.21	5.49±0.14	38.1
V	1.28±0.11	4.76±0.18	44.2
VI	1.52±0.09	4.75±0.21	72.7

[a] Including standard error of the mean.
Note: Cholesterol occurs in testes and lungs primarily in the unesterified condition, whereas in the adrenal it is primarily esterified.

TABLE VII

Component Fatty Acids of Hepatic Cholesterol Esters of Rats Fed Various Oils

Fatty acid	Group number					
	I	II	III	IV	V	VI
	%	%	%	%	%	%
14:0	trace	1.8	2.3	0.8	0.8	0.7
14:1	.....	.....	.....	.....	.....	trace
16:A[a]	.....	.....	.....	.....	.....	trace
16:0	34.9	19.5	32.0	31.3	24.8	17.1
16:1	6.5	3.0	6.8	11.1	4.9	17.7
18:A[a]	.....	1.1	0.9	0.4	0.4	1.8
18:0	6.2	4.2	3.4	2.5	2.2	2.0
18:1	44.0	21.6	32.7	53.7	33.7	36.6
18:2	3.6	28.0	2.6	trace	30.8	2.7
18:3	trace	trace	5.7	.....	trace	4.9
20:1	.....	.....	.....	.....	.....	1.7
20:3	4.7	.....	.....	.....	.....	.....
20:4	.....	20.9	2.7	.....	2.6	2.6
20:5[a]	.....	.....	11.1	.....	.....	12.3

[a] No standards available for comparison. These are calculated values.

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