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A comparison of the effectiveness of soy protein isolate and fish oil for reducing the severity of retinoid-induced hypertriglyceridemia

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Abstract

The effectiveness of soy protein isolate (SPI) to reduce the severity of retinoid-induced hypertriglyceridemia has been demonstrated in the rat, but not in human subjects. Because fish oil has been demonstrated to be effective at lowering serum triglyceride concentration in human subjects undergoing retinoid therapy, a study was conducted to compare the ability of SPI with that of fish oil to reduce the severity of retinoid-induced hypertriglyceridemia in the rat. Male Fischer 344 rats, $n = 8$/group, were fed one of four isonitrogenous, isoenergetic diets, consisting of a control diet containing 24% casein +20% corn oil (C), and three 13-cis retinoic acid (13cRA)-supplemented diets containing 24% casein +20% corn oil (R), 24% SPI +20% corn oil (SR), and 24% casein +15% fish oil and 5% corn oil (FR). There was no effect of diet on food intake or final body weight. Serum triglyceride concentration for group R was higher ($P < 0.001$) than for groups C, SR, and FR (7.20 vs. 2.50, 2.84 , and 2.02 mmol/L, respectively); values for groups SR and FR did not differ for this parameter. The serum concentration of 13cRA for group R did not differ from that for groups SR and FR. Thus, SPI was as effective as fish oil in reducing the severity of retinoid-induced hypertriglyceridemia in an animal model, suggesting that it may be effective for this purpose in human subjects.

Keywords: Soy protein; Fish oil; Retinoids; Hypertriglyceridemia

1. Introduction

Retinoids, vitamin A and related compounds, have been used in the prevention and treatment of cancer and in the treatment of dermatologic conditions such as acne and psoriasis [1–7]. Side effects of retinoid therapy include myalgia, cheilitis, hypercalcemia, and hypertriglyceridemia [2–4, 8–10]. The latter condition is of concern as hypertriglyceridemia can increase the risk of developing cardiovascular disease or precipitate an attack of pancreatitis [11–13]. The condition of retinoid-induced hypertriglyceridemia can be simulated in the rat by incorporating 13-cis retinoic acid (13cRA) into purified diets having casein as the source of protein and corn oil as the source of lipid [14–16]. In this animal model, the severity of retinoid-induced hypertriglyceridemia can be reduced by replacing dietary casein with soy protein isolate (SPI) [14,15]. This finding raises the possibility that SPI may be useful in the control of retinoid-induced hypertriglyceridemia in human subjects and may offer an alternative to the use of lipid-lowering drugs for this purpose [4,17], thereby avoiding the side effects of these drugs [18]. Fish oil is effective at lowering serum triglyceride concentrations in patients receiving retinoids, including 13cRA [19–21], although its effectiveness for this purpose in rats fed 13cRA has not been investigated. However, fish oil has been shown to lower serum triglyceride concentration in rats fed retinyl acetate [22,23], suggesting that this oil may exert a triglyceride-lowering action in rats fed 13cRA. It was, therefore, of interest to compare the effect of fish oil with that of SPI on serum triglyceride concentration in rats fed 13cRA, so that the potential of using SPI to lower serum triglyceride concentration in human subjects receiving retinoid therapy could be assessed.

2. Materials and methods

2.1. Animals and diets

Five-week-old male Fischer 344 rats (mean weight of 118 g; Harlan Sprague-Dawley, Houston, TX) were housed

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Table 1
Composition of the experimental diets

Ingredient (g/kg diet)	Diets			
	C[1]	R	SR	FR
Casein[2,3]	240	240		240
Soy protein isolate[4]			240	
Cornstarch	150	150	150	150
Sucrose	290.9	290.8	290.8	290.8
Corn oil[2]	200	200	200	50
Fish oil[5]				150
Vitamin mix[6]	12.0	12.0	12.0	12.0
Mineral mix[7]	42.0	42.0	42.0	42.0
Choline bitartrate	2.4	2.4	2.4	2.4
Cellulose	60.0	60.0	60.0	60.0
13-cis Retinoic acid[2,8]		0.1	0.1	0.1
dl-α-Tocopherol	1.5	1.5	1.5	1.5
Tenox 20[9]	1.2	1.2	1.2	1.2

[1] Abbreviations used: C, control; R, retinoid; SR, soy protein isolate and retinoid; FR, fish oil and retinoid.
[2] Sigma Chemical, St. Louis, MO.
[3] Contained 142 g N/kg.
[4] Bio-Serv, Nutley, NJ; contained (g/kg): N, 141; daidzein, 0.23; genistein, 0.55; glycitein, 0.03. (Values for isoflavones were provided by Dr. E. C. Henley, Protein Technologies International.)
[5] Menhaden oil, Zapata Haynie Corp., Reedville, VA.
[6] AIN-76A vitamin mixture.
[7] AIN-76 mineral mix.
[8] 13-cis retinoic was dissolved in 10 g of ethanol and stirred into dietary oils; after the retinoid had dissolved, ethanol was removed by bubbling nitrogen through the mixture, and dl-α-tocopherol and Tenox 20 were incorporated into the mixture.
[9] Food grade antioxidant (Eastman Products, Kingsport, TN). Contained (g/kg): tertiary butyl hydroquinone, 200; anhydrous citric acid, 100; propylene glycol, 700.

individually in suspended stainless steel cages with mesh wire floors and kept in a windowless room at 22 ± 1°C with a 12:12-hr light-dark cycle. Animal care conformed with guidelines established by the Texas Woman's University Animal Care and Use Committee. Four experimental groups were used (n = 8/group); each was fed one of four purified diets. These consisted of a control diet, which contained casein and corn oil (C), and three 13cRA-supplemented diets, which contained casein and corn oil (R), SPI and corn oil (SR), and casein, fish oil, and corn oil (FR). (Corn oil was included in diet FR in order to meet requirements for linoleic acid [24].) The compositions of the diets and the dietary oils used are given in Tables 1 and 2, respectively. With the exception of the dietary oils, 13cRA and dietary antioxidants, all ingredients were prepared in bulk and stored at −20°C. Fresh batches of diets were formulated three times per week and stored in polyethylene bags, flushed with nitrogen and stored at −20°C. Fresh food was given daily, and all unused food was discarded. After 14 days of consuming the diets, and beginning three hours into the light cycle, animals were anesthetized with diethyl ether and exasanguinated by cardiac puncture. Serum, prepared by centrifuging blood at 4°C for 20 min, was stored at −20°C.

Table 2
Composition of the dietary oils

Fatty acid	Corn oil	Fish oil
g/100 g total fatty acids		
14:0		6.6
16:0	11.5	20.1
16:1	0.1	11.0
18:0	2.0	4.4
18:1n-9	25.7	16.0
18:2n-6	59.7	1.8
18:3n-3	0.9	0.5
20:4n-6		1.4
20:5n-3		22.8
22:6n-3		15.4

2.2. Fatty acid composition of dietary oils

Fatty acid analysis was performed according to Lepage and Roy [25], with fatty acid methyl esters being quantified by chromatography using a 30-m fused-silica column with an internal diameter of 0.25 mm (Supelco, Bellafonte PA). Peaks were identified by comparing retention times with those of authentic fatty acid methyl ester standards (Alltech, Deerfield, IL). The percentage of each fatty acid was determined by the integration of the peak area.

2.3. Determinations on serum

Triglycerides were determined after hydrolysis with lipase [26]. Total serum cholesterol (TC) was determined by sterol esterase and cholesterol oxidase [27]. High-density lipoprotein-cholesterol (HDL-C) was determined after low-density lipoproteins plus very-low-density lipoproteins (LDL + VLDL-C) were precipitated with phosphotungstic acid and magnesium chloride [28]. Serum retinoid concentration was determined by high-performance liquid chromatography [29].

2.4. Statistical analysis

For all parameters, values were compared by analysis of variance; this was followed by Tukey's post hoc test to detect specific intergroup differences; $P < 0.05$ was considerd to be statistically significant.

3. Results

No among group differences were noted for initial body weight, final body weight, or food intake; serum concentrations of 13cRA were unaffected by diet for groups receiving this retinoid (Table 3). Among group differences were noted for serum triglyceride concentration. For example, the value for group R was higher ($P < 0.001$) than for groups C, SR, and FR by 188, 153, and 256%, respectively (Table 4). Among group differences were also noted for TC, HDL-C,

Table 3
Body weight, food intake, and serum 13-cis retinoic acid concentration of rats fed purified diets

	Diet[1]			
	C	R	SR	FR
Body weight (g)[2]:				
Initial	117 ± 8[a,3]	117 ± 9[a]	119 ± 9[a]	119 ± 8[a]
Final	187 ± 5[a]	188 ± 7[a]	183 ± 6[a]	187 ± 6[a]
Food intake (g/day)	14.2 ± 1.3[a]	14.0 ± 1.2[a]	14.7 ± 1.4[a]	14.6 ± 1.4[a]
13-cis retinoic acid (nmol/L)	ND[4]	447 ± 62[a]	469 ± 60[a]	434 ± 52[a]

[1] Abbreviations used: C, control; R, retinoid; SR, soy protein isolate and retinoid; FR, fish oil and retinoid.
[2] Data shown are mean ± SD, n=8.
[3] Values with the same superscript within a row are not significantly different.
[4] Not detectable.

LDL + VLDL-C and the TC: HDL-C ratio (Table 4). For example, the value for LDL + VLDL-C was higher for group R than for all other groups ($P < 0.05$ vs. group SR and $P < 0.001$ vs. groups C and FR).

4. Discussion

As previously demonstrated [14–16], the incorporation of 13cRA into a diet having casein as the source of protein and corn oil as the source of lipid induced a large rise in serum triglycerides, with the value for group R being 188% higher than for group C (7.20 vs. 2.50 mmol/L). The higher value for serum triglycerides was accompanied by a higher value for low-density plus very-low-density lipoprotein cholesterol (LDL + VLDL-C), but a lower value for high-density lipoprotein cholesterol (HDL-C). Similar effects of retinoids on serum lipid concentrations have been noted in human subjects [10]. The present study demonstrates, for the first time, that use of dietary fish oil can reduce the severity of 13cRA-induced hypertriglyceridemia in an animal model of this condition, as serum triglyceride concentration for group FR was considerably lower than for group R (2.02 vs. 7.20 mmol/L). Moreover, serum concentration of 13cRA was unaffected by the use of fish oil. Since fish oil has been shown to reduce serum triglyceride concentration in human subjects given 13cRA [21], this finding suggests that the animal model of retinoid-induced hypertriglyceridemia used here may be useful in determining if other nonpharmacologic approaches (for example, use of SPI) may be feasible for lowering serum triglyceride concentration in human subjects. In keeping with two previous studies [14,15], replacement of dietary casein with SPI was effective at reducing the severity of 13cRA-induced hypertriglyceridemia, as the concentration of serum triglycerides for group SR was lower than for group R (2.84 vs. 7.20 mmol/L). Furthermore, the serum triglyceride concentration for group SR was not different from that of FR. Thus, the use of dietary SPI proved as effective as the use of fish oil for lowering serum triglycerides in rats given 13cRA. Studies designed to compare the effectiveness of SPI with that of fish oil in human subjects treated with either 13cRA or other therapeutically useful retinoids (for example, bexarotene [4]) appear to be justified, particularly since use of SPI rather than in casein did not affect the serum concentration (and thus the pharmacologic effectiveness) of 13cRA. (The lack of effect of replacing dietary casein

Table 4
Serum lipids of rats fed purified diets

	Diet[1]			
	C	R	SR	FR
Triglycerides (mmol/L)[2]	2.50 ± 0.47[a,3]	7.20 ± 1.57[b]	2.84 ± 0.88[a]	2.02 ± 0.25[a]
TC (mmol/L)[4]	2.22 ± 0.12[a]	2.38 ± 0.12[a]	1.97 ± 0.14[b]	1.76 ± 0.13[c]
HDL-C (mmol/L)	1.37 ± 0.07[a]	1.12 ± 0.06[b]	0.94 ± 0.07[c]	0.96 ± 0.05[c]
LDL + VLDL-C (mmol/L)	0.84 ± 0.16[a]	1.27 ± 0.15[b]	1.03 ± 0.18[a]	0.82 ± 0.15[a]
TC: HDL-C	1.62 ± 0.14[a]	2.14 ± 0.19[b]	2.10 ± 0.27[b,c]	1.84 ± 0.17[a,c]

[1] Abbreviations used for diets: C, control; R, retinoid; SR, soy protein and retinoid; FR, fish oil and retinoid.
[2] Data shown are mean ± SD, n=8.
[3] Values with the same superscript within a row are not significantly different.
[4] Abbreviations used for serum lipids: TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL + VLDL-C, low-density + very-low-density lipoprotein cholesterol.

with SPI on serum 13cRA concentration has been noted previously [15].)

Possible mechanisms by which retinoids induce hypertriglyceridemia include induction of increased rates of hepatic triglyceride synthesis and release [30], decreased hepatic rates of removal of VLDL [31], and decreased levels of lipoprotein lipase activity in skeletal muscle [32]. Fish oil may exert a triglyceride-lowering effect by decreasing the hepatic rate of triglyceride synthesis [33,34] and by increasing the activity of lipoprotein lipase in both heart and skeletal muscle [35,36]. The triglyceride-lowering action of fish oil appears to be due to the presence of long chain polyunsaturated omega-3 fatty acids [37,38], which constituted 38% (by weight) of the total fatty acids in the fish oil used in the present study. SPI may also exert its triglyceride-lowering effect by decreasing rates of triglyceride synthesis and removal [39–42]. The triglyceride-lowering effect of SPI may be due to the presence of isoflavones [42] (for example, daidzein and glycitein, which were present at levels of 230 and 550 mg/kg, respectively, in the SPI used in this study) and/or the higher concentration of arginine, a triglyceride-lowering amino acid, in SPI than in casein [16].

The lower serum concentrations of triglycerides in groups SR and FR relative to group R were accompanied by lower concentrations of LDL + VLDL-C. This finding is consistent with the action of fish oil and soy protein on serum lipids in human subjects [43,44]. Total cholesterol (TC) concentration was lowered by the incorporation of fish oil and SPI into the 13cRA-containing diets. This finding is consistent with the effects of dietary interventions involving the incorporation of soy protein and fish into the diets of human subjects, although the effect of fish oil on TC is influenced by the percentage of energy from fat in the diet [45,46]. Both SPI and fish oil reduced HDL-C concentration, as the value for this parameter was lower for groups SR and FR than group R. However, due to concomitant decreases in values for LDL + VLDL-C, the TC:HDL-C ratio for group SR was not different from that of group R, and the value for group FR was lower than for group R. A depressant action of both SPI and fish oil on HDL-C concentration in the rat has been noted in other studies [16,47,48]. However, neither soy protein nor fish oil lowers HDL-C concentration in human subjects [43–46]. Thus, as pointed out previously, this inter-species difference in the effect of some dietary manipulations on HDL-C suggests that changes in LDL + VLDL-C in the rat may be more indicative of their effects in human subjects than changes in HDL-C [16].

The severity of retinoid-induced hyperlipidemia has been decreased by pharmacologic means. For example, gemfibrozil (a fibric-acid derivative) has been used to lower elevated triglyceride concentrations caused by the use of 13cRA in the treatment of chronic myelogenous leukemia [17], and atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitor, has been used for the same purpose in patients receiving bexarotene for the treatment of T-cell lymphoma [4]. There are, however, side effects associated with the pharmacologic control of hyperlipidemia. For example, fibric acid derivatives may cause abdominal discomfort, flatulence, increased lithogenicity of bile, and HMGCoA reductase inhibitors may cause rhabdomyolysis, a condition associated with necrosis or disintegration of skeletal muscle; both classes of lipid-lowering drugs may elevate serum levels of liver enzymes and creatine kinase [18]. Futhermore, use of a single lipid-lowering drug may not be sufficient to control retinoid-induced hypertriglyceridemia. For example, atorvastatin was only partially effective at lowering serum triglyceride concentration in cancer patients receiving bexarotene. Smaller doses of the retinoid were required to prevent hypertriglyceridemia, reducing the effectiveness of this anticancer agent [4]. Use of two lipid-lowering drugs (such as a fibric acid derivative and an HMGCoA reductase inhibitor) can sometimes result in rhabdomyolysis [49]. Thus, there may be a role for the use of nonpharmacologic agents (such as soy protein and fish oil) together with lipid-lowering drugs to lower serum lipids in patients receiving retinoids, rather than relying on drugs alone to achieve this end. The use of atorvastatin and omega-3 fatty acids for the treatment of combined hyperlipidemia demonstrates the feasibility of this approach [50]. Although the effect of SPI on drug-induced hyperlipidemia has not been determined, replacement of casein by SPI in liquid formula diets lowered serum triglyceride concentration (from 8.04 to 5.41 mmol/L) in patients with hyperlipidemia [51].

In conclusion, SPI was as effective as fish oil for the control of retinoid-induced hypertriglyceridemia in an animal model. Since fish oil has been shown to be effective for this purpose in human subjects, this finding raises the possibility that SPI, either singly or in combination with either fish oil or a lipid-lowering drug, may have the potential to reduce the unwanted side effect of hypertriglyceridemia in patients receiving retinoids, thereby allowing these drugs to be used more effectively. Hypertriglyceridemia is a side effect that occurs with the use of drugs other than retinoids, and there may be a role for the use of dietary SPI in the control of other drug-induced dyslipidemias [52,53].

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