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Use of Arginine to Reduce the Severity of Retinoid-Induced Hypertriglyceridemia

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Abstract: *Previous research with an animal model of retinoid-induced hypertriglyceridemia, rats fed a 13-*cis *retinoic acid-containing diet in which casein was the dietary protein, has demonstrated that replacement of dietary casein with soy protein isolate can reduce the severity of this condition. A depressant action of soy protein vs. that of casein on serum triglyceride concentration has also been demonstrated in rats fed purified diets without supplemental retinoid. Because this action of soy protein appears to be due to its having a higher arginine-to-lysine ratio than casein, a study was done to determine how feeding a casein-containing diet supplemented with sufficient arginine, to give a dietary arginine-to-lysine ratio equivalent to that of soy protein, would affect the development of retinoid-induced hypertriglyceridemia. Groups of five-week-old male Fischer 344 rats (*n = *7/group) were fed a control diet containing casein or one of three 13-*cis *retinoic acid-containing diets in which dietary nitrogen was provided as casein, casein + arginine, or soy protein. Incorporation of dietary 13-*cis *retinoic acid resulted in hypertriglyceridemia, with serum triglyceride concentrations of 2.00 and 7.23 mmol/l, or 177 and 640 mg/dl, for groups fed the control and casein + 13-*cis *retinoic acid diets, respectively. For animals fed the 13-*cis *retinoic acid-containing diets, serum triglyceride levels for the casein + arginine and soy protein diet groups (4.75 and 2.92 mmol/l, or 421 and 258 mg/dl, respectively) were significantly lower than for the casein group (*$p < 0.05$*); however, the value was significantly lower for the group fed the soy protein diet than for the group fed the casein + arginine diet. Serum and dietary arginine-to-lysine ratios were highly correlated (*$r = 0.93$, $p < 0.0001$*). Thus, supplementing dietary casein with arginine reduced the severity of retinoid-induced hypertriglyceridemia, but not as effectively as replacing casein with soy protein.*

Introduction

Hypertriglyceridemia is a common side effect resulting from the use of retinoids in the prevention or treatment of cancer (1,2). The condition is also induced when retinoids are used in the treatment of dermatologic conditions such as acne (3). The triglyceride-raising action of retinoids is of concern, because hypertriglyceridemia can increase the risk of developing cardiovascular disease or precipitate an attack of pancreatitis (4,5). The condition of retinoid-induced hypertriglyceridemia can be simulated in the rat by incorporating retinoids (e.g., retinyl acetate and 13-*cis* retinoic acid) into purified diets in which casein is the source of protein (6,7). In this animal model, the severity of retinoid-induced hypertriglyceridemia can be reduced by replacing dietary casein with oilseed proteins (e.g., cottonseed protein and soy protein) (7–9). The ability of oilseed proteins to reduce the severity of retinoid-induced hypertriglyceridemia has been attributed to these proteins having higher levels of arginine, a triglyceride (TG)-lowering amino acid (10,11), than casein (7,9). This suggests that arginine may have the potential to be used to control the severity of retinoid-induced hypertriglyceridemia. Although the effect of feeding rats a retinoid-containing diet supplemented with arginine on serum TG concentration has not been investigated, Vahouny and associates (10), using growing rats, demonstrated that, relative to their casein diet, a diet in which casein was supplemented with arginine exerted a TG-lowering effect comparable to that of a diet in which soy protein isolate was the dietary nitrogen source. In their study, the level of arginine supplementation resulted in the supplemented diet having an arginine-to-lysine ratio equal to that of soy protein. A major objective of the study was to investigate how dietary differences in this ratio might influence the distribution of lipids among the serum lipoproteins, thereby complementing the work of Sugano and co-workers (12), which indicated that the arginine-to-lysine ratio of dietary proteins may play a role in regulating serum TG concentration.

In view of the pharmacological usefulness of retinoids, the undesirability of hypertriglyceridemia as a side effect of retinoid therapy, and the availability of an animal model to test the potential of dietary manipulations (e.g., increasing the dietary level of arginine) to reduce the severity of retinoid-induced hypertriglyceridemia, the following study was done to determine the effect of supplementing dietary casein with arginine on serum TG concentration in rats fed diets containing 13-*cis* retinoic acid. The effectiveness of arginine for this

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purpose was compared with that of soy protein [which has previously been demonstrated to be effective at reducing the severity of retinoid-induced hypertriglyceridemia (7,9)]. This was accomplished by determining serum TG concentration in rats fed casein-containing diets with or without 13-*cis* retinoic acid, as well as in rats fed retinoid-containing diets in which casein was supplemented with arginine or in which soy protein was the dietary nitrogen source. In addition to determining serum TG concentrations, we also determined the levels of other serum lipids [i.e., low-density + very-low-density lipoprotein cholesterol (LDL-C + VLDL-C), high-density lipoprotein cholesterol (HDL-C), and phospholipids (PLs)] and serum amino acids to describe more fully the impact of the dietary treatments on lipid status and to determine whether differences in dietary amino acid content would be reflected by among-group differences in the serum concentrations of amino acids and the arginine-to-lysine ratio.

Materials and Methods

Animals and Diets

Five-week-old male Fischer 344 rats (mean wt 127 g; Harlan Sprague Dawley, Houston, TX) were housed individually in suspended stainless steel cages with mesh wire floors and kept in a windowless room at 22 ± 1°C with a 12:12-hour light-dark cycle. Animal care conformed with guidelines established by the Texas Woman's University Animal Care and Use Committee. Four experimental groups (n = 7/group) were used; each was fed one of the four open-formula diets (i.e., a control diet containing casein and three 13-*cis* retinoic acid-containing diets in which casein, casein + arginine, or soy protein isolate was the dietary nitrogen source, designated Diets C, CR, CAR, and SR, respectively). The compositions of the diets, the method of incorporation of 13-*cis* retinoic acid into the diets, and the amino acid compositions of the dietary proteins are given in Tables 1 and 2. The arginine-to-lysine ratio was 0.48:1 for Diets C and CR and 1.23:1 for Diets CAR and SR.

Throughout the experimental period, food and tap water were allowed ad libitum. Fresh diets were formulated twice weekly 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 exsanguinated by cardiac puncture. Serum, prepared by centrifuging blood at 800 *g* at 4°C for 20 minutes, was stored at –20°C.

Table 1. Composition of the Experimental Diets on a Weight Basis[a]

	Diets[b]			
	C	CR	CAR	SR
Casein[c]	24.0	24.0	24.0	
Soy protein isolate[d]				24.2
Arginine			1.4	
Cornstarch	15.0	15.0	14.52	14.93
Sucrose	29.09	29.08	28.16	28.95
Corn oil	20.0	20.0	20.0	20.0
Vitamin mix[e]	1.2	1.2	1.2	1.2
Mineral mix[f]	4.2	4.2	4.2	4.2
Choline bitartrate	0.24	0.24	0.24	0.24
Cellulose	6.0	6.0	6.0	6.0
13-*cis* Retinoic acid[g]		0.01	0.01	0.01
dl-α-Tocopherol	0.15	0.15	0.15	0.15
Tenox 20[h]	0.12	0.12	0.12	0.12

a: Values are expressed as g/100 g diet.
b: Abbreviations are as follows: C, casein; CR, casein + retinoid (13-*cis* retinoic acid); CAR, casein + arginine + retinoid; SR, soy + retinoid.
c: Casein (Sigma Chemical, St. Louis, MO) contained 14.2 g N/100 g.
d: Soy protein isolate (Bio-Serv, Nutley, NJ) contained (g/100 g) 14.1 N, 0.023 daidzein, 0.055 genistein, 0.003 glycitein. (Values for levels of isoflavones were provided by Dr. E. C. Henley, Protein Technologies International.)
e: AIN-76A vitamin mixture.
f: AIN-76 mineral mixture.
g: 13-*cis*-Retinoic acid (Sigma Chemical) was dissolved in 10 g of ethanol and stirred into corn oil; after retinoid had dissolved, ethanol was removed by bubbling nitrogen through mixture, and *dl*-α-tocopherol and Tenox 20 were incorporated into mixture.
h: Tenox 20, a food-grade antioxidant (Eastman Products, Kingsport, TN) contained (g/100 g) 20 tertiary butyl hydroquinone, 10 anhydrous citric acid, 70 propylene glycol.

Determination of Dietary Nitrogen Content and Amino Acid Composition of Dietary Proteins

Proteins were analyzed for nitrogen as described by Radcliffe and Morrison (13). With the exception of cysteine, methionine, and tryptophan, all amino acids were deter-

Table 2. Amino Acid Composition on a Weight Basis and Arginine-to-Lysine Ratio for Dietary Proteins[a]

	Protein	
Amino Acid	Casein[b]	Soy protein isolate[c]
Alanine	2.77	3.93
Arginine	3.52	7.66
Aspartic acid	6.11	10.71
Cysteine	0.45	1.46
Glutamic acid	20.56	19.10
Glycine	1.74	4.22
Histidine	3.45	3.27
Isoleucine	5.42	4.71
Leucine	8.70	8.16
Lysine	7.36	6.22
Methionine	2.60	1.27
Phenylalanine	4.72	4.98
Proline	10.26	5.37
Serine	5.44	5.28
Threonine	4.46	4.03
Tryptophan	1.16	1.22
Tyrosine	5.02	3.79
Valine	6.24	4.65
Arginine-to-lysine ratio	0.48:1	1.23:1

a: Values (except ratios) are expressed as g/100 g amino acids.
b: To convert to g/16 g N, multiply by 1.17.
c: To convert to g/16 g N, multiply by 1.03.

Table 3. Body Weight and Food Intake of Rats Fed Experimental Diets[a]

	Diet			
	C	CR	CAR	SR
Body wt, g				
Initial	127 ± 6	127 ± 6	126 ± 4	126 ± 4
Final	196 ± 8	192 ± 9	193 ± 6	186 ± 4
Food intake, g/day	14.1 ± 1.4	14.2 ± 0.7	13.2 ± 0.6	13.9 ± 1.3

a: Values are means ± SD of 7 rats/group. See Table 1 footnote for explanation of diet groups.

mined on a hydrolysate produced by heating the proteins with 6 N HCl at 100°C for 24 hours in evacuated, sealed tubes; norleucine served as an internal standard. With use of performic acid oxidation (14), cysteine and methionine were converted to cysteic acid and methionine sulfone, which were hydrolyzed with 6 N HCl as described above. Tryptophan was determined after alkaline hydrolysis, with 3-nitrotyrosine used as an internal standard (15,16). Hydrolysates were analyzed using the ninhydrin reaction after fractionation by ion-exchange chromatography.

Determinations on Serum

TGs were determined after hydrolysis with lipase (17). Total cholesterol (TC) was determined by using sterol esterase and cholesterol oxidase (18). HDL-C was determined after lipoproteins containing apolipoprotein B (LDLs and VLDLs) were precipitated with phosphotungstic acid and magnesium (19). PLs were determined by using phospholipase and choline oxidase (20). Amino acids were determined by high-performance liquid chromatography (21).

Statistical Analysis

For all parameters, values were compared by analysis of variance; where appropriate, this was followed by the Tukey's post hoc test to detect specific intergroup differences; $p < 0.05$ was considered to be statistically significant. Correlations between variables were done using simple regression analysis.

Results

Body Weight and Food Intake

As indicated in Table 3, there were no among-group differences in initial body weight, final body weight, or food intake.

Serum Lipid Levels

As indicated in Table 4, differences between groups fed the control and 13-*cis* retinoic acid-containing casein diet (i.e., Diets C and CR) were found for all lipids other than TC; with the exception of HDL-C, all values were lower for Diet C than for Diet CR. There were differences among groups fed the 13-*cis* retinoic acid-containing diets; for example, Diet CR had significantly higher concentrations of TGs and TC than Diets CAR and SR and significantly higher concentrations of all other lipids (HDL-C, LDL + VLDL-C, and PLs) than Diet SR.

Amino Acids and the Arginine-to-Lysine Ratio in Serum

For five amino acids (citrulline, glutamic acid, isoleucine, taurine, and tryptophan), no among-group differences were noted (Table 5). There was only one difference between groups fed the control and retinoid-supplemented casein-containing diets, with the value for histidine being lower for Diet C than for Diet CR. For five amino acids (e.g., alanine and methionine), the values for Diet CR were higher than for Diet CAR, whereas the values for two amino acids (arginine and ornithine) were lower. Differences between Diets CR and SR were noted for 12 amino acids, with a higher value for Diet CR for 7 of these (e.g., alanine and methionine) and lower for the others (e.g., arginine and ornithine). Differences between the arginine-supplemented and soy groups were noted for seven amino acids, with higher

Table 4. Serum Lipid Levels of Rats Fed Experimental Diets[a–d]

	Diet			
Lipid	C	CR	CAR	SR
TGs	2.00 ± 0.40*	7.23 ± 0.89†	4.75 ± 0.63‡	2.92 ± 0.68*
TC	2.28 ± 0.16*,†	2.40 ± 0.06*	2.22 ± 0.07†	1.69 ± 0.10‡
HDL-C	1.53 ± 0.11*	1.23 ± 0.15†	1.15 ± 0.16†	0.93 ± 0.08‡
LDL + VLDL-C	0.76 ± 0.10*	1.18 ± 0.15†	1.07 ± 0.15†	0.76 ± 0.17*
PLs	2.43 ± 0.19*	2.91 ± 0.23†	2.62 ± 0.17*	2.10 ± 0.10‡

a: Values (means ± SD of 7 rats/group) are expressed in mmol/l.
b: See Table 1 footnote for explanation of diet groups.
c: Abbreviations are as follows: TGs, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL + VLDL-C, low-density + very-low-density lipoprotein cholesterol; PLs, phospholipids.
d: Values with common symbols (*, †, ‡) are not significantly different.

Table 5. Serum Amino Acid Levels and Arginine-to-Lysine Ratio of Rats Fed Experimental Diets[a–c]

Amino Acid	Diet			
	C	CR	CAR	SR
Alanine	837 ± 35*,†	944 ± 64*	763 ± 95†	831 ± 89†
Arginine	167 ± 14*	174 ± 23*	275 ± 28†	276 ± 28†
Asparagine	82 ± 11*	82 ± 17*	64 ± 15*	117 ± 17†
Aspartic acid	38 ± 7*,†	44 ± 8*	38 ± 8*,†	30 ± 7†
Citrulline	84 ± 2	89 ± 3	88 ± 5	89 ± 7
Glutamic acid	191 ± 21	195 ± 42	189 ± 23	178 ± 27
Glutamine	738 ± 62*	700 ± 92*,†	599 ± 58†	603 ± 69†
Glycine	112 ± 7*,†	121 ± 18*	94 ± 17†	170 ± 19‡
Histidine	86 ± 4*	95 ± 6†	83 ± 2*	89 ± 6*,†
Isoleucine	162 ± 19	167 ± 22	144 ± 11	161 ± 18
Leucine	266 ± 25*	259 ± 35*,†	226 ± 14†,‡	202 ± 24‡
Lysine	573 ± 14*	531 ± 61*,†	485 ± 44†	507 ± 42†
Methionine	90 ± 7*	82 ± 9*	69 ± 4†	39 ± 7‡
Ornithine	38 ± 2*	46 ± 5*	79 ± 10†	87 ± 17†
Phenylalanine	67 ± 7*	60 ± 10*,†	55 ± 7†	58 ± 5*,†
Proline	756 ± 121*	774 ± 111*	698 ± 170*	380 ± 46†
Serine	359 ± 113*	371 ± 39*	293 ± 29*	584 ± 48†
Taurine	218 ± 26	203 ± 23	186 ± 34	180 ± 35
Threonine	601 ± 54*	643 ± 48*	489 ± 29†	660 ± 61*
Tryptophan	129 ± 14	131 ± 19	113 ± 12	109 ± 16
Tyrosine	150 ± 14*	143 ± 24*†	123 ± 11†,‡	104 ± 17‡
Valine	414 ± 33*	404 ± 59*	360 ± 21*	287 ± 38†
Arginine-to-lysine ratio	0.29 ± 0.02*	0.33 ± 0.05*	0.56 ± 0.06†	0.55 ± 0.06†

a: Values (means ± SD of 7 rats/group) are expressed in μmol/l (except for ratios).
b: See Table 1 footnote for explanation of diet groups.
c: Values with common symbols (*, †, ‡) are not significantly different.

values for Diet CAR than for Diet SR for three amino acids (methionine, proline, and valine) but lower for the others (e.g., asparagine and glycine).

Discussion

By using an animal model of retinoid-induced hypertriglyceridemia, we have demonstrated, for the first time, that arginine can be used to reduce the severity of this condition, inasmuch as the serum TG concentration for Diet CAR was lower than that for Diet CR (4.75 vs. 7.23 mmol/l, or 421 vs. 640 mg/dl). Supplementing casein with arginine was not, however, as effective as replacing dietary casein with soy protein isolate in reducing the severity of this condition, inasmuch as serum TG concentration was higher for Diet CAR (by 63%) than for Diet SR.

The induction of hypertriglyceridemia in rats fed retinoids incorporated into diets in which casein was the protein source, illustrated in the present study by a higher serum TG concentration for Diet CR than for Diet C (7.23 vs. 2.00 mmol/l, or 640 vs. 177 mg/dl), has been reported previously by others, e.g., Imrhan and associates (22) and Radcliffe and Czajka-Narins (7), who used retinyl acetate and 13-*cis* retinoic acid, respectively, as the dietary retinoid. Retinoid-induced hypertriglyceridemia has also been induced in rats fed nonpurified, closed-formula diets (23), which have protein of animal and vegetable origin (24). Possible mechanisms by which retinoids cause hypertriglyceridemia include their induction of increased rates of hepatic TG synthesis and release (25), decreased hepatic rates of VLDL removal (26), and decreased levels of lipoprotein lipase activity in skeletal muscle (27). The effects of retinoids on TG metabolism appear to be mediated, at least in part, by retinoic acid receptors and the retinoid X receptor (28,29).

One possible reason for the lowering of serum TG concentration as a result of supplementation with dietary arginine is that changes in serum amino acid concentrations accompanying supplementation brought about changes in TG metabolism. These changes included higher serum values for arginine and the arginine-to-lysine ratio for Diet CAR than for Diet CR, with differences being +58% and 70%, respectively. According to Sanchez and Hubbard (30), differences in dietary amino acid content can cause differences in serum amino acid concentrations, which, in turn, can result in a change in the insulin-to-glucagon ratio, considered to be a major determinant of the balance between lipogenesis and lipolysis. Furthermore, Sanchez and Hubbard pointed out that, at levels of arginine found in dietary proteins like soy, an increased intake of arginine results in a higher serum concentration of this amino acid, which could result in a decrease in the insulin-to-glucagon ratio. This decrease in the insulin-to-glucagon ratio could then result in a decrease in the activity of hepatic lipogenic enzymes.

Because the serum values for arginine and the arginine-to-lysine ratio were the same for Diets CAR and SR, some

difference between the supplemented diet and the soy protein diet on serum amino acid pattern other than on this ratio may be responsible for differences in these diets on serum TG concentration. These differences include, for example, higher concentrations of methionine, proline, and valine in Diet CAR than in Diet SR. A nonprotein constituent of soy protein may also be responsible for the lower serum TG concentration for Diet SR than for Diet CAR. One possibility includes the isoflavones [e.g., daidzein and genistein (31)] found in soy protein isolate. (The levels of these isoflavones in the soy protein used in the present study are given in Table 2.) Recently, Crouse and associates (32) showed that soy isoflavones can lower serum TG concentration in subjects with elevated LDL-C levels.

Although the effects of supplementing dietary casein with arginine and replacing casein with soy protein on the metabolism of TGs or TG-rich lipoproteins (e.g., VLDLs) have not been studied in rats fed 13-*cis* retinoic acid-containing diets, the effects of soy protein vs. casein on these aspects of lipid metabolism have been studied in rats fed purified diets. For instance, when soy protein replaces casein as the protein source, rates of TG synthesis and VLDL secretion are reduced (33,34) and the rate of VLDL removal is increased (35). The decreased rates of hepatic TG synthesis may be caused by a suppression of hepatic lipogenic enzymes (e.g., acetyl-CoA carboxylase and fatty acid synthase) (36). Diet-induced decreases in the activity of these enzymes may be responsible for the depressant action of Diets CAR and SR on serum TG concentration in the present study, inasmuch as decreased hepatic TG production could lead to decreased secretion of VLDLs.

The supplementation of casein with arginine resulted in a decrease in TC concentration, with the value for Diet CAR being 8% lower than for Diet CR. This contrasts with the lack of effect of arginine supplementation on TC in a study by Vahouny and associates (10) with rats but agrees with a study by Hurson and associates (37) with human subjects, in which 18 days of arginine supplementation resulted in a 10% decrease in TC. The way in which arginine supplementation leads to a decrease in TC is not known, although, according to Sanchez and Hubbard (30), changes in the dietary intake of amino acids can alter cholesterol metabolism by, for example, affecting the synthesis of cholesterol.

In agreement with studies by Radcliffe and Czajka-Narins (7) and Radcliffe and associates (9), the use of soy protein rather than casein in 13-*cis* retinoic acid-containing diets resulted in decreased TC values, with a value 30% lower for Diet SR than for Diet CR. A depressant action of soy protein vs. casein has also been demonstrated by others for rats fed purified diets (10,38) This cholesterol-lowering action of soy protein is thought to be related to its amino acid composition [including its higher level of arginine and lower level of methionine (39)] and the fact that it contains nonprotein constituents like saponins (40,41). These compounds, which are widely distributed in many plant species (40), may increase the excretion of cholesterol and interrupt the enterohepatic circulation of bile acids (42). In compensation, this interruption may lead to an increased hepatic rate of bile acid synthesis, resulting in an increased removal of cholesterol from the blood via the LDL receptor (42,43).

Previous research, in the rat and humans, has shown that soy protein exerts a cholesterol-lowering effect on LDL-C and VLDL-C, as well as on TC (9,38,44,45). Consistent with this finding, the LDL + VLDL-C concentration was lower (−36%) for Diet SR than for Diet CR. In contrast to the similarity of action of soy protein on TC and the LDL-C and LDL + VLDL-C fractions in rats and humans, soy protein can exert a lowering action on HDL-C in rats (38), but not in humans, where this protein does not affect HDL-C concentration (45). In the present study, soy protein lowered HDL-C concentration for the retinoid-treated rats, with a lower value for Diet SR than for Diet CR. Because of this interspecies difference in the effect of soy protein on HDL-C, changes in TC and LDL + VLDL-C resulting from the use of soy protein to replace casein, or from using arginine to supplement casein, may be more indicative of their effects in humans than are accompanying changes in HDL-C.

As in previous studies where soy protein was used to replace casein in control or 13-*cis* retinoic acid-containing diets (7,9), serum PLs were lower as a result of using soy protein rather than casein, with the difference (SR vs. CR) being −26%. The decrease in serum PL concentration is consistent with the demonstration of Ide and associates (46) that rats fed soy protein have lower hepatic rates of phosphatidylcholine synthesis than those fed casein.

Serum amino acid concentrations, as well as the arginine-to-lysine ratio, were altered as a result of dietary treatments. Some of these alterations can be explained in terms of differences in amino acid composition between the diets resulting from differences in amino acid composition between casein and soy protein and from the incorporation of arginine into Diet CAR (e.g., increased serum levels of arginine for Diets CAR and SR relative to Diets C and CR and an increased concentration of methionine for Diets C, CR, and CAR relative to Diet SR), but others cannot. These unexplained differences include a higher value for histidine for Diet CR than for Diet C and higher levels of methionine and threonine for Diet CR than for Diet CAR. Although an effect of 13-*cis* retinoic acid on histidine concentration has not been reported previously (there appear to have been no previous studies reporting the effect of this retinoid on serum amino acids), a depressant action of arginine supplementation on serum concentrations of methionine and threonine has been reported for human subjects given arginine as arginine aspartate (37). The arginine-to-lysine ratios in serum and diets were positively correlated: $y = 0.33 \times x + 0.15$, where y and x are arginine-to-lysine ratios in serum and diets, respectively ($r = 0.93$, $p < 0.0001$).

The findings from the present study, which demonstrate that arginine supplementation can be used to reduce the severity of retinoid-induced hypertriglyceridemia and confirm that the replacement of dietary casein with soy protein isolate can reduce the severity of this condition, may be relevant to the development of nutritional strategies to lower TG

levels in humans being treated with retinoids. Although the effect of arginine on hypertriglyceridemia in humans has not, apparently, been studied, Hurson and associates (37) demonstrated a decrease in serum TG concentration (from 1.82 to 1.50 mmol/l, or 161 to 133 mg/dl) after 14 days of arginine supplementation in elderly subjects. The effect of soy protein on hypertriglyceridemia (although not of iatrogenic origin) in humans has been reported by Grundy and Abrams (47), who demonstrated that giving hypertriglyceridemic subjects their dietary protein as soy protein, rather than casein, resulted in a decrease in serum TG level (from 8.05 to 5.41 mmol/l, or 713 to 479 mg/dl).

The findings from the present study, taken together with those from the two previously mentioned studies with human subjects (37,47), suggest that the use of arginine or soy protein may help reduce the severity of retinoid-induced hypertriglyceridemia in patients being treated with retinoids. Because hypertriglyceridemia is an unwanted side effect arising from the use of many pharmacological agents [e.g., corticosteroids, thiazide-type diuretics, and nonselective β-blockers (48)], the concept of using arginine and soy protein to reduce the severity of iatrogenically induced hypertriglyceridemia may be applicable not only to retinoids but to other drugs as well.

Acknowledgments and Notes

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References

1. Lee, JS, Lippman, SM, Benner, SE, Lee, JJ, Ro, JY, et al.: Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. *J Clin Oncol* **12,** 937–945, 1994.
2. Triozzi, PL, Walker, MJ, Pellegrini, AE, and Dayton, MA: Isotretinoin and recombinant interferon alfa-2a therapy of metastatic malignant melanoma. *Cancer Invest* **14,** 293–298, 1996.
3. Peck, GL, Olsen, TG, Yoder, FW, Strauss, JS, Downing, DT, et al.: Prolonged remissions of cystic and conglobate acne with 13-*cis* retinoic acid. *N Engl J Med* **300,** 329–333, 1979.
4. Jeppesen, J, Hem, HO, Suadicani, P, and Gyntelberg, F: Triglyceride concentration and ischemic heart disease. An eight-year follow-up in the Copenhagen Male Study. *Circulation* **97,** 1029–1036 1998.
5. Cameron, JL: Lipid abnormalities and acute pancreatitis. *Hosp Pract* **12**(4), 95–101, 1977.
6. Gerber, LE, and Erdman, JW, Jr: Effect of retinoic acid and retinyl acetate upon lipid metabolism in adrenalectomized rats. *J Nutr* **109,** 580–589, 1979.
7. Radcliffe, JD, and Czajka-Narins, DM: Partial replacement of casein with soy protein isolate can reduce the severity of retinoid-induced hypertriglyceridemia. *Plant Foods Hum Nutr* **52,** 97–108, 1998.
8. Radcliffe, JD, and Glass, AC: Dietary cottonseed protein can reduce the severity of retinoid-induced hypertriglyceridemia. *Cancer Detect Prev* **18,** 401–406, 1994.
9. Radcliffe, JD, Imrhan, VL, and Hsueh, AM: The use of soy protein isolate to reduce the severity of 13-*cis* retinoic acid-induced hypertriglyceridemia. *Cancer Detect Prev* **22,** 526–532, 1998.
10. Vahouny, GV, Adamson, I, Chalcartz, W, Satchithanandam, S, Muesing, R, et al.: Effects of casein and soy protein on hepatic and serum lipids and lipoprotein lipid distributions in the rat. *Atherosclerosis* **56,** 127–137, 1985.
11. Eklund, A, and Sjoblom, L: Effects of the source of dietary protein on serum lower density lipoprotein (VLDL + LDL) and tocopherol levels in female rats. *J Nutr* **110,** 2321–2335, 1980.
12. Sugano, M, Ishiwaki, N, Nagata, Y, and Imaizumi, K: Effects of arginine and lysine addition to casein and soya bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. *Br J Nutr* **48,** 211–221, 1982.
13. Radcliffe, JD, and Morrison, SM: Protein quality, food intake and growth in normal and Walker 256 carcinosarcoma-bearing rats. *J Nutr* **110,** 2182–2189, 1980.
14. Weidner, K, and Eggum, BO: Protein hydrolysis: a description of the method used at the Department of Animal Physiology in Copenhagen. *Acta Agric Scand* **16,** 115–119, 1966.
15. Hugli, TE, and Moore, S: Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. *J Biol Chem* **247,** 2828–2834, 1972.
16. Zarkadas, CG, Zarkadas, GC, Karatzas, CN, Khalili, AD, and Nguyen, Q: Rapid method for determining desmosine, isodesmosine, 5-hydroxylysine, tryptophan, lysinoalaline, and the amino sugars in proteins and tissues. *J Chromatogr* **378,** 67–76, 1986.
17. Bucolo, G, and David, H: Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* **19,** 476–482, 1973.
18. Siedel, J, Hagele, EO, Ziegenhorn, J, and Wahlefeld, AW: Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem* **29,** 1075–1080, 1983.
19. Lopes-Virella, MF, Stone, P, Ellis, S, and Colwell, JA: Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem* **23,** 882–884, 1977.
20. Takayama, M, Itoh, S, Nagasaki, T, and Tanimizu, I: A new enzymatic method for determination of serum choline-containing phospholipids. *Clin Chim Acta* **79,** 93–98, 1977.
21. Sarwar, G, and Botting, HG: Rapid analysis of nutritionally important free amino acids in serum and organs (liver, brain, and heart) by liquid chromatography of precolumn phenylisothiocyanate derivatives. *J Assoc Off Anal Chem* **73,** 470–475, 1990.
22. Imrhan, V, Radcliffe, J, Hsueh, A, and Czajka-Narins, D: Soy protein isolate can reduce the severity of retinyl acetate-induced hypertriglyceridemia (abstr). *J Nutr* **125,** 803S, 1995.
23. Radcliffe, JD: Effect of *N*-4-hydroxyphenyl retinamide on lipid metabolism in rats. *Nutr Rep Int* **28,** 799–803, 1983.
24. American Institute of Nutrition: Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. *J Nutr* **107,** 1340–1348, 1977.
25. Gerber, LE, and Erdman, JW, Jr: Hyperlipidemia in rats fed retinoic acid. *Lipids* **16,** 496–501, 1981.
26. Gustafson, S, Vahlquist, C, Sjoblom, L, Eklund, A, and Vahlquist, A: Metabolism of very-low-density lipoproteins in rats with isotretinoin (13-*cis* retinoic acid)-induced hyperlipidemia. *J Lipid Res* **31,** 183–190, 1990.
27. Vahlquist, C, Lithell, H, Michaelsson, G, Selinus, I, Vahlquist, A, et al.: Plasma fat elimination, tissue lipoprotein lipase activity and plasma fatty acid composition during sequential treatment with etretinate and isotretinon. *Acta Derm Venereol Suppl (Stockh)* **67,** 139–144, 1987.
28. Standeven, AM, Beard, RL, Johnson, AT, Boehm, MF, Escobar, M, et al.: Retinoid-induced hypertriglyceridemia in rats is mediated by retinoic acid receptors. *Fundam Appl Toxicol* **33,** 264–271, 1996.
29. Vu-Dac, N, Gervois, P, Torra, IP, Fruchart J-C, Kosykh, V, et al.: Retinoids increase human apo C-111 expression at the transcriptional level via the retinoid X receptor. *J Clin Invest* **102,** 625–632, 1998.
30. Sanchez, A, and Hubbard, RW: Plasma amino acids and the insulin/glucagon ratio as an explanation for the dietary protein modulation of atherosclerosis. *Med Hypotheses* **36,** 27–32, 1991.

31. Setchell, KDR: Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* **68** Suppl, 1333S–1346S, 1998.
32. Crouse, JR, Terry, JG, Morgan, TM, McGill, BL, Davis, DH, et al.: Soy protein containing isoflavones reduces plasma concentrations of lipids and lipoproteins (abstr). *Circulation* **97,** 816, 1998.
33. Iritani, N, Suga, A, Fukuda, H, Katsurada, A, and Tanaka, T: Effects of dietary casein and soybean protein on triglyceride turnover in rat liver. *J Nutr Sci Vitaminol* **34,** 309–315, 1988.
34. Pfeuffer, M, and Barth, CA: Modulation of very low-density lipoprotein secretion by dietary protein is age dependent in rats. *Ann Nutr Metab* **30,** 281–288, 1986.
35. Cohn, JS, Kimpton, WG, and Nestel, PJ: The effect of dietary casein and soy protein on cholesterol and very-low-density lipoprotein metabolism in the rat. *Atherosclerosis* **52,** 219–231, 1984.
36. Iritani, N, Hosomi, H, Fukuda, H, Tada, K, and Ikeda, H: Soybean protein suppresses hepatic lipogenic enzyme gene expression in Wistar fatty rats. *J Nutr* **126,** 380–388, 1996.
37. Hurson, M, Regan, MC, Kirk, SJ, Wasserkrug, HL, and Barbul, A: Metabolic effects of arginine in a healthy elderly population. *J Parent Enteral Nutr* **19,** 227–230, 1995.
38. Morita, T, Oh-hashi, A, Takei, K, Ikai, M, Kasaoka, S, et al.: Cholesterol-lowering effects of soybean, potato, and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *J Nutr* **127,** 470–477, 1997.
39. Carroll, KK, and Kurowska, EM: Soy consumption and cholesterol reduction: review of animal and human studies. *J Nutr* **125,** 594S–597S, 1995.
40. Price, KR, Johnson, IT, and Fenwick, GR: The chemistry and biological significance of saponins in foods and feedingstuffs. *Crit Rev Food Sci Nutr* **26,** 27–135, 1987.
41. Sidhu, GS, and Oakenfull, DG: A mechanism for the hypocholesterolaemic activity of saponins. *Br J Nutr* **55,** 643–649, 1986.
42. Potter, SM: Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr* **125,** 606S–611S, 1995.
43. Baum, JA, Teng, H, Erdman, JW, Jr, Weigel, RM, Klein, BP, et al.: Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *Am J Clin Nutr* **68,** 545–551, 1998.
44. Wong, WW, O'Brian-Smith, E, Stuff, JE, Hatchey, DL, Heird, WC, et al.: Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am J Clin Nutr* **68** Suppl, 1385S–1389S, 1998.
45. Anderson, JW, Johnstone, BM, and Cook-Newell, ME: Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* **333,** 276–282, 1995.
46. Ide, T, Murata, M, and Sunada, Y: Soybean protein-dependent changes in triacylglycerol synthesis and concentration of diacylglycerol in the liver microsomes of fasted rats. *Ann Nutr Metab* **36,** 87–96, 1992.
47. Grundy, SM, and Abrams, JJ: Comparison of actions of soy protein and casein on metabolism of plasma lipoproteins and cholesterol in humans. *Am J Clin Nutr* **38,** 245–252, 1983.
48. Henkin, Y, Jackson, AC, and Oberman, A: Secondary dyslipidemia: inadvertent effects of drugs in clinical practice. *JAMA* **267,** 961–968, 1992.