Effects of Simultaneous Dietary Fish Oil Ingestion and Sulfur Amino Acid Supplementation on the Lipid Metabolism in Hepatoma-Bearing Rats with Hyperlipidemia

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Summary The effects of simultaneous dietary fish oil ingestion and sulfur amino acid (L-methionine and L-cystine) supplementation on serum lipid concentrations and various parameters related to the lipid metabolism were studied in Donryu rats subcutaneously implanted with an ascites hepatoma cell line, AH109A. A diet containing 10% fish oil was found to reduce serum triglyceride, total cholesterol, (very-low-density lipoprotein plus low-density lipoprotein)-cholesterol, phospholipid and nonesterified fatty acid (NEFA) concentrations in these animals, and dietary supplementation of 1.2% L-methionine and L-cystine also suppressed these serum lipid concentrations. Hepatic fatty acid synthesis and the availability of serum NEFA were decreased, and epipleymal adipose tissue lipoprotein lipase (LPL) activity was elevated by dietary fish oil, while LPL activity in various tissues and hepatic fatty acid oxidation were increased by dietary sulfur amino acids, resulting in a reduction in the serum triglyceride concentration by dietary fish oil and sulfur amino acids, respectively. Dietary fish oil suppressed the hepatoma-induced increase in cholesterogenesis in the host liver, and dietary methionine and cystine enhanced bile acid excretion into feces, which were the causes of the hypocholesterolemic effect. In these serum lipid concentrations, there were significant effects of fish oil ingestion and sulfur amino acid supplementation, but no significant interaction between these two factors was seen. These results indicate that dietary fish oil and sulfur amino acid, L-methionine and L-cystine, have hypolipidemic effects in cancer-related hyperlipidemia, and that the effects of these two factors on the decrease in these serum lipid concentrations are additive; these two factors may affect the lipid metabolism via different pathways and mechanisms.

Key Words dietary fish oil, dietary sulfur amino acid, hepatoma, hyperlipidemia, rats

Various cancers affect serum lipoprotein profiles in humans (1–4). Hepatoma also induces an abnormal serum lipid metabolism in humans (5) and animals (6). Rats subcutaneously implanted with an ascites hepatoma cell line of AH109A cells show hyperlipidemia (7) with a notable decrease in high-density lipoprotein (HDL) fraction and an enormous increase in the very-low-density lipoprotein plus low-density lipoprotein (VLDL+LDL) fraction during growth of the hepatoma. AH109A-bearing rats thus provide an endogenously hyperlipidemic animal model that is quite distinct from the cholesterol-loaded animals widely used as an exogenously hyperlipidemic model.

Dietary fish oil, which is rich in n-3 polyunsaturated fatty acids, is well known to suppress concentrations of serum lipids such as triglyceride, cholesterol, phospholipid and nonesterified fatty acid (NEFA) in normal rats (8, 9), rats with cancer-related hyperlipidemia (10), non-insulin dependent diabetes mellitus mice (11), and hyperlipidemic patients (12). Dietary supplementation of amino acids and their derivatives have been demonstrated to affect the lipid metabolism. Sulfur amino acids such as methionine (13) and cystine (13), and basic amino acids such as arginine (14), have been shown to reduce hepatoma-induced hyperlipidemia in hepatoma-bearing rats. Taurine was found to improve ovariectomy-induced hypercholesterolemia (15), and to reduce serum cholesterol concentration in rats fed a cholesterol-containing diet supplemented with bile acid (16).

We previously reported that in AH109A-bearing rats, dietary fish oil reduces hyperlipidemia with the suppression of the hepatoma-induced increase in hepatic cholesterogenesis and fatty acid mobilization from adipose tissue (17). A diet supplemented with sulfur amino acids such as L-methionine and L-cystine also improved hepatoma-induced hypertriglyceridemia and hypercholesterolemia with suppression of fatty acid synthesis in the host liver and restoration of decreased bile acid excretion into feces, respectively (18).

In the present study, AH109A-bearing rats were simultaneously fed two hypolipidemic nutrients, dietary
fish oil and sulfur amino acid, and their effects on serum lipid concentrations and various parameters related to the lipid metabolism were investigated.

**MATERIALS AND METHODS**

*Animals and diets.* Animal experiments were conducted in accordance with criteria established by the Animal Care and Use Committee at Tokyo Noko University. Male Donryu rats (3 wk old) obtained from NRC Haruna (Gunma, Japan) were individually housed in stainless steel cages with wire bottoms and kept on a stock pellet diet (CE-2; CLEA Japan Inc., Tokyo, Japan) in an air-conditioned room with a temperature of 22 ± 2°C, a relative humidity of 60 ± 5%, and an 0800 h to 2000 h light cycle. On the 4th day of the stock pellet feeding, the rats were divided into two groups with similar body weights and for 21 d were fed a basal diet (20% casein diet) containing 10% corn oil (Hayashi Chemicals Co., Tokyo, Japan) or fish oil (San-omega EPA-S18GA, the generous gift of NOF Corporation, Tokyo, Japan). The fish oil contained 0.3% vitamin E, so the corn oil diet was also supplemented with extra vitamin E (Riken E Oil 800; Riken Vitamin Co., Tokyo, Japan) to equalize the vitamin E content of the two diets (350 mg/kg diet). No side effects have been noted at this dose of vitamin E in rats (19). Other components of the diets were purchased from Oriental Yeast Co., Ltd., Tokyo, Japan (casein and cellulose powder), Nihon Nosan Kogyo Co., Yokohama, Japan (α-cornstarch, mineral mixture and vitamin mixture), Nissin Sugar Manufacturing Co., Tokyo, Japan (sucrose) and Wako Pure Chemical Industries, Ltd., Osaka, Japan (choline bitartrate). The fatty acid composition of each oil is shown in Table 1. After being fed the basal diet for 21 d, all rats received a subcutaneous implantation of 5 × 10⁵ AH109A cells (provided by SRL, Tokyo, Japan) in the back to produce a solid hepatoma (7). The corn oil and fish oil groups of rats were each divided into 3 groups with equal body weights and given the basal diet (control group), or the basal diet supplemented with 1.2% L-methionine (Ajinomoto Co., Inc., Tokyo, Japan) or L-cystine (Ajinomoto Co., Inc.). All 6 groups were maintained for an additional 14 d on these diets. The compositions of the experimental diets are shown in Table 2. Water and the diet were available at all times. Animals were deprived of their diet at 0900 h on day 14 but allowed free access to water until killing, which was performed 4 h later by decapitation. Blood was collected and left to clot at room temperature to obtain serum. The liver, epididymal adipose tissue, cardiac muscle, gastrocnemius and solid hepatoma were quickly removed, washed with cold 0.9% NaCl, and blotted on filter paper. Aliquots of these tissues were frozen in liquid nitrogen and stored at −70°C until analysis.

**Lipid analyses.** Total lipids were extracted according to the procedure of Folch et al. (20) from the liver and solid hepatoma. After portions of the chloroform phase had been dried under nitrogen, cholesterol (21), triglyceride (22) and phospholipid (23) were determined as previously described (24). The serum triglyceride and phospholipid concentrations were also determined as described above. The serum NEFA concentration was measured by the method of Kushiro et al. (25). The serum lipoproteins were separated into HDL and VLDL+LDL fractions by the precipitation method (7). The total cholesterol of unfractionated serum and HDL

| Table 1. Fatty acid composition of corn and fish oils.                                      |
|-----------------------------------------------|---------------------|---------------------|
| Fatty acid | Corn oil (g/100 g fatty acids) | Fish oil |
| 14:0      | trace                           | 6.1      |
| 16:0      | 11.5                             | 14.2     |
| 16:1      | trace                           | 7.1      |
| 18:0      | 2.3                             | 2.0      |
| 18:1      | 31.3                            | 11.9     |
| 18:2 n-6  | 53.3                             | 1.4      |
| 18:3 n-3  | trace                           | 1.0      |
| 18:4 n-3  | trace                           | 3.6      |
| 20:5 n-3  | trace                           | 17.7     |
| 22:6 n-3  | trace                           | 11.8     |

| Table 2. Composition of experimental diets (g/kg).                                      |
|-----------------------------------------------|---------------------|---------------------|
| Ingredient | Corn oil | Fish oil |
| Basal | Methionine | Cystine | Basal | Methionine | Cystine |
| Casein | 200 | 200 | 200 | 200 | 200 | 200 |
| α-Cornstarch | 462.7 | 450.7 | 450.7 | 463 | 451 | 451 |
| Sucrose | 170 | 170 | 170 | 170 | 170 | 170 |
| Cellulose powder | 20 | 20 | 20 | 20 | 20 | 20 |
| Corn oil | 100 | 100 | 100 | — | — | — |
| Fish oil | — | — | — | 100 | 100 | 100 |
| Mineral mixture (AIN 76 composition) | 35 | 35 | 35 | 35 | 35 | 35 |
| Vitamin mixture (AIN76 composition) | 10 | 10 | 10 | 10 | 10 | 10 |
| Vitamin E | 0.3 | 0.3 | 0.3 | — | — | — |
| Choline bitartrate | 2 | 2 | 2 | 2 | 2 | 2 |
| L-Methionine | — | 12 | — | 12 | — | — |
| L-Cystine | — | — | 12 | — | — | 12 |
(HDL-cholesterol) were determined by an enzymatic method using a cholesterol C-test kit (Wako Pure Chemical Industries, Ltd.), and the difference between total cholesterol concentration and HDL-cholesterol concentration was regarded as (VLDL+LDL)-cholesterol concentration.

**Measurement of tissue lipolytic activities.** Lipoprotein lipase (LPL) was extracted from epididymal adipose tissue, cardiac muscle and gastrocnemius by the method of Noguchi et al. (26): the tissues weighing 300 mg were homogenized in 0.2 mol/L Tris-HCl buffer (pH=8.5) containing 10 U/mL of sodium heparin (Wako Pure Chemical Industries, Ltd.). Hepatic triglyceride lipase (HTGL) was also extracted from the liver and solid hepatoma in the same way. Lipase (LPL and HTGL) activities were then estimated as described previously (27, 28). Briefly, 0.1 mL of the above-mentioned enzyme source was mixed with 0.1 mL of substrate solution (arabic gum emulsified Tris-HCl buffer, 0.2 mol/L and pH=8.0, containing rat serum to supply adequate apolipoprotein C-II as a LPL activator for LPL, 0.2 mol/L and pH=8.5 for HTGL) containing 9.25 Bq (≈55,500 dpm)/μmol/assay of [carboxyl-14C]triolein (original specific radioactivity=4.1 GBq/mmol, NEN Research Products, Boston, MA). The mixture was incubated at 37˚C for 1 h, followed by the extraction and counting of hydrolyzed [14C]oleic acid. Hormone-sensitive lipase (HSL) activity in adipose tissue was also determined. From epididymal adipose tissue, HSL was extracted with two volumes of 0.2 mol/L phosphate buffer (pH=7.0) by homogenization (29). The enzyme suspension (0.1 mL) previously incubated with 1 mol/L NaCl for 10 min was mixed with 0.1 mL of substrate solution (arabic gum-emulsified 0.2 mol/L phosphate buffer, pH=7.0) containing 9.25 Bq/μmol/assay of [carboxyl-14C]triolein and the mixture was incubated at 30˚C for 1 h. At the end of the incubation period, hydrolyzed [14C]oleic acid was extracted and radioactivity was counted after adding Insta-Gel (Packard Instrument Co., Inc., Meriden, CT). One unit of lipase activity was defined as 1 μmol of fatty acid released per hour.

**Hepatic fatty acid oxidation.** Fatty acid oxidation was estimated using liver slices (30). Liver slices weighing 200 mg were placed in 2 mL of Krebs-Ringer phosphate buffer (pH=7.4) containing 37 kBq/μmol/assay of [1-14C]palmitic acid (original specific radioactivity=310 MBq/mmol, NEN Research Products). Each tube was gassed with 100% O2, stopped tightly with a screwcap, and incubated at 37˚C for 2 h. At the end of the incubation period, 1 mL of 15% ethanolic KOH was added and the mixture was saponified at 75˚C for 2 h. Nonsaponifiable lipids were extracted three times with petroleum ether, and digi-tonin-precipitable sterols were formed, isolated, washed, dissolved in methanol, and counted after addition of a toluene-based scintillator. The residual aqueous layer was acidified to pH=2 with concentrated HCl, and total fatty acids were extracted three times with petroleum ether. Pooled extracts were dried under nitrogen, dissolved in chloroform, and washed twice with water. The chloroform layer was quantitatively transferred to a scintillation vial, dried, and counted as mentioned above.

**Fecal steroid excretion.** Feces were collected for 2 d before sacrifice (day 12–14). Neutral steroids and bile acids were extracted from dried and ground feces after saponification according to the procedure of Yamanaka et al. (32). Fecal 3β-hydroxy neutral steroids and 3α-hydroxy bile acids were determined by enzymatic methods (33), using commercial kits as described previously (34).

**Statistical analysis.** Results were expressed as mean±SE. Data were analyzed by two-way ANOVA with main effects (oil ingestion and sulfur amino acid supplementation) and their interaction using the SPSS Statistics, version 17.0 (SPSS Japan Inc., Tokyo, Japan). Groups were compared by Student’s t-test (between oil groups) or Dunnett’s pairwise multiple comparison t-test (among sulfur amino acid groups) in the case of no interaction being investigated. Significance was assigned to p values <0.05.

**RESULTS**

Both food intake and body weight gain during the 21 d of preliminary feeding were almost identical between the corn oil and fish oil groups (food intakes for these groups were 312.0±5.0 and 299.5±5.3 g, respectively, and body weight gains were 137.9±3.2 and 135.1±3.4 g, respectively).

Initial body weight, food intake and body weight gain for the duration of the 14 d of feeding the experimental diet, and the weights of the liver and solid hepatoma of the rats at the end of the experimental diet period after AH109A implantation are shown in Table 3. The food intake and liver weight of the rats 14 d after AH109A implantation were also similar between the oil diet groups and among the sulfur amino acid groups. Body weight gain was significantly higher in rats fed a cystine-supplemented diet than in those on the basal diet.
Table 3. Initial body weight, food intake, body weight gain, and weights and lipid contents of liver and hepatoma in hepatoma-bearing rats fed corn or fish oil diet supplemented with sulfur amino acids.1

| Measurement                        | Corn oil          | Fish oil         | ANOVA2     |
|------------------------------------|-------------------|------------------|------------|
|                                    | Basal Methionine | Basal Methionine |            |
| Initial body weight (g)            | 228.8±8.0         | 229.1±6.4        | 226.3±7.8 |
|                                    | 229.1±6.3         | 226.2±6.9        | 226.1±7.2 |
| Food intake (g/14 d)               | 191.9±9.3         | 193.2±12.0       | 204.3±8.3 |
|                                    | 201.0±12.7        | 206.3±2.8        | 209.2±9.4 |
| Body weight gain (g/14 d)          | 40.3±5.9          | 52.6±5.7         | 50.4±5.2  |
|                                    | 62.3±8.2*         | 59.3±2.6         | 62.1±3.9* |
| Liver weight (g)                   | 9.74±0.48         | 10.64±0.51       | 10.38±0.52|
|                                    | 10.92±0.46        | 10.70±0.31       | 11.03±0.41|
| Hepatoma weight (g)                | 32.1±5.0          | 32.4±2.5         | 23.5±2.4* |
|                                    | 35.6±4.2          | 21.7±2.8*        | 28.2±3.8* |
| Liver lipid content (μmol/g of liver) |                   |                  |            |
| Triglyceride                       | 4.46±0.62         | 4.83±0.25        | 2.72±0.62*|
|                                    | 4.09±0.25         | 3.84±0.50*       | 2.97±0.12*|
| Cholesterol                        | 6.47±0.49         | 6.39±0.18        | 5.72±0.34*|
|                                    | 6.23±0.52         | 5.87±0.18*       | 5.79±0.10*|
| Phospholipid                       | 34.9±1.2          | 34.1±1.0         | 38.0±1.7* |
|                                    | 32.9±0.9          | 39.5±1.4*        | 38.2±0.4* |
| Hepatoma lipid content (μmol/g of hepatoma) |       |                  |            |
| Triglyceride                       | 1.49±0.25         | 1.49±0.25        | 1.11±0.12*|
|                                    | 1.73±0.12         | 1.36±0.25*       | 1.24±0.12*|
| Cholesterol                        | 7.19±0.44         | 8.17±0.34        | 7.63±0.26 |
|                                    | 6.72±0.28         | 7.01±0.36        | 7.47±0.62 |
| Phospholipid                       | 14.2±1.7          | 14.7±1.9         | 16.5±2.5  |
|                                    | 15.4±1.7          | 17.7±1.4         | 18.5±1.5  |

1Each value represents the mean±SE of six rats. *Significantly different from the corn oil group at p<0.05 by Student’s t-test. #Significantly different from the basal diet group at p<0.05 by Dunnett’s pairwise multiple comparison t-test.

2 Analyzed by two-way ANOVA.

NS: no significance.
Hepatoma weight was significantly lower in the fish oil groups than in the corn oil groups.

Serum lipid concentrations are shown in Fig. 1. Serum triglyceride, total cholesterol, (VLDL+LDL)-cholesterol, phospholipid and NEFA concentrations were significantly lower in the fish oil groups than in the corn oil groups. Rats on diets supplemented with both methionine and cystine also showed significantly reduced serum triglyceride, total cholesterol, (VLDL+LDL)-cholesterol, phospholipid and NEFA concentrations compared with those on the basal diet. Serum HDL-cholesterol concentration showed almost identical values between the oil diet groups and among the sulfur amino acid groups.

Tissue lipid contents are also shown in Table 3. Liver triglyceride, cholesterol and phospholipid contents, and hepatoma triglyceride content were significantly lower in the fish oil groups than in the corn oil groups. Hepatoma cholesterol and phospholipid contents were not significantly different between the oil diet groups or among the sulfur amino acid groups.

Table 4 shows various parameters related to the triglyceride metabolism. LPL activity in epididymal adipose tissue was significantly higher in rats fed the fish oil diet than in those fed the corn oil diet. Both the methionine- and cystine-supplemented diets significantly increased cardiac muscle and gastrocnemius LPL activity, while only the methionine-supplemented diet significantly increased epididymal adipose tissue LPL and hepatoma HTGL activity. Epididymal adipose tissue HSL activity was significantly lower in the fish oil groups than in the corn oil groups. Dietary fish oil significantly lowered hepatic fatty acid synthesis as compared with corn oil. Hepatic total fatty acid oxidation was significantly higher in the fish oil groups than in the corn oil groups. The production of ketone bodies was significantly higher in rats fed the cystine-supplemented diet than in those fed the basal diet. CO2 production was not significantly different between the oil diet groups or among the sulfur amino acid groups. Total fatty acid oxidation was significantly higher in rats fed the cystine-supplemented diet than in those fed the basal diet.

Table 5 shows various parameters related to the cholesterol metabolism. Hepatic cholesterol synthesis was significantly lower in rats fed the fish oil diet than in those fed the corn oil diet. Excretion of feces was not significantly different between the oil diet groups or among the sulfur amino acid groups. Neutral steroid excretion into feces was significantly lower in rats fed the fish oil diet than in those fed the corn oil diet. Bile acid excretion into feces was significantly higher in rats fed diets supplemented with both methionine and cystine than in rats fed the basal diet.

**DISCUSSION**

In the present study, we examined the effect of simul-
Table 4. Tissue lipolytic activities, hepatic fatty acid synthesis, and hepatic fatty acid oxidation in hepatoma-bearing rats fed corn or fish oil diet supplemented with sulfur amino acids. 1

| Measurement                           | Basal Corn Oil | Methionine Corn Oil | Cystine Corn Oil | Basal Fish Oil | Methionine Fish Oil | Cystine Fish Oil | ANOVA2 |
|---------------------------------------|----------------|---------------------|------------------|----------------|---------------------|------------------|--------|
| Lipoprotein lipase activity (U/g of tissue) | 1.51±0.17     | 1.94±0.34           | 1.66±0.19        | 2.20±0.42      | 3.31±0.39           | 2.35±0.28        | 0.05   |
| Epididymal adipose tissue             | 0.46±0.03      | 0.34±0.01           | 0.65±0.04        | 0.34±0.01      | 0.29±0.01           | 0.05±0.01        | p<0.05 |
| Cardiac muscle                        | 4.46±0.71      | 0.71±0.55           | 0.71±0.55        | 0.71±0.55      | 0.71±0.55           | 0.71±0.55        | NS     |
| Gastrocnemius                         | 0.92±0.06      | 0.65±0.04           | 0.65±0.04        | 0.65±0.04      | 0.65±0.04           | 0.65±0.04        | NS     |
| Hepatoma                              | 1.06±0.01      | 1.06±0.01           | 1.06±0.01        | 1.06±0.01      | 1.06±0.01           | 1.06±0.01        | NS     |
| Hepatic triglyceride lipase activity (U/g of tissue) | 0.46±0.03      | 0.34±0.01           | 0.34±0.01        | 0.34±0.01      | 0.34±0.01           | 0.34±0.01        | p<0.05 |
| Hepatic fatty acid synthesis (dpm/100 g liver) | 39.5±5.0       | 52.6±7.8            | 52.6±7.8         | 52.6±7.8       | 52.6±7.8            | 52.6±7.8         | NS     |
| Hepatic fatty acid oxidation (dpm/10−1/2 g liver) | 39.5±5.0       | 52.6±7.8            | 52.6±7.8         | 52.6±7.8       | 52.6±7.8            | 52.6±7.8         | NS     |
| CO2                                   | 6.54±3.2       | 6.54±3.2            | 6.54±3.2         | 6.54±3.2       | 6.54±3.2            | 6.54±3.2         | NS     |
| Ketone bodies                         | 37.1±4.6       | 37.1±4.6            | 37.1±4.6         | 37.1±4.6       | 37.1±4.6            | 37.1±4.6         | NS     |

1 Each value represents the mean ± SE of six rats. * Significantly different from the basal diet group at p<0.05 by Student's t-test. # Significantly different from the basal diet group at p<0.05 by Dunnett's pairwise multiple comparison test. 2 Analyzed by two-way ANOVA. 3 NS: no significance. 4 One unit of lipase activity was defined as 1 μmol of fatty acid released per hour.

Table 5. Hepatic cholesterol synthesis, and fecal weight and steroid excretion in hepatoma-bearing rats fed corn or fish oil diet supplemented with sulfur amino acids. 1

| Measurement                           | Basal Corn Oil | Methionine Corn Oil | Cystine Corn Oil | Basal Fish Oil | Methionine Fish Oil | Cystine Fish Oil | ANOVA2 |
|---------------------------------------|----------------|---------------------|------------------|----------------|---------------------|------------------|--------|
| Hepatic cholesterol synthesis (dpm/10−1/2 g liver) | 7.70±1.31     | 5.5±1.14            | 6.82±1.38        | 7.20±1.38      | 5.5±1.14            | 6.82±1.38        | NS     |
| Fecal weight (g/2 d)                  | 5.5±1.14       | 6.82±1.38           | 5.5±1.14         | 6.82±1.38      | 5.5±1.14            | 6.82±1.38        | NS     |
| Steroid excretion (mol/24 h)          | 0.30±0.078     | 0.86±0.133          | 0.96±0.096       | 0.56±0.23      | 0.86±0.133          | 0.96±0.096       | NS     |
| Neutral steroids (mol/24 h)           | 15.2±2.46      | 17.02±2.05          | 17.02±2.05       | 17.02±2.05     | 15.2±2.46           | 17.02±2.05       | NS     |
| Bile acids                            | 13.2±1.4       | 26.6±5.5            | 31.8±3.4         | 31.8±3.4       | 13.2±1.4            | 26.6±5.5         | NS     |

1 Each value represents the mean ± SE of six rats. * Significantly different from the corn oil group at p<0.05 by Student’s t-test. # Significantly different from the basal diet group at p<0.05 by Dunnett’s test. 2 Analyzed by two-way ANOVA. 3 NS: no significance.
taneous dietary fish oil ingestion and sulfur amino acid reduced by dietary fish oil in normal rats (38). Reduc-
and acid reduce the AH109A-induced increase in serum triglyceride, total cholesterol, (VLDL+LDL)-cholesterol, phospholipid and NEFA concentrations.

Hypertriglyceridemia was improved by dietary fish oil and methionine- and cystine-supplemented diets in hepatoma-bearing rats. This hypotriglyceridemic effect of dietary fish oil is probably due to decreased formation of VLDL by the liver. Dietary fish oil inhibited VLDL-apo-
protein B synthesis (35) and VLDL-triglyceride syn-
thesis (36). In addition, hepatic fatty acid synthesis was suppressed by dietary fish oil in the present study, which may result in decreased VLDL formation. The second possible mechanism of hypotriglyceridemic effect by dietary fish oil is increased removal of VLDL. Increased LPL may be a factor in increased VLDL removal in the periphery. In the present study, we observed that dietary fish oil increased epididymal adipose tissue LPL activity, which may result in increased removal of VLDL. The third possible explanation for the reduced serum trigly-
seride concentration is that the availability of NEFA is decreased in rats fed dietary fish oil; we observed a reduced serum NEFA concentration in rats fed the fish oil diet. It is possible that mobilization of fatty acid from adipose tissue is decreased. In the present study, HSL activity was reduced by dietary fish oil ingestion. NEFA is released by HSL from adipose tissue to serum. Dietary fish oil suppressed HSL activity and thus suppressed adipose tissue triglyceride hydrolysis. It is possible that the observed decreased serum NEFA availability may be due to a general increase in fatty acid oxidation. In the present study, fish oil ingestion enhanced hepatic fatty acid oxidation, as other investigators have reported in normal rats (37), which reduces the pool of hepatic fatty acid available for esterification to triglyceride.

The hypotriglyceridemic effect of methionine and cystine may be due to an elevation of tissue LPL activity and to increased fatty acid mobilization from adipose tissue. In the present study, we observed that the methionine-supplemented diet increased epididymal adipose tissue LPL activity, and cardiac muscle and gastrocnemius LPL activity were also increased by the methionine- and cystine-supplemented diets. The elevation of these tissue LPL activities further hydrolyzes serum triglyceride and reduces its concentration. Serum NEFA concentration decreased significantly in rats fed the methionine- and cystine-supplemented diets compared with that in rats fed the basal diet. This decreased fatty acid mobilization from adipose tissue is thought to be another cause of the hypotriglyceridemic effect of methionine and cystine.

AH109A-induced hypercholesterolemia was im-
proved by dietary fish oil. In the present study, dietary fish oil suppressed hepatic cholesterol synthesis and decreased neutral steroid excretion into feces. Cholesterol biosynthesis is regulated by 3-hydroxy-3-methylglu-
taryl-CoA (HMG-CoA) reductase. Other investigators have found that hepatic HMG-CoA reductase activity is reduced by dietary fish oil in normal rats (38). Reduc-
tion of serum cholesterol concentration may be accom-
panied by a decrease in cholesterol secretion from the liver to serum resulting from the suppression of hepatic cholesterol synthesis, and a decrease in neutral steroid excretion into feces may be caused by the suppression of hepatic cholesterol synthesis.

Hypercholesterolemia in hepatoma-bearing rats was also improved by the methionine- and cystine-supple-
mented diets. In hepatoma-bearing rats, supplementation with both methionine and cystine increased the excretion of bile acid into feces in the corn oil diet (39). In the present study, supplementation with both methionine and cystine increased bile acid excretion into feces; we fed the fish oil diet as well as in rats fed the corn oil diet. Bile acid biosynthesis is rate-
limited by cholesterol 7α-hydroxylase (CYP7A1). It is thought that methionine and cystine may stimulate CYP7A1 activity and/or its protein level, and promote bile acid excretion.

In conclusion, dietary fish oil was found to reduce serum lipid (triglyceride, total cholesterol, (VLDL+LDL)-cholesterol, phospholipid and NEFA) concentra-
tions as compared with corn oil, and these serum lipid concentrations were also suppressed by diets supple-
mented with both methionine and cystine. There were significant effects of fish oil ingestion and sulfur amino acid supplementation, but no significant interaction between these two factors was seen in these serum lipid concentrations. In most of the mechanisms of these serum lipid concentration lowering effects investigated in the present study, there was a significant effect of either fish oil ingestion or sulfur amino acid supple-
mentation. These results indicate that the effects of these two factors on the decrease in these serum lipid concentrations are additive; these two factors may affect the lipid metabolism via different pathways and mecha-

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