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Nutritional value of the marine invertebrates *Anemonia viridis* and *Haliothis tuberculata* and effects on serum cholesterol concentration in rats☆

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Abstract

The purpose of this study was to determine the nutritional value of diets with protein from two marine species (*Haliotis tuberculata* and *Anemonia viridis*) as compared to a high-quality protein reference based on casein or casein supplemented with olive oil. We also investigated the effects of these diets on serum lipid levels. Male rats were fed these diets for 23 days. Protein quality indicators (true digestibility, net protein utilization, biological value) were similar to those obtained for casein-based feeds except for lower true digestibility and net protein utilization values for the *Anemonia viridis* feed. HDL-cholesterol level was significantly higher ($p < 0.05$) in the groups fed marine species or casein supplemented with olive oil than in the casein group. Total-cholesterol level was higher in the group fed *Haliotis tuberculata* fed than in the other groups. These results suggest that these marine species are a good protein source, and that they may have positive effects on serum cholesterol level.

Keywords: Cholesterol; Nutritional value; *Anemonia viridis; Haliotis tuberculata;* Casein

1. Introduction

Fish and marine invertebrates constitute an important food source, supplying proteins of high nutritional value, polyunsaturated fatty acids, vitamins and minerals for human nutrition [1]. However, the availability of traditional seafood products has undergone significant changes in recent decades; particularly as a result of over-fishing. Increasing interest is thus being paid to the identification of non-traditional species with potential food value. Previous studies have reported that marine oil and marine protein modify serum lipid levels [2,3] and have a therapeutic role in the prevention and treatment of coronary artery disease [4,5].

The role of dietary protein in lipid metabolism has become the focus of increasing attention over the last decade [6]. Previous investigations have found that dietary protein level may influence hepatic fatty acids synthesis [7], possibly through the modulation of hepatic delta 6 desaturase activity [8], and VLDL secretion [9]. Hurley *et al*. [10] observed an interaction between dietary proteins and carbohydrates, which produced higher postprandial insulin levels with marine protein; therefore, it is possible that proteins and carbohydrates may influence fasting and postprandial lipid and glucose metabolism through plasma insulin levels. Larson *et al.* [11] suggested that protein affects blood lipid levels, HMG CoA reductase activity, glucagon, cortisol, and weight gain. It is also known that the amino acid composition of dietary proteins is an important determinant of their cholesterolemic properties [12].

Dietary fat is one of the key determinants of serum cholesterol level, and the cholesterol-lowering effect of dietary polyunsaturated fatty acids has been well documented [13,14]. Fats from marine species contain highly polyunsaturated very–long-chain fatty acids and can inhibit the growth of atherosclerotic plaques, checking the tendency to thrombosis and protecting the myocardium from fatal arrhythmia [15]. Moreover, long-chain polyunsaturated fatty acids are essential for normal development. Postnatally, human milk provides long-chain polyunsaturated fatty acids to the newborn. Maternal long-chain polyunsaturated fatty

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Table 1
Composition of the feeds (% dry weight) supplied to the rats in each group

	Casein	Casein + olive oil	*H. tuberculata*	*A. viridis* fried
Protein	12	12	12	12
Cellulose	8	8	8	8
Olive oil	4	14.53	3.29	12.66
Marine oil	—	—	0.71	1.87
Mineral complex[1]	5	5	5	5
Vitamin complex[2]	5	5	5	5
Sucrose	33	33	33	33
Starch	33	33	33	33

In all diets vitamin A and D intakes were 30 Ul/day and 10 Ul/day respectively.

[1] Content (g/kg complex): 330 sodium chloride, 334 dibasic calcium phosphate, 219 potassium hydrogen carbonate, 72 di-potassium hydrogen phosphate, 38 magnesium carbonate, 1.18 manganese sulphate, 0.16 cupric sulphate, 0.08 aluminium-potassium sulphate, 0.08 calcium chloride, 0.04 potassium iodate, 0.08 sodium fluoride, 0.04 zinc carbonate and 9.30 iron II sulphate.

[2] Content (mg/kg complex): 1500 thiamine, 200 riboflavin, 2200 nicotinamide, 200 pyridoxol hydrochloride, 300 calcium pantothenate. Made up to 1000 g with starch.

acids reserves depend upon diet and can be improved by supplementation of docosahexaenoic acid and arachidonic acid during pregnancy and lactation [16].

In the present study, we investigated the nutritional value of two little known marine species, the sea *Anemonia viridis* and the abalone *Haliotis tuberculata*. Specifically, we performed analyses of basic nutritional composition and amino acid contents, and assessed protein quality and effects on serum lipid profile on the basis of feeding trials in rats.

2. Materials and methods

Recently weaned male Sprague-Dawley rats weighing between 50–60 g were maintained in individual metabolic cages. These cages were kept in a room at constant temperature and humidity, with a 12:12 h light:dark cycle. The rats were divided into four groups of eight rats which were given the corresponding feed and water *ad libitum* for 23 days.

The diets were prepared by the Thomas-Mitchell method [17]. The composition of each feed are detailed in Table 1. Protein was determined by the Kjeldahl method, and water content measured with a Scaltec SMO 01moisture analyzer. Lipid content was determined by diethyl ether extraction for 5 h in a Shoxlet. Amino acid content were determined by fluorescence detection reverse phase HPLC with 9-fluorenylmethylchloroformat as derivator agent [18]. Cysteine determinations were performed after sample oxidation. The results obtained are shown in Table 3.

Total lipid contents in control diet and in the *Haliotis tuberculata* diet were adjusted to 4% with olive oil. For the *Anemonia viridis* diet, the raw product was first fried in olive oil to detoxify its toxic polypeptides. Lipid content was then determined by extraction in a Shoxlet; total lipid content in control + oil diet was adjusted to the same value (14,53%) with olive oil. Both control diets were additionally supplemented with 0.6% DL-methionine, since casein is deficient in this amino acid.

Table 2
Basic compositions of the marine species (%)

	A. viridis fresh	*A. viridis* fried	*H. tuberculata*
Water	83.82	58.00	72.48
Protein	11.12	27.81	26.61
Lipids	1.87	14.53	0.71

The protein quality of the four diets was determined by the Thomas-Mitchell [19] technique, based on nitrogen balance in growing rats. Specifically, the rate of nitrogen uptake was measured and compared to the rate of excretion in faeces and urine. The nitrogen contents of diets, urine and faeces were determined using the Kjeldahl method. Protein quality was assessed by calculation of the indicators True Digestibility (TD), Nitrogen Balance (NB), Biological Value (BV), and Net Protein Utilization (NPU) [19].

At the end of the experimental period and after a 24 h fast, the rats were killed by jugular incision and blood samples were collected into heparinized tubes. Serum total cholesterol and HDL-cholesterol were determined with a Spotchem SP-4410 autoanalyzer from MENARINI (a fully automated dry chemistry system). Serum LDL-cholesterol was calculated as: total-cholesterol–HDL-cholesterol. The atherogenic index was calculated as: total-cholesterol/HDL-cholesterol.

Data were subjected to one-way ANOVA, and differences between means were assessed by Dunnett's test and were considered statistically different at $p < 0.05$.

3. Results

The water, protein and fat composition (%) of two marine species are listed in Table 2. Fresh *Haliotis tuberculata* has a higher protein content than fresh *Anemonia viridis* but a slightly lower protein content than fried *Anemonia viridis*. Amino acids compositions are shown in Table 3. The marine species studied have higher glycine, arginine and cysteine contents than the control feed and similar aspartic acid and alanine contents. The remaining amino acids were presents at higher concentration in the casein than in the marine species. Frying of *Anemonia viridis* reduced amino acid content except for serine, histidine, proline, methionine and cysteine.

Table 4 shows food intake and protein quality indicators in each group. Neither mean food intake nor mean weight gain varied significantly among groups. Protein quality indicators for the *Haliotis tuberculata* feed were similar to

Table 3
Amino acid contents of the protein sources (mg amino acid/g protein), after methionine supplementation

Amino acids	Casein	*H. tuberculata*	*A. viridis* fried	*A. viridis* fresh
Aspartic acid	27.52	29.95	25.98	35.82
Glutamic acid	122.98	64.67	54.12	73.50
Serine	29.00	18.18	18.24	23.35
Glycine	8.95	27.73	45.62	61.39
Histidine	32.08	12.17	18.61	24.37
Threonine	58.05	36.07	12.80	37.03
Alanine	20.56	29.62	22.08	31.38
Arginine	26.73	59.50	31.55	44.80
Proline	64.42	17.94	21.73	23.14
Tyrosine	46.47	32.92	15.40	28.76
Valine	67.22	26.94	11.76	32.30
Methionine	31.82	23.66	11.56	19.53
Cysteine	2.90	4.29	4.67	4.26
Isoleucine	53.98	31.32	9.92	25.92
Leucine	94.89	63.14	18.16	45.60
Phenylalanine	52.26	26.28	13.65	43.60
Lysine	62.88	36.48	43.79	58.74

(Other amino acids and non-proteic nitrogen until 1 g)

those for the corresponding control group. True Digestibility and Net Protein Utilization for the *Anemonia viridis* feed were significantly lower than for the corresponding control feed; however, Nitrogen Balance and Biological Value were similar to those obtained for the control diet.

Serum total-cholesterol level in rats fed *Anemonia viridis* decreased in comparison with the other groups (Table 5). In contrast, the animals fed *Haliotis tuberculata* showed an increase in serum total-cholesterol. Mean HDL-cholesterol levels in the groups fed the marine species or casein supplemented with olive oil were significantly higher than in casein no olive oil group. Mean LDL-cholesterol levels showed a significant decrease in rats fed *Anemonia viridis* by comparison with rats that received the control feeds. Mean LDL-cholesterol concentration was likewise significantly lower in rats fed *Haliotis tuberculata* than in the corresponding control group. The atherogenic index in rats fed the marine species was in both cases significantly lower than in the corresponding control group.

4. Discussion

Fresh *Haliotis tuberculata* has a higher protein content than fresh *Anemonia viridis*. The protein content of fried *Anemonia viridis* was higher than that of the fresh product due to water loss. Fresh *Anemonia viridis* has a higher lipid concentration than fresh *Haliotis tuberculata*.

Generally, the lipids of marine animals are the most unsaturated of the animal kingdom. These lipids contain a high proportion of polyunsaturated fatty acids, principally omega-3 fatty acids [20]. The lipid composition of sea anemones is similar to that of other marine invertebrates, with a high proportion of polyunsaturated fatty acids, notably eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Typically, marine animals contain significant amounts of several phospholipids and triglycerides. The phospholipids present at highest concentration in the species studied here are phosphatidylcholine and phosphatidylethanolamine. Phosphatidylinositol content is also high [21]. In the case of the prosobranch genus *Haliotis*, Dustan *et al.* [22] observed that 47–49% of fatty acids are polyunsaturated fatty acids, and that tissues contain a significant amount of cholesterol.

Haliotis tuberculata and fried *Anemonia viridis* are rich in arginine, glycine and cysteine, and have similar aspartic acid and alanine content to the casein. Most amino acids were present at lower concentration in fried *Anemonia viridis* than in the fresh product, probably as a result of the temperatures reached during frying. Amino acid contents in fresh *Anemonia viridis* were similar to those in *Haliotis tuberculata*. Fresh *Anemonia viridis* is rich in aspartic acid, serine, glycine, alanine, arginine and cysteine.

True Digestibility and Net Protein Utilization were significantly lower for the rats fed *Anemonia viridis* than for the corresponding control group. The difference in digestibility may be attributable to other components that accompany the protein, such as lipids, metals, nucleic acids, and polysaccharides [23]. The True Digestibility values obtained (97% for *H. tuberculata*, 92% for *A. viridis*) are similar to the values typically obtained for fish (70–100%), egg (90–100%) and meat (80–100%)[24]. The lower net protein utilization values for the anemone diet than for the

Table 4
Protein quality indicators determined for the different feeds on the basis of feeding trials

	Casein	Casein + olive oil	*H. tuberculata*	*A. viridis* fried
Food intake (g/rat)	113.13 ± 8.56	114.36 ± 7.33	106.55 ± 11.88	123.55 ± 7.33
True digestibility (%)	97.57 ± 0.65	97.30 ± 0.43	96.85 ± 0.91	92.27 ± 0.78^{a,b,c}
Net protein utilization (%)	94.42 ± 1.88	89.39 ± 5.84	89.99 ± 2.72	88.29 ± 2.67^{a}
Nitrogen balance (mg/day)	114.56 ± 16.13	117.70 ± 17.53	93.16 ± 12.46	129.46 ± 17.02^{c}
Biological value (%)	96.78 ± 2.16	91.87 ± 5.92	92.91 ± 2.47	95.68 ± 2.82

Values are means ± SD for eight rats in each group. Values with different superscripts are significantly different according to ANOVA and Dunnett's test; $p < 0.05$: [a] significantly different from casein group; [b] significantly different from casein supplemented with olive oil group; [c]significantly different from the *Haliotis tuberculata* group.

Table 5
Cholesterol levels (mg/dl)

	Casein	*H. tuberculata*	Casein + olive oil	*A. viridis* fried
Total-cholesterol	102.50 ± 9.99	128.43 ± 20.35	108.43 ± 15.01	83.20 ± 12.95[b]
HDL-cholesterol	18.70 ± 10.81	52.11 ± 8.35[a]	53.00 ± 8.45[a]	53.75 ± 11.22[a]
LDL-cholesterol	83.80	76.32	55.43	29.45
Atherogenic index	5.48	2.40	2.04	1.54

Total and HDL-cholesterol values are means ± SD for eight rats in each group.

Values with different superscripts are significantly different according to ANOVA and Dunnett's test; $p < 0.05$: [a] significantly different from the casein group; [b] significantly different from the *Haliotis tuberculata* group.

Serum LDL-cholesterol and atherogenic index values were calculated with the mean values of each group.

corresponding control diet may be attributable to the lower concentration of some amino acids as a result of frying. The NPU values obtained for the anemone and the abalone meals were higher than for other marine products, soy or meat protein [25]. It has been reported that differences in NPU are attributable to differences in amino acid content [26]. The Biological Value of the *Anemonia viridis* and *Haliotis tuberculata* diets were similar to those of the control feeds. Brewer *et al.* [27] observed that the relative amounts of the different essential amino acids may be more important than absolute amounts, because of the complex relationships that exist between them. Also, the presence of a certain amount of nitrogen is required for synthesis of non essential amino acids [26]. The Biological Value obtained for the *Anemonia viridis* and *Haliotis tuberculata* diets can be considered satisfactory, since it is generally considered that a protein with a Biological Value >70% is able to meet nitrogen requirements during growth [26]. Protein quality indicators obtained for these marine species can therefore be considered good, and are similar to those of cod and beef, both of which are considered high-quality protein sources [24,28].

Marine animals are not only valuable as a source of proteins and a wide variety of minerals, but also of lipids, with a high proportion of polyunsaturated fatty acids. Several studies have observed the influence of these polyunsaturated fatty acids on serum lipid levels [29,30] and it is also thought that marine-species proteins may reduce serum cholesterol [31]. The combination of the two effects may therefore accentuate the response.

In rats fed with *Anemonia viridis*, we observed that serum total-cholesterol level was lower than in rats fed with casein or casein supplemented with olive oil diets. This may be attributable to the relatively high polyunsaturated fatty acid content of this species, since it is known that consumption of these fatty acids may increase nitric oxide synthesis [32]. Nitric oxide decreases serum total cholesterol and LDL-cholesterol levels [33,34], and may even help to increase HDL-cholesterol level [35]. Furthermore, polyunsaturated fatty acids may decrease the activity of the hepatic enzyme HMG-CoA, which is the major regulatory enzyme for cholesterol biosynthesis [36]. Polyunsaturated fatty acids may also modify the expression of several genes related to this enzyme [37,38].

Polyunsaturated fatty acids may induce changes in the metabolic pathways and transport of cholesterol in rat liver [39]. Specifically, polyunsaturated fatty acids have been reported to increase biliary excretion of cholesterol [40].

In animals that received the *Haliotis tuberculata* meal, total cholesterol level increased with respect to the other groups due to the presence of significant amounts of cholesterol in the tissues of this mollusc [22,41].

However, HDL-cholesterol was significantly higher in the groups fed marine species than in the control-feed group. Several previous studies have reported an increase in HDL-cholesterol with polyunsaturated fat consumption [42, 43]. Another factor that may have contributed to the high HDL-cholesterol level in these groups is the relatively high arginine contents in the marine species. Arginine is a precursor of nitric oxide. Furthermore, polyunsaturated fats may increase nitric oxide levels [44,45]. Nitric oxide is a beneficial agent in endothelium [46,47], which may increase HDL-cholesterol concentration in serum [35]. Rats that received the casein feed supplemented with olive oil likewise showed increased HDL-cholesterol in serum as expected given the known influence of olive oil intake on HDL-cholesterol levels [48].

LDL-cholesterol in rats fed *Anemonia viridis* was lower than in the other groups, which is attributable to the presence of polyunsaturated fats [49] and olive oil [50] in this feed and to the high cysteine and arginine contents [51,52].

The atherogenic index in rats fed the marine species was lower than in rats fed the casein meal. Low values of the atherogenic index are associated with a low risk of coronary and atherosclerotic diseases [39,53,54]. The atherogenic index in animals fed *Haliotis tuberculata* was below the normal range, reflecting the high HDL-cholesterol levels in serum.

In conclusion, the marine species considered in this study are good sources of high quality protein. The *Anemonia viridis* feed reduced total-cholesterol level in serum while *Haliotis tuberculata* feed increased HDL-cholesterol level; this latter species could thus be beneficial for reducing cardiovascular risk. Both species may be suitable for human consumption, in view of protein content, both quantity and quality, as well as the likewise positive effect on cholesterol levels.

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References

[1] F. Soriguer, S. Serna, E. Valverde, J. Hernando, A. Martin-Reyes, M. Soriguer, A. Pareja, F. Tinahores, I. Esteva, Lipid, protein, and molluscs commonly eaten in the South of Spain, Eur. J. Epidemiol. 13 (1997) 451–463.
[2] K. Tanaka, T. Sakai, I. Ikeda, K. Imaizumi, M. Sugano M, Effects of dietary shrimp, squid and octopus on serum and liver lipid levels in mice, Biosci. Biotechnol. Biochem. 62 (1998) 1369–1375.
[3] M.L. Garg, T. Li, The importance of dietary eicosapentanoic acid ratio in modulation of serum lipid and arachidonic acid levels, Nutr. Res. 14 (1994) 1572–1582.
[4] J.S. Charnock, Omega-3 polyunsaturated fatty acids and ventricular fibrillation: the possible involvement of eicosanoids, Prostaglan. Leukot. Essent. Fatty Acids 61 (1999) 243–247.
[5] S. Nair, J.W. Leitch, J. Falconer, M.L. Grag, Prevention of cardiac arrhythmia by dietary n-3 polyunsaturated fatty acids and their mechanisms of action, J. Nutr. 127 (1997) 383–393.
[6] N.S. Aljawad, E.B. Fyer, H.C. Fryer, Effects of casein, soy, and whey proteins and amino acid supplementation on cholesterol metabolism in rats, J. Nutr. Biochem. 2 (1991) 150–155.
[7] L. Sjoblom, A. Eklund, Dietary protein modifies oxidized cholesterol-induced alterations of linoleic acid and cholesterol metabolism in rats, Biochem. Biophys. Acta 1044 (1990) 187–192.
[8] K. Osada, T. Kodama, K. Minehira, K. Yamada, M. Mugano, Dietary protein modifies oxidized cholesterol-induced alterations of linoleic acid and cholesterol metabolism in rats, J. Nutr. 126 (1996) 1635–1643.
[9] A.D. Kalopissis, G. Griffaton, D. Fau, Inhibition of hepatic very-low-density lipoprotein secretion in obese Zucker rats adapted to a high-protein diet, Metabolism 44 (1995) 19–29.
[10] C. Hurley, I. Galibois, H. Jacques, Fasting and postpandrial lipid and glucose metabolism are modulated by dietary proteins and carbohydrates: role of plasma insulin concentrations, J. Nutr. Biochem. 6 (1995) 540–546.
[11] M.R. Larson, S.M. Donovan, S.M. Potter, Effects of dietary protein source on cholesterol metabolism in neonatal pigs, Nutr. Res. 16 (1996) 1563–1574.
[12] I. Giroux, E.M. Kurowska, K.K. Carrol, Role of dietary lysine, methionine, and arginine in the regulation of hypercholesterolemia in rabbits, J. Nutr. Biochem. 10 (1999) 166–171.
[13] P. Weber, D. Raedertorff, Triglyceride-lowering effect of omega 3 LC-polyunsaturated fatty acids-a review, Nutr. Metab. Cardiovasc. Dis. 10 (2000) 28–37.
[14] I.C. Torres, L. Mira, C.P. Ornelas, A. Melim, Study of effects of dietary fish intake on serum lipids and lipoproteins in two populations with different dietary habits, Br. J. Nutr. 83 (2000) 371–379.
[15] S.L. Connor, W.E. Connor, Are fish oils beneficial in the prevention and treatment of coronary artery disease? Am. J. Clin. Nutr. 66 (1997) 1020S–1031S.
[16] M. Homsh, N. Salem, Long-chain polyunsaturated fatty acids, Biol. Neonate. 74 (1998) 106–120.
[17] H.H. Mitchell, A method of determining the biological value of proteins, J. Biol. Chem. 58 (1924) 873–905.
[18] M.L. Corrales, J.J. Sanchez, Determination of amino acids by High Pressure liquid chromatography (HPLC) in reverse phase, with 9-Fluorenylmethyl chloroformat (9-FMOC) as derivator agent using fluorescence detector. *Afinidad.* XLVI, (1987) 515–518.
[19] D.A. Bender, A.C. Bender, Protein nutrition, in: Nutrition: a reference handbook. Oxford University Press, New York, 1997, pp. 159–192.
[20] R.G. Ackman, Variability of fatty acids and lipids in seafoods, Omega-3 News, 4 (1990) 1.
[21] K.L. Stefanov, W.W. Christie, E.Y. Brechan, S.S. Poppov, S.Andrev, Lipid composition of the red and green forms of *Actinia equina* from the Black Sea, Comp. Biochem. Physiol., 103B (1992) 687–690.
[22] G.A. Dunstan, H.J. Baillie, S.M. Barrett, J.K. Volkman, Effect of diet on the lipid composition of wild and cultured abalone, Aquaculture 140 (1996) 115–127.
[23] J.A.F. Tresguerres, Fisiología del sistema digestivo, in: Fisiología humana (Ed.), Interamericana, Mc Graw-Hill, Madrid, 1993, pp. 698–776.
[24] FAO (1981). Amino-acid content of food and biological data on proteins. Expert Committee. Rome.
[25] O. Moreiras-Varela, B. Ruiz-Roso, Utilización nutritiva del pescado graso, Divulgación 6 (1986) 11–21.
[26] Santidrián, A.S, Digestión de las proteínas y absorción y metabolismo de los aminoácidos, Offarm., (1989) 59–70.
[27] M.F. Brewer, J.D. Halvorson, H.E. Clark, Nitrogen retention of young men who consumed selection patterns of essential amino acids at a constant nitrogen intake, Am. J. Clin. Nutr. 21 (1978) 1–4.
[28] S. Abdel Aziz, L. Hussein, S. Esmail, N. El-Awadi, In vivo rat assay for true protein digestibility and protein quality of beef and meat products extended with soy protein, Int. J. Food Sci. Nutr. 48 (1997) 51–56.
[29] H.O. Bang, J. Dyerberg, H.M. Sinclair, The composition of Eskimo food in north western Greenland, Am. J. Clin. Nutr. 33 (1980) 2657–2661.
[30] J. Dyebenrg, H.O. Bang, E. Stoffersen, S. Moncada, J.R. Vane, Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis, Lancet 2 (1978) 117–119.
[31] I. Demonty, Y. Deshaies, H. Jaques, Dietary proteins modulate the effects of fish oil on triglyceridemia in the rat, Lipids 33 (1998) 913–921.
[32] N. Sepe, L. Pentrocellis, F. Montanaro, G. Cimio, V. DiMarzo, Bioactive long chain N-acylethanolamines in five species of edible bivalve molluscs. Possible implications for mollusc physiology and seafood industry, Biochim. Biophys. Acta. 1389 (1998) 101–111.
[33] G.E. Fraser, Nut consumption, lipids, and risk of a coronary event, Clin. Cardiol. 22 (1999) 11S–15S.
[34] E.M. Kurowska, K.K. Carrol, Hypocholesterolemic properties of nitric oxide. *In vivo* and *in vitro* studies using nitric oxide donor, Biochim. Biophys. Acta. 78 (1998) 303–326.
[35] A. Khedara, Y. Kawai, J. Kayashita, N. Kato, Feeding rats the nitric oxide synthase inhibitor, L-N^Wnitroarginine, elevates serum triglyceride and cholesterol and lowers hepatic fatty acid oxidation, J. Nutr. 126 (1996) 2563–2567.
[36] A. El-Sohemy, M.C. Archer, Regulation of mevalonate synthesis in rat mammary glands by dietary n-3 and n-6 polyunsaturated fatty acids, Cancer Res. 57 (1997) 3685–3687.
[37] S.D. Clarke, D.B. Jump, Regulation of gene transcription by polyunsaturated fatty acids, Prog. Lipid. Res. 3 (1993) 139–149.
[38] D. Jump, S. Clarke, Regulation of gene expression by dietary fat, Ann. Rev. Nutr. 10 (1999) 63–90.
[39] M.J. Smith, A,M. Temmerman, H. Wolters, F. Kuipers, A. Beynen, R.J. Vonk, Dietary fish oil-induced changes in intrahepatic cholesterol transport and bile acid synthesis in rats, J. Clin. Invest. 88 (1991) 943–951.
[40] M. Fukushima, T. Ohashi, M. Sekikawa, M. Nakano, Comparative hypocholesterolemic effects of five animal oils in cholesterol-fed rats, Biosci. Biotechnol. Biochem. 63 (1999) 202–205.

[41] T. Okita, M. Sugano, Effects of dietary protein levels and cholesterol on tissue lipids of rats, J. Nutr. Sci. Vitaminol. 36 (1990) S151–S156.
[42] W.S. Harris, Fish and plasma lipid and lipoprotein metabolism in humans: a critical review, J. Lipid. Res. 30 (1989) 785–807.
[43] T.A. Sanders, M. Vickers, A.P. Haines, Effect on blood lipids and haemostasis of a supplement of cod-liver, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men, Clin. Sci. 61 (1981) 317–324.
[44] J. Soucy, J.,J. LeBlanc, The effects of a beef and fish meal on plasma amino acids, insulin and glucagon, Nutr. Res. 19 (1999) 17–24.
[45] A. Nordoy, L.F. Hatcher, S.M. Goodight, G.A. Fitzgerald, W.E. Connor, Effects of dietary fat content, saturated fatty acids and fish oil on eicosanoid production and hemostatic parameters in normal men, J. Lab. Clin. Med. 123 (1994) 914–920.
[46] A. Calver, J. Collier, P. Vallance, Nitric oxide and cardiovascular control, Exp. Physiol. 78 (1993) 303–326.
[47] I. Sonaka, Y. Futami, T. Kobayashi, T. Umezawa, T. Maki, Effects of protein restriction on nitrogen balance and cardiovascular functions in aged rats, J. Gerontol. Biol. Sci. 48 (1993) B145–B150.
[48] P. Mata, J.A. Garrido, J.M. Ordovás, E. Blazquez, L.A. Alvarez-Sala, J. Rubio, R. Alonso, M. De Oya, Effect of dietary monosaturated fatty acid on plasma lipoproteins and apolipoproteins in women, Am. J. Clin. Nutr. 56 (1992) 77–83.
[49] N.W. Chang, P.C. Haung, Effects of the ratio of polyunsaturated and monounsaturated fatty acids to saturated fatty acid on rat plasma and liver lipid concentrations, Lipids. 33 (1998) 481–487.
[50] E. Vognild, E.O. Elvevoll, J. Brox, R.L. Olse, H. Barstad, M. Aursand, B. Østervd, Effect of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses and serum lipids in Healthy Humans, Lipids. 33 (1998) 427–436.
[51] C. Moundras, C. Demigne, C. Morand, M.A. Leurat, C. Remesy, Lipid metabolism and lipoprotein susceptibility to peroxidation are affected by a protein-deficient diet in the rat, Nutr. Res. 17 (1997) 125–135.
[52] S. Kubow, N. Goyette, K.G. Kosk, Tissue lipid peroxidation and serum lipoproteins in hamsters are affected by dietary protein composition, Nutr. Res. 17 (1997) 271–281.
[53] K. Ando, K. Nagata, M. Beppu, K. Kikugawa, Effect of n-3 fatty acid supplementation on lipid peroxidation and protein aggregation in rat erythrocyte membranes, Lipids. 33 (1998) 505–512.
[54] S.A. Kobayashi, Scientific basis for the longevity of Japanese in relation to diet and nutrition, Nutr. Rev. 50 (1992) 353–354.