British Journal of Nutrition (2006), **95**, 696–702
DOI: 10.1079/BJN20051682

Differential effects of konbu and nori seaweed dietary supplementation on liver glutathione status in normo- and hypercholesterolaemic growing rats

Aránzazu Bocanegra[1], Juana Benedí[2] and Francisco J. Sánchez-Muniz[1]*

[1]*Departamento de Nutrición y Bromatología I (Nutrición), Facultad de Farmacia, Universidad Complutense de Madrid, 28040-Madrid, Spain*
[2]*Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense de Madrid, 28 040-Madrid, Spain*

(Received 10 June 2005 – Revised 24 November 2005 – Accepted 24 November 2005)

The effects of six balanced diets for 3 weeks on dietary intake, growth, liver weight and fat, plasma cholesterol, total antioxidant capacity, liver glutathione status and antioxidant enzymes in growing male Wistar rats were studied. Ten rats per group were fed casein- and soyabean-based diets with or without 2·4 % cholesterol-raising agent. Seven percent of the diet consisted of a cellulose–wheat starch mix (35:65; control diets), freeze-dried nori (nori diets) or konbu (konbu diets). The 7 % dietary supplement of seaweeds was well accepted and induced normal growth rates in rats. Except for food intake, total and reduced glutathione and total antioxidant capacity, dietary cholesterol addition significantly affected (at least $P<0·05$) all parameters studied. Alga consumption affected total and reduced glutathione, glutathione reductase activity, plasma cholesterol, and total and cholesterol-adjusted total antioxidant capacity (at least $P<0·05$). A significant cholesterol–alga interaction was found for liver weight, total glutathione peroxidase (GSH-Px) and the Se-dependent GSH-Px:total GSH-Px ratio (at least $P<0·05$). GSH-Px activity increased in cholesterol-fed nori rats mainly as Se-dependent GSH-Px, while in konbu and control groups the GSH-Px activity was related to increases in both non-Se-dependent and Se-dependent GSH-Px activities. The decrease in the antioxidant status of konbu rats was related to the high As content of this alga, which led to a compensatory increase in glutathione reductase activity in these animals. In conclusion, although some antioxidant compounds are present in algae, other dietary compounds, such as As, induced poor antioxidant status in rats.

Algae: Food intake: Hypercholesterolaemic rats: Liver: Antioxidant status

In the last decade, consumption of algae has considerably increased in industrialised countries (Rupérez & Saura-Calixto, 2001). Due to their composition, algae have been recommended as dietary supplements, especially for vegetarians (Conquer & Holub, 1996). The protein content in seaweed is significant, generally being higher in red than in brown algae (Fleurence, 1999). Algal fat content is low, but 20–50 % of the total fatty acid content consists of *n*-3 fatty acids (Jeong *et al.* 1993). Seaweeds are rich in polysaccharides, vitamins, minerals and trace elements (Ergueta Martínez, 2001), but data regarding their bioavailability are limited (Bocanegra *et al.* 2003). These marine vegetables are rich in minor compounds such as phytosterols (Ergueta Martínez, 2001) and constitute potential sources of dietary fibre that differ chemically and physico-chemically from those of land plants, for which reason their physiological effects on man may differ from those of terrestrial plant foods (Lahayen, 1991; Jiménez-Escrig & Sánchez-Muniz, 2000). As alga fibre is a powerful cation exchanger (Figueira *et al.* 2000), these plants can contain high amounts of heavy metals (Phaneuf *et al.* 1999) and thus represent a potential health risk (Ho, 1990).

Moreover, seaweeds may present antioxidant properties (Jiménez-Escrig & Goñi, 1999), but their effect on glutathione status and on antioxidant enzymes has not been extensively studied. Maruyama *et al.* (1991) demonstrated that the activity of glutathione peroxidase (GSH-Px) and the Se concentration in livers of female rats fed 2 % kelp (*Laminaria religiosa*) were slightly lower than those of untreated control rats. GSH-Px activity in the brain and liver decreased in ageing mice treated with the red alga *Porphyra haitanesis* (Zhang *et al.* 2004). According to Zhang *et al.* (2003) the sulfated polysaccharides of *P. haitanesis* can be used in mice to compensate for the decline in total antioxidant capacity (TAOC) and antioxidant enzyme activities, and thereby reduce the risk of lipid peroxidation.

Cholesterol-rich diets have different effects on lipid peroxidation, with hypercholesterolaemic animals presenting higher peroxidation values than normocholesterolaemics (Mahfouz & Kummerow, 2000). Various investigations have reported that seaweeds, their water-soluble fraction or isolated algal polysaccharides induce hypocholesterolaemic effects in experimental animals (Koseki *et al.* 1990; for a review, see Jiménez-Escrig & Sánchez-Muniz, 2000). Thus, it can be

Abbreviations: ABTS, 2,2′-azino-di-3 ethylbenzothiazoline-6-sulfonate; GSH-Px, glutathione peroxidase; TOAC, total antioxidant capacity.

* **Corresponding author:** Professor Dr Francisco J. Sánchez-Muniz, fax +34 91 3941810, email frasan@farm.ucm.es

hypothesised that seaweeds exert both hypocholesterolaemic and antioxidant effects. Nonetheless, paradoxically, algae contain high amounts of organic As (Ergueta Martínez, 2001), which may affect glutathione reductase activity and therefore glutathione status (Nikaido *et al.* 2003). Furthermore, the mid-to-long-term effects of diets containing relatively large amounts of algae have not been studied in growing animals. It has been suggested that the adverse effects associated with herbal medications include, among others, liver failure, toxic hepatitis and death (Ernst, 2003).

Taking into account the increasing consumption of seaweeds by the young, and because of recently published information regarding the serious adverse effects of unconventional herbal therapies in children and adolescents (Ernst, 2003), the present study was designed to determine the effect in normo- and hypercholesterolaemic growing Wistar rats of a dietary supplement of seaweeds (nori (*Porphyra*) and konbu (*Laminaria*)) on (i) growth, (ii) liver size and composition, (iii) plasma cholesterol and plasma TAOC, and (iv) liver glutathione status.

Materials and methods

Materials

The red seaweed (*Rhodophyceae*) konbu (*L. digitata*) and the brown seaweed (*Phaeophyceae*) nori (*P. tenera*) were obtained from a local supplier (Algamar C.B., Redondela, Pontevedra, Spain). These commercial marine seaweeds were freeze-dried and milled, using a cyclotic mill (Tecator 1093; Foss Tecator, Hoeganaes, Sweden), to a particle size of $<1{\cdot}0$ mm before use. The composition of the edible seaweeds employed in the present study has been previously reported (Rupérez & Saura-Calixto, 2001). Both seaweeds consist of a matrix of soluble, insoluble, and total dietary fibre (dry weight) of 9·15, 26·98 and 36·12 g/100 g respectively, in konbu and 14·56, 19·22 and 33·78 g/100 g respectively, in nori. The protein content (dry weight) of konbu was 10·7 g/100 g and that of nori, 28·29 g/100 g, while fat content (dry weight) was 1·83 g/100 g in konbu and 1·64 g/100 g in nori.

Diet preparation and experimental design

Six experimental semi-synthetic diets were prepared: (a) the control diet without added cholesterol (normo-control) was obtained by homogeneously mixing 93 % of a rodent diet (AIN-93M purified rodent diet; DYETS, Inc., Bethlehem, PA, USA) with 7 % of a cellulose–wheat starch mix (35:65, w/w); (b) the normo-konbu diet consisted of a mixture of the rodent diet (93 %) with freeze-dried *Laminaria* (7 %); (c) the normo-nori diet consisted of a mixture of the rodent diet (93 %) with freeze-dried *Porphyra* (7 %); (d) the hyper-control diet (identical to the normo-control diet but with 2 % cholesterol and 0·4 % sodium cholate instead of maize starch); (e) the hyper-konbu diet (the hyper-control diet but with 7 % freeze-dried konbu); (f) the hyper-nori diet (same as the hyper-control diet but with 7 % freeze-dried nori) (Table 1).

Animals and maintenance

Sixty male growing Wistar rats with a body weight of approximately 127 g at the outset were distributed in six groups of ten animals each, according to average body weight. The rats were obtained from the breeding centre at the Facultad de Farmacia (Universidad Complutense de Madrid, Spain) and handled

Table 1. Composition of the control, nori (*Porphyra tenera*) and konbu (*Laminaria digitata*) normocholesterolaemic and hypercholesterolaemic experimental diets

	Normocholesterolaemic diet		Hypercholesterolaemic diet	
93 % Diet AIN-93M + 7 % supplement (g/kg)	Normo-control	Normo-algae	Hyper-control	Hyper-algae
Casein	130·2	130·2	130·2	130·2
Maize starch	433·09	433·09	410·72	410·72
Dyetrose*	144·15	144·15	144·15	144·15
Sucrose	93	93	93	93
Microcrystalline cellulose	46·5	46·5	46·5	46·5
Salt mix no. 210050†	32·55	32·55	32·55	32·55
Vitamin mix no. 310025‡	9·3	9·3	9·3	9·3
L-Cystine	1·674	1·674	1·674	1·674
Choline bitartrate	2·325	2·325	2·325	2·325
t-Butylhydroquinone	0·007	0·007	0·007	0·007
Soyabean oil	37·2	37·2	37·2	37·2
Cellulose–maize starch (35:65)	70	–	70	–
Nori or konbu (freeze-dried)	–	70	–	70
Cholesterol	–	–	18·6	18·6
Cholic acid Na salt	–	–	3·72	3·72

* Dyetrose (carbohydrate composition) (g/kg): monosaccharides, 10; disaccharides, 40; trisaccharides, 50; tetrasaccharides and higher, 900.

† Mineral mix contained AIN-93M mineral mix (g/kg): calcium carbonate, 357·00; potassium phosphate monobasic, 250·00; potassium citrate.H_2O, 28·00; sodium chloride, 74·00; potassium sulfate, 46·60; magnesium oxide, 24·00; ferric citrate U.S.P, 6·06; zinc carbonate, 1·65; manganous carbonate, 0·63; cupric carbonate, 0·30; potassium iodate, 0·01; sodium selenate, 0·01025; ammonium paramolybdate.$4H_2O$, 0·00795; sodium metasilicate.$9H_2O$, 1·45; chromium potassium sulfate.$12H_2O$, 0·275; lithium chloride, 0·0174; boric acid, 0·0815; sodium fluoride, 0·0635; nickel carbonate, 0·0318; ammonium vanadate, 0·0066; finely powdered sucrose, 209·806.

‡ AIN-93VX vitamin mixture (g/kg): niacin, 3·00; calcium pantothenate, 1·60; pyridoxine HCl, 0·70; thiamine HCl, 0·60; riboflavin, 0·60; folic acid, 0·20; biotin, 0·02; vitamin E acetate (500 IU/g), 15·00; vitamin B_{12} (0·1 %), 2·50; vitamin A palmitate, (500 000 IU/g), 0·80; vitamin D_3 (400 000 IU/g), 0·25; vitamin K_1–dextrose mix (10 mg/g), 7·50; sucrose, 967·23.

according to the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council (1985). The animals were fed the six experimental diets for 3 weeks and housed individually in metabolic cells in a temperature-controlled room (22·3 ± 18°C) with a 12 h light–dark cycle. The present study was approved by the Spanish Science and Technology Advisory Committee (Comisión Asesora de Ciencia y Tecnología) and by an ethics committee of the Facultad de Farmacia of the Universidad Complutense de Madrid (Spain).

Dietary treatments

After weaning, rats were fed with commercial rat pellets (Panlab, Barcelona, Spain) and switched to the experimental diets following a 1-week period of adaptation to environmental conditions. Diets contained approximately 13 % protein, 4 % fat and 7 % total dietary fibre (Table 1). Water and food were provided *ad libitum* over the 3-week experimental period.

Food intake, growth rate and liver weights

Food intake was checked daily and body-weight variations were measured on alternate days. At the end of the experiment, one animal at a time was taken at random from each of the six groups in turn, anaesthetised with an intraperitoneal injection of sodium pentobarbital (45 mg/kg body weight) and killed, in non-fasting conditions, by extracting blood from the descending aorta with a syringe. The liver was removed, weighed and stored in liquid N_2 until analysis.

Dietary arsenic

Dietary As content was measured by inductively coupled plasma atomic emission spectrometry (Ródenas de la Rocha *et al.* 2002).

Liver fat

Liver fat was extracted from the cystic lobe according to the Bligh & Dyer (1959) method.

Liver enzymes

The right lateral lobe was homogenised in phosphate buffer (50 mM, pH 7·4). Homogenates were centrifuged at 3000 rpm (1500 ***g***) at 4°C for 15 min. The resulting supernatant fraction was used to determine enzyme activities and glutathione concentrations.

Liver glutathione reductase activity (nmol NADPH/min per mg protein) was assessed by the method of Barga de Quiroga *et al.* (1990). This enzymic activity was monitored at 340 nm by following the rate of reduction of $NADP^+$ to NADPH in the presence of GSSG.

GSH-Px activity was determined as Se-dependent GSH-Px and total GSH-Px. Se-dependent GSH-Px activity was assessed by the method of Paglia & Valentine (1967), using H_2O_2 as substrate and including azide as catalase inhibitor. Total GSH-Px was measured according to the Lawrence & Burk (1976) method, using cumene hydroperoxides.

Glutathione was assayed by an enzymic recycling procedure in which it was sequentially oxidised by 5,5′-dithiobis-(2-nitrobenzoic acid) and reduced by NADPH in the presence of glutathione reductase (Griffith, 1980). The 2-nitro-5-thiobenzoic acid formation rate was monitored at 412 nm. GSSG was determined selectively by masking the reduced glutathione with 2-vinylpyridine.

All spectrophotometric measurements were carried out in an Uvikon 930 UV spectrophotometer (Kontron Instruments, Munich, Germany) with 1·0 ml quartz cuvettes with a light path of 1·0 cm. Specific activities were expressed as mmol/min per mg protein. Liver protein concentrations were determined by the Lowry *et al.* (1951) method.

Plasma total antioxidant capacity

TAOC of plasma, as an estimation of the total amount of antioxidants present in plasma, was assessed by the method described by Miller *et al.* (1993) using total antioxidant status kits (Randox Laboratories Ltd, Crumlin, Co Antrim, UK). In this method, 2,2′-azino-di-3 ethylbenzothiazoline-6-sulfonate (ABTS) was incubated with peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ($ABTS^+$), whose absorbance was measured at 600 nm. The ability of antioxidants contained in the sample to inhibit this reaction was measured and compared with standard 6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox® Chemexper sprl, Court-St-Etienne, Belgium). In brief, 20 μl plasma were mixed with 1 ml chromogen (metmyoglobin (6·1 μmol/l) plus ABTS (610 μmol/l)) and 200 μl H_2O_2 (1250 μmol/l). The sample was incubated at 37°C and absorbance at 600 nm was read after 3 min. Results are expressed in mmol Trolox®/l.

Cholesterol adjustment for antioxidant vitamin concentrations (for example, tocopherol) is widely performed. Thus, looking for more realistic information about plasma antioxidant defence in hypercholesterolaemic rats, we performed cholesterol adjustment for the TAOC.

Plasma cholesterol

Plasma cholesterol was determined by the enzymic colorimetric method of Boehringer Mannheim (Mannheim, Germany).

Statistical analyses

Results are expressed as mean values and standard deviations. Two-way ANOVA (cholesterol and alga effects) was performed. When significant cholesterol–alga interaction was found, the alga effect was separately tested in normo and hyper groups by *post hoc* analysis (Bonferroni and Welch robust tests). The effect of cholesterol consumption (hyper diets *v.* their corresponding normo diets) was tested by the unpaired Student's *t* test. Results were considered significant at $P<0·05$. Statistical analyses were conducted using the SPSS version 11 statistical analysis packages (SPSS Inc., Chicago, IL, USA).

Results

Food and arsenic intakes

Food intake was not significantly affected by cholesterol or alga intakes. Arsenic intake was significantly higher ($P<0·001$) in normo- and hyper-alga-diet rats than in the respective control animals (Table 2).

Body-weight gain

Body-weight gain (in g and %) decreased due to cholesterol feeding ($P<0·01$ and $P<0·05$ respectively). The alga effect was of borderline statistical significance ($P=0·07$, $P=0·06$ respectively) (Table 2).

Liver weight and liver fat

Liver weight increased significantly as a result of cholesterol feeding ($P<0·001$). A significant cholesterol–alga interaction for liver weight was found ($P<0·05$). The *post hoc* test showed that hyper-nori and hyper-konbu rats had higher liver weights ($P<0·05$) than their respective normo counterparts (Table 2). Liver fat (in absolute terms (g) and in relative terms (g fat/100 g liver)) increased significantly by cholesterol feeding ($P<0·001$) (Table 2).

Plasma cholesterol

Table 3 shows that plasma cholesterol was significantly affected by cholesterol ($P<0·001$) and alga ($P<0·05$) consumption.

Total antioxidant capacity

TAOC was significantly affected by alga consumption. Cholesterol and alga feeding significantly influenced ($P<0·001$ and $P<0·01$ respectively) the adjusted TAOC. A significant cholesterol–alga interaction ($P<0·05$) was also found. The adjusted TAOC of normo-konbu rats was significantly lower ($P<0·05$) than that of the other normo-diet rats (Table 3).

Liver enzymes

Glutathione reductase activity was significantly affected by cholesterol ($P<0·001$) and alga ($P<0·05$) consumption (Table 4). Cholesterol feeding significantly increased the activity of both total and Se-dependent GSH-Px ($P<0·001$). A significant cholesterol–alga interaction ($P<0·05$) on total GSH-Px was found. The hyper-nori diet induced lower GSH-Px activity than the hyper-control diet ($P<0·05$).

The Se-dependent GSH-Px:total-GSH-Px ratio was affected by cholesterol consumption ($P<0·01$). A significant cholesterol–alga interaction ($P<0·001$) was found for this ratio.

Glutathione status

Total and reduced glutathione concentrations decreased significantly due to alga consumption ($P<0·001$). GSSH increased as a result of cholesterol feeding ($P<0·05$).

Discussion

Food intake

Diets supplemented with konbu and nori were well accepted (Wong *et al.* 1999). Data from the present study concur with those of other authors who report that alga and control diets were consumed at similar rates (Wong *et al.* 1999).

Body-weight gain

The present results are in line with those reported by Wong *et al.* (1999), who found that body-weight gains of rats fed seaweed-based diets were similar to those of others given basal diets. Alga supplements counterbalance the negative effect on body-weight gain observed in the hyper-control

Table 2. Food intake, body-weight gain, liver weight, and liver fat in rats consuming control, nori (*Porphyra tenera*) and konbu (*Laminaria digitata*) normocholesterolaemic (Normo) and hypercholesterolaemic (Hyper) experimental diets

(Mean values and standard deviations)

		Control (*n* 10)		Nori (*n* 10)		Konbu (*n* 10)		ANOVA		
		Mean	SD	Mean	SD	Mean	SD	Cholesterol effect	Alga effect	Interaction
Food intake (g/d)	Normo	21·28	1·51	21·08	1·17	21·13	1·20	NS	NS	$P=0·07$
	Hyper	20·19	1·67	21·96	1·18	20·33	1·84			
As intake (μg/d)	Normo	0·17	0·01	36·46	2·03	47·33	2·71	NS	$P<0·001$	$P=0·09$
	Hyper	0·16	0·01	38·00	2·06	45·55	4·12			
Body-weight gain (g)	Normo	155·7	16·2	158·02	14·1	147·4	11·5	$P<0·01$	$P=0·07$	NS
	Hyper	125·2	21·6	150·3	18·1	139·1	19·08			
Body-weight gain (%)	Normo	54·70	2·35	55·40	3·52	53·78	0·9	$P<0·05$	$P=0·06$	NS
	Hyper	49·43	7·30	54·61	3·52	52·11	3·46			
Liver weight (g)	Normo	13·10	1·81	12·40	1·14	12·52	0·82	$P<0·001$	NS	$P<0·05$
	Hyper	14·67	2·62	16·41*	2·06	16·27*	1·49			
Liver fat (g fat/100 g liver)	Normo	5·33	1·75	3·71	1·22	3·80	0·80	$P<0·001$	NS	NS
	Hyper	12·41	2·38	12·2	3·31	13·47	2·61			
Total liver fat (g)	Normo	0·69	0·24	0·45	0·14	0·47	0·10	$P<0·001$	NS	NS
	Hyper	1·77	0·18	2·02	0·62	2·17	0·33			

* Mean value was significantly different from that for the rats fed the normocholesterolaemic diet ($P<0·05$; Student's *t* test).

Table 3. Plasma cholesterol and total antioxidant capacity (TAOC) in rats consuming control, nori (*Porphyra tenera*) and konbu (*Laminaria digitata*) normocholesterolaemic (Normo) and hypercholesterolaemic (Hyper) experimental diets

(Mean values and standard deviations)

		Control (*n* 10)		Nori (*n* 10)		Konbu (*n* 10)		Statistical significance (two-way ANOVA)		
Plasma		Mean	SD	Mean	SD	Mean	SD	Cholesterol effect	Alga effect	Interaction
Cholesterol (mmol/l)	Normo	1·62	0·27	1·68	0·21	1·88	0·18	$P<0·001$	$P<0·05$	NS
	Hyper	5·20	1·78	4·33	0·64	5·67	1·46			
TAOC (IU)	Normo	0·75	0·16	0·66	0·07	0·65	0·05	NS	$P<0·05$	NS
	Hyper	0·71	0·07	0·64	0·07	0·71	0·07			
Adjusted TAOC (IU/mmol plasma cholesterol)	Normo	0·48[a]	0·14	0·39[a]	0·03	0·35[b]	0·04	$P<0·001$	$P<0·01$	$P<0·05$
	Hyper	0·15*	0·05	0·15*	0·02	0·13*	0·03			

* Mean value was significantly different from that for the rats fed the normocholesterolaemic diet ($P<0·05$; Student's *t* test; *post hoc* analysis).
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Bonferroni and Welch robust tests).

group by substituting the cholesterol-raising agent with 2·4 % (w/w) in carbohydrates.

Liver weight and liver fat

Cholesterol-enriched diets produced hepatomegaly and steatosis and increased the somatic index (data not shown), all of which are related to fatty liver induction. These results are in agreement with those of Fukushima *et al.* (2001), Sánchez-Muniz *et al.* (1996) and Sánchez-Muniz *et al.* (2003), but not with those of Wong *et al.* (1999), who did not find any significant changes in liver weights in different diets containing algae and cholesterol. According to Viejo *et al.* (2003), hepatic fat content increases as a result of cholesterol feeding, mainly in the form of triacylglycerols and esterified cholesterol.

Plasma cholesterol

Cholesterol supplementation induced moderate hypercholesterolaemia in all groups. This finding concurs with others studies (Sánchez-Muniz *et al.* 1996; Viejo *et al.* 2003) in which casein diets containing cholesterol significantly increased plasma cholesterol levels. The hypocholesterolaemic effect of fibre is related to the plasma cholesterol level, with hypercholesterolaemic animals being the most affected (Kritchevsky & Story, 1993). The fact that the hyper-nori diet exhibited the least hypercholesterolaemic effect is related to the alga fibre itself. In fact, the nori diet contained more soluble fibre than the konbu and control diets (Rupérez & Saura-Calixto, 2001). Sodium alginate, funoran, porphyran, and carrageenan exhibited significant hypocholesterolaemic activity when included in the cholesterol-enriched basal diets, while agar was almost inactive (Ito & Tsuchiya, 1972). Alginates, fucans and cellulose constitute the main cell-wall polysaccharides of Phaeophyceae (nori), whereas sulfated galactans, xylans, mannans and cellulose compose the cell walls of Rhodophyceae (Laminaria) (Lahayen, 1991). Nori contains more porphyrans, alginates and furans than konbu, explaining, at least partially, the present results.

Liver enzymes and glutathione status

Antioxidants have been closely linked with the preservation of health and longevity in both mice and rats (Rao *et al.* 1990). GSH-Px is one of the major enzymes directly involved in *in vivo* antioxidant defence. Hyper diets increased GSH-Px activity, coinciding with the findings of Mahfouz & Kummerow (2000). The latter authors demonstrated that cholesterol feeding in rats (1 % for 8 weeks) did not increase lipid peroxidation in the liver due to the significant increase in hepatic GSH-Px activity, and suggested that this enzyme may decrease lipid peroxide and oxysterol formation. Cholesterol feeding led to a 3-fold increase in total GSH-Px activity in controls, but to a much lower increase in alga-fed rats (1·3-fold in nori rats and 2-fold in konbu animals), suggesting that algae induced lower increments of non-Se-dependent GSH-Px. Comparing results of total GSH-Px, Se-dependent GSH-Px and their respective ratio, the increase in GSH-Px activity due to cholesterol feeding seems mainly related to the increase in Se-dependent GSH-Px activity in the nori diet, while in control and konbu diets the increase was higher for non-Se-dependent GSH-Px than for its Se-dependent counterpart. We are far from knowing the reason for these results but they could be related to the dietary level and bioavailability of Se. This hypothesis will be tested in future studies.

Reduced and total glutathione concentrations were lower in normo- and hyper-konbu rats than in their control counterparts. This explains the negative effect of konbu seaweed upon TAOC. According to Nikaido *et al.* (2003), As blocks the production of reduced and total glutathione. Konbu rats had high As intakes (45·55 μg/d), which would have contributed to the low reduced and total glutathione concentrations found in these animals. We suggest that the increase in glutathione reductase activity displayed by konbu rats could partially compensate for the decrease in glutathione, partially helping to restore the impaired antioxidant system.

According to Menone & Pflugmache (2005), the elevation of microsomal and cytosolic glutathione S-transferase showed the utilisation of reduced glutathione in the formation of thioethers, called glutathione S-conjugates. Due to this, reduced glutathione is decreased and a decrease in the total reduced glutathione pool could be expected. On the other hand, depletion of the reduced glutathione pool will sooner or later lead to activation of glutathione reductase, an enzyme involved in maintenance of a suitable high reduced glutathione level.

Nonetheless, although nori also contains high amounts of As, no negative effect on total and reduced glutathione was

Table 4. Liver glutathione concentrations, glutathione peroxidase (GSH-Px) and glutathione reductase activities in rats consuming control, nori (*Porphyra tenera*) and konbu (*Laminaria digitata*) normocholesterolaemic (Normo) and hypercholesterolaemic (Hyper) experimental diets
(Mean values and standard deviations)

		Control (n 10)		Nori (n 10)		Konbu (n 10)		Statistical significance (two-way ANOVA)		
Liver		Mean	SD	Mean	SD	Mean	SD	Cholesterol effect	Alga effect	Interaction
Total glutathione (μmol/g liver)	Normo	6·47	1·78	5·00	2·52	4·08	1·77	NS	$P<0·00$ 1	NS
	Hyper	6·15	1·88	6·95	1·94	3·91	1·49			
GSSG (μmol/g liver)	Normo	0·29	0·22	0·29	0·18	0·26	0·19	$P<0·05$	NS	NS
	Hyper	0·33	0·14	0·52	0·17	0·31	0·19			
Reduced glutathione (μmol/g liver)	Normo	6·21	1·76	4·72	2·51	3·82	1·88	NS	$P<0·00$ 1	NS
	Hyper	5·81	1·90	6·43	1·93	3·60	1·37			
Se-dependent GSH-Px (nmol NADPH/min per mg protein)	Normo	790·3	145·1	971·4	237·0	1047·2	338·0	$P<0·00$ 1	NS	NS
	Hyper	1761·8	762·3	1528·3	291·4	1686·4	433·7			
Se-dependent GSH-Px:total GSH-Px ratio	Normo	$0·77^{a,b}$	0·07	$0·70^{a}$	0·11	$0·82^{b}$	0·03	$P<0·01$	$P=0·08$	$P<0·001$
	Hyper	0·57*	0·13	$0·79^{b}$	0·14	$0·67^{a,b}$	0·16			
Total GSH-Px (nmol NADPH/min per mg protein)	Normo	1034·4	181·8	1450·3	477·5	1264·8	379·0	$P<0·00$ 1	NS	$P<0·05$
	Hyper	$3214·2^{a*}$	1469	$1980·9^{b}$	420·2	$2589·6^{a,b*}$	743·0			
Glutathione reductase (nmol NADPH/min per mg protein)	Normo	52·76	23·8	45·36	12·82	72·83	22·10	$P<0·00$ 1	$P<0·05$	NS
	Hyper	97·10	32·7	96·32	23·24	119·69	29·72			

*Mean value was significantly different from that for the rats fed the normocholesterolaemic diet ($P<0·05$; Student's t test; *post hoc* analysis).
a,bMean values within a row with unlike superscript letters were significantly different ($P<0·05$; Bonferroni and Welch robust tests).

observed in the present study. We are far from knowing the reasons behind the differences due to nori and konbu effects. Ismail & Siew Hong (2002) found that seaweeds available in the Malaysian supermarket differ in their total antioxidant and free radical-scavenging activities. Rabbani *et al.* (2003) suggest that exogenous antioxidants such as polyphenols and supplements of vitamins, Zn and Se are useful for As detoxification.

In conclusion, the addition of algae to the diet did not significantly change total food intake or body-weight gain of growing male rats. The present findings suggest that although some antioxidant compounds are present in algae, other dietary compounds, such as As, induced poor antioxidant status in rats. Further studies should be designed to confirm the positive and negative effects of large-scale konbu and nori consumption.

Acknowledgements

We are indebted to Dr Isabel Goñi, Dr Fulgencio Saura and Laura Barrios for their assistance. We also thank Dr María Teresa Larrea from the Centro Nacional de Investigaciones Metalúrgicas (CSIC), Madrid, Spain, for As determination in the diets. The present study was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología, project ALI 98-0830, and by Dirección General de Investigación del Ministerio de Educación y Ciencia, project AGL2005-07204-C02-01/ALI.

References

Barga de Quiroga G, Perez de Campo R & López-Torres M (1990) Antioxidant defences and peroxidation in liver and brain of aged rats. *Biochem J* **272**, 247–250.

Bligh EC & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.

Bocanegra A, Nieto A, Blas A & Sánchez-Muniz FJ (2003) Diets containing a high percentage of nori or konbu algae are well-accepted and efficiently utilised by growing rats but induce different degrees of histological changes in the liver and bowel. *Food Chem Toxicol* **41**, 1473–1480.

Conquer JA & Holub BJ (1996) Supplementation with an alga source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. *Nutrition* **126**, 3032–3039.

Ergueta Martínez A (2001) Análisis elemental de algas empleadas en alimentación, mediante espectrometrías ICP. PhD Thesis, , Facultad de Farmacia, Universidad Complutense de Madrid.

Ernst E (2003) Serious adverse effects of unconventional therapies for children and adolescents: a systematic review of recent evidence. *Eur J Ped* **162**, 81–83.

Figueira MM, Volesky B, Ciminelli VST & Roddick FA (2000) Biosorption of metals in brown seaweed biomass. *Water Res* **34**, 196–204.

Fleurence J (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci Tech* **10**, 25–28.

Fukushima M, Ohhashi T, Ohno S, Saitoh H, Sonoyama K, Shimada K, Sekikawa M & Nakano M (2001) Effects of diets enriched in n-6 or n-3 fatty acids on cholesterol metabolism in older rats chronically fed a cholesterol-enriched diet. *Lipids* **36**, 261–266.

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**, 207–212.

Ho YB (1990) Metals in Ulva lactuca in Hong Kong intertidal waters. *Bull Mar Sci* **47**, 79–85.
Ismail A & Siew Hong T (2002) Antioxidant activity of selected commercial seaweeds. *Mal J Nutr* **8**, 167–177.
Ito K & Tsuchiya Y (1972) The effect of algal polysaccharides on the depressing of plasma cholesterol levels in rats. In *Proceeding of The Seventh International Seaweed Symposium*, pp. 451–454 Tokyo: Tokyo University Press.
Jeong BY, Cho DM, Moon SK & Pyeum JH (1993) Quality factors and functional components in the edible seaweeds. I. Distribution on n-3 fatty acids in 10 species of seaweeds by their habitats. *J Korean Soc Food Nutr* **22**, 612–628.
Jiménez-Escrig A & Goñi I (1999) Evaluación nutricional y efectos fisiológicos de macroalgas marinas comestibles (Nutritional evaluation and physiological effects of edible marine algae). *Arch Latinoam Nutr* **49**, 114–120.
Jiménez-Escrig A & Sánchez-Muniz FJ (2000) Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr Res* **20**, 585–598.
Koseki M, Tsuji K, Kazama M, Kitabatake N & Doi E (1990) Interaction between dietary cholesterol or fatty acids and water-soluble fibers and increase in cholesterol excretion by pectin in rats. *Nippon Shokuhin Kogyo Gakkaishi* **37**, 559–564.
Kritchevsky D & Story JA (1993) Influence of dietary fibre on cholesterol metabolism in experimental animals. In *Handbook of Dietary Fiber in Human Nutrition*, pp. 163–178 [GA Spiller, editor]. Boca Raton, CA: CRC Press.
Lahayen M (1991) Marine algae as sources of fibres: determination of soluble and insoluble dietary fibre contents in some sea vegetables. *J Sci Food Agric* **54**, 587–594.
Lawrence R & Burk R (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **7**, 952–958.
Lowry OH, Rosebrough NJ, Farr Al & Randall RL (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**, 265–275.
Mahfouz MM & Kummerow FA (2000) Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides and antioxidant enzymes in rats and rabbits. *J Nutr Biochem* **11**, 293–302.
Maruyama H, Watanabe K & Yamamoto I (1991) Effect of dietary kelp on lipid peroxidation and glutathione peroxidase activity in livers of rats given breast carcinogen DMBA. *Nutr Cancer* **15**, 221–228.
Menone ML & Pflugmache S (2005) Effects of 3-chlorobyfphenyl on photosynthetic oxygen production glutathione content in the detoxication enzymes in the acuatic macrocytes *Ceratophyllum demersum*. *Chemosphere* **60**, 79–84.
Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V & Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (London)* **84**, 407–412.
National Research Council (1985) *Guide for the Care and Use of Laboratory Animals*. Publication no. 85-23 (revision). Washington, DC: NIH.
Nikaido M, Pi J, Jumagai Y, Yamauchi H, Taguchi K, Horiguchi S, Sun Y, Sun G & Shimojo N (2003) Decreased enzyme activity of hepatic thioredoxin reductase and glutathione reductase in rabbits by prolonged exposure to inorganic arsenate. *Environ Toxicol* **18**, 306–311.
Paglia D & Valentine W (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70**, 158–169.
Phaneuf D, Côté I, Dumas P, Ferron LA & LeBlanc A (1999) Evaluation of the contamination of marine algae (seaweed) from the St. Lawrence river and likely to be consumed by humans. *Environ Res* **80A**, S175–S182.
Rabbani GH, Saha SK, Akhtar M, Marni F, Mitra AK, Ahmed S, Alauddin M, Bhattacharjee M, Sultana S & Chowdhury AK (2003) Antioxidants in detoxification of arsenic-induced oxidative injury in rabbits: preliminary results. *J Environ Sci Health* **38A**, 273–287.
Rao G, Xia E, Nadakavukaren MJ & Richardson A (1990) Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. *J Nutrition* **120**, 602–609.
Ródenas de la Rocha S, Ergueta Martínez A, Sánchez Muniz FJ & Larrea Marín MT (2002) Análisis elemental de algas por ICP-AES (Elemental analysis of algae for ICP-AES). *Schironia* **1**, 10–15.
Rupérez P & Saura-Calixto F (2001) Dietary fiber and physicochemical properties of edible Spanish seaweeds. *Eur Food Res Technol* **212**, 349–354.
Sánchez-Muniz FJ, Cava F, Viejo JM, Bastida S, Higón E & Marcos A (1996) Olive oil-fried sardines in the prevention of dietary hypercholesterolemia in rats. Effects on some serum lipids and cell-damage marker enzymes. *Nutr Res* **16**, 111–121.
Sánchez-Muniz FJ, García Linares MC, García Arias MT, Bastida S & Viejo J (2003) Fat and protein from olive oil fried sardines interact to normalize serum lipoproteins and liver lipids in hypercholesterolemic rats. *J Nutr* **133**, 2302–2308.
Viejo J, García-Linares MC, Bastida S, García-Arias MT & Sánchez-Muniz FJ (2003) Effect of olive oil-fried sardine consumption on liver lipid composition and fatty acid esterification in hypercholesterolemic rats. *Food Sci Technol Int* **9**, 329–338.
Wong KH, Sam SW, Cheung PCK & Ang PO (1999) Changes in lipid profiles of rats fed with seaweed-based diets. *Nutr Res* **19**, 1519–1527.
Zhang Q, Ning L, Gefei Z, Xiaolan L & Zuhong X (2003) In vivo antioxidant activity of polysaccharide fraction from Porphyra haitanensis (Rhodephyta) in aging mice. *Pharmacol Res* **48**, 151–155.
Zhang Q, Ning L, Xiguang L, Zengqin Z, Zhien L & Zuhong X (2004) The structure of a sulfated galactan from Porphyra haitanensis and its in vivo antioxidant activity. *Carbohyd Res* **339**, 105–111.