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ORIGINAL PAPER

Wakame and Nori in Restructured Meats Included in Cholesterol-enriched Diets Affect the Antioxidant Enzyme Gene Expressions and Activities in Wistar Rats

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Abstract The effects of diets including restructured meats (RM) containing Wakame or Nori on total liver glutathione status, and several antioxidant enzyme gene expressions and activities were tested. Six groups of ten male growing Wistar rats each were fed a mix of 85% AIN-93 M diet and 15% freeze-dried RM for 35 days. The control group (C) consumed control RM, the Wakame (W) and the Nori (N) groups, RM with 5% Wakame and 5% Nori, respectively. Animals on added cholesterol diets (CC, CW, and CN) consumed their corresponding basal diets added with cholesterol (2%) and cholic acid (0.4%). Alga and dietary cholesterol significantly interact ($P<0.002$) influencing all enzyme expressions but not activities. The cholesterol supplement decreased most enzyme expression and activity. W-RM *vs.* C-RM increased ($P<0.05$) expression of GPx, GR, Mn-SOD, and Cu,Zn-SOD and decreased that of catalase. N-RM *vs.* C-RM increased ($P<0.05$) expression of catalase and Mn-SOD. GR activity increased in W-RM rats while SOD activity increased, but that of Se-GPx decreased in N animals. W-RM increased total and reduced glutathione and decreased the redox index. CN diet induced significantly lower plasma cholesterol levels ($P<0.001$) than the CW diet. In conclusion, Nori-RM is a hypocholesterolemic food while Wakame-RM is an antioxidant food. This should be taken into account when including this kind of RM as potential functional foods in human.

Keywords Nori · Wakame · Functional meats · Antioxidant enzymes · Glutathione · Gene-expression

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Introduction

Meat and meat products, which concentrate and supply a large number of valuable nutrients -protein, fat, vitamins, and minerals-, have traditionally been basic components of the human diet. However, epidemiological associations between consumption of meat and meat derivatives and some of the major degenerative diseases such as coronary heart disease (CHD), cancer, high blood pressure, and obesity have influenced nutritional thinking and dietary guidelines over the last few years [1]. For this reason, the meat industry is presently employing various strategies to introduce qualitative and/or quantitative modifications in meat and/or meat derivatives to create "functional" products [1, 2].

Most physiologically active substances come from plants, and combined with other foods such as meat derivatives can transform them in functional foods. Consumption of marine algae, which are a traditional component of the Asian diet, has increased considerably in the Western world over the last decade [3]. Some species of

Undaria and *Porphyra* contain high levels of fiber, several minerals, and vitamins, and their lipid content is normally <1.0%. Furthermore, those seaweeds have recently been reported to contain several minor compounds with beneficial biological activities [4]. Hypercholesterolemia is associated with increased oxidative stress in animals [5] and humans [6], which in turn has been linked to degenerative diseases such as atherosclerosis. It has been suggested that increased dietary intake of antioxidants may reduce the progression of these diseases [7]. Our group has tested the effect of diets containing Nori and Konbu on the antioxidant status of Wistar rats consuming high dietary levels of cholesterol [8].

However, no studies are available on the effect of diets enriched in restructured meat (RM) containing algae on the antioxidant status. The inclusion of alga in meat would have a double-edged effect because meat is rich in iron, and high levels of this metal are known to increase oxidative stress [9]. Due to the growing demand for alternative treatments for CHD, this study given a dietary cholesterol supplement aimed to investigate the effect of diets enriched in restructured pork meat containing Wakame and Nori for five weeks on antioxidant substrate concentrations and antioxidant enzyme activities and gene expression in growing Wistar rats.

Materials and Methods

Restructured Meat Preparation

Meat raw materials (post-rigor pork meat and pork backfat), seaweeds: Wakame (*Undaria pinnatifida*) and Nori (*Porphira umbilicalis*), and additives (sodium chloride, sodium tripolyphosphate (STP) and sodium nitrite) were used. Fresh marine seaweeds were collected on the Atlantic coast, dried in the shade and packed in polyethylene plastic bags for commercial distribution (Algamar C.B., Redondela, Pontevedra, Spain). These seaweeds were ground in a mill (ZM 200, Retsch GmbH and Co., KG, Haan, Germany), passed through a screen with an aperture of 0.25 mm and stored in plastic flasks at 4±2 °C until used. Details of the RM preparation and composition have been already published [2]. Briefly, raw meat material was homogenized and ground for 1 min in a chilled cutter (2 °C) (Stephan Universal Machine UM5, Stephan, Sóhne GmbH and Co., Hameln, Germany). All the fat and half of the seaweeds, NaCl (2.0% for control samples and 0.5% for samples with added seaweed), STP and sodium nitrite were added to the ground meat and the whole was mixed for 1 min; then the rest of the ingredients were added and the mixture homogenized for 1 min. Finally, the whole meat batter was homogenized under vacuum for 2 min. Each sample was prepared in duplicate. N-RM and W-RM contained lower Na than the C-RM (385.5, 626.7 and 873.8 mg/100 g RM, respectively). As stated in that article [2], this was the result of using less NaCl in the formulations, because the use of seaweeds helped to overcome technological problems associated with low-salt products [2].

Animals and Treatments

Sixty male growing Wistar rats with a body weight of approximately 90 g at the outset were obtained from Harlan Laboratories Models, SL, Barcelona (Spain). The animals were 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 (project AGL 2005-07204-C02-01/ALAI,) and by an ethics committee of the Universidad Complutense de Madrid (Spain). After weaning, rats were fed commercial rat pellets (Panlab, Barcelona, Spain) during a 1-week period of adaptation to environmental conditions and then distributed into six groups of 19 animals each, according to average body weight. Experimental diets contained approximately 20.7% protein, 8.7% fat, and 4.2% total dietary fiber. Water and food were provided *ad libitum* over the 5-week experimental period. Six experimental semi-synthetic diets (Table 1) were prepared: (a) the control diet (C) contained 85% rodent feed (AIN-93 M, purified rodent diet; Dyets #180729, DYETS, Inc., Bethlehem, PA, USA) and 15% freeze-dried control pork meat to which 4% cellulose had been added; (b) the Wakame diet (W) consisted of a mixture of AIN-93 M #180729 feed (85%) and freeze-dried restructured Wakame meat (15%); (c) the Nori diet (N) consisted of a mixture of AIN-93 M #180729 feed (85%) and freeze-dried restructured Nori meat (15%); (d) the cholesterol control diet (CC) was identical to the C diet but with 2% cholesterol (95%–98% purity) and 0.4% cholic acid (>98% purity) substituting an equal amount of starch (AIN-93 M # 180730 diet); (e) the cholesterol-Wakame diet (CW) was the Wakame diet enriched with cholesterol and cholic acid, and (f) the cholesterol-Nori diet (CN) consisted of the Nori diet enriched with cholesterol and cholic acid.

Food intake was checked daily and body-weight variations were measured on alternate days. At the end of the experiment, the rats were sacrificed by cervical dislocation. Blood was collected and processed for the biochemical estimations. Liver tissue (0.5 g) was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4), and centrifuged at 6,500 rpm (3,800*g*) at 4 °C for 20 min. All spectrophotometric measurements were carried out in an Uvikon 930 UV spectrophotometer (Kontron Instruments, Munich, Germany).

Table 1 Composition of the Control, Wakame *(Undaria pinnatifida)*, and Nori *(Porphyra umbilicalis)*, normocholesterolaemic and hypercholesterolaemic experimental diets[a]

	Non-added cholesterol diet		Added cholesterol diet	
	Control	Nori or Wakame	Control	Nori or Wakame
Diet AIN-93 M				
Casein	127.5	127.5	127.5	127.5
Maize starch	382.75	382.75	362.35	362.35
Dyetrose[b]	131.75	131.75	131.75	131.75
Sucrose	85	85	85	85
Microcrystalline cellulose	42.50	42.50	42.50	42.50
Salt mix nº 210050[c]	29.75	29.75	29.75	29.75
Vitamin mix nº 10025as[d]	12.16	12.16	12.16	12.16
L-Cystine	1.53	1.53	1.53	1.53
Choline bitartrate	3.06	3.06	3.06	3.06
t-Butylhydroquinone	0.0068	0.0068	0.0068	0.0068
Soybean oil	34	34	34	34
Cholesterol			17	17
Cholic acid			3.4	3.4
Pork meat (freeze-dried)[e]				
Control-meat[e]	150		150	
Wakame-meat[e] or Nori-meat[e]		150		150

[a] Ingredients (g/kg)

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

[c] Mineral mix contained AIN-93 M 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

[d] 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 B12 (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

[e] Protein (g), fat (g), minerals (g) (ash) and fibre (g) present in the 150 g of Control, Wakame and Nori freeze-dried restructured-meats; Control meat: 76.99, 51.52, 15.61 and 4 (microcrystalline cellulose), respectively; Nori-meat: 73.94, 53.10, 17.40 and 3.0, respectively; Wakame-meat: 73.94, 53.10, 17.40 and 3.45

Enzymes Assays

Liver glutathione reductase (GR) activity (nmol NADPH/min/per mg protein) was assessed by following the rate of reduction of NADP + to NADPH in the presence of oxidized glutathione (GSSG) by the method of Barga de Quiroga et al. [10]. Glutathione peroxidase (GPx) activity (nmol NADPH/min/per mg protein) was determined as Se-dependent GPx and total GPx activities. Se-dependent GPx activity was assessed, using H_2O_2 as substrate and including azide as catalase inhibitor, by the method of Paglia and Valentine [11]. Total GPx activity was measured, using cumene hydroperoxides, according to the Lawrence and Burk method [12]. Catalase (CAT) activity was determined by measuring the decrease in absorbance at 240 nm using hydrogen peroxide as substrate [13]; one CAT unit is defined as the amount of enzyme that transforms 1 μmol of hydrogen peroxide per minute at 25 °C. Catalase activity was expressed as international units per milligram protein. Total superoxide dismutase activity (SOD) was determined using the method described by Marklund [14], based on the capacity of pyrogallol to autoxidize, a process highly dependent on superoxide radicals. SOD activity was expressed as international units per milligram protein. Liver protein concentrations were determined using bovine serum albumin as the standard, by the Bradford method [15].

Glutathione Status and Lipid Peroxide Assay

Reduced glutathione (GSH) content was determined using a slight modification of the Hissin and Hilf method [16], in which GSH was sequentially oxidized by o-phthaldialdehyde and reduced by NADPH in the presence of GR. The

oxidized glutathione level (GSSG) was determined by masking GSH with N-ethylmaleimide. Glutathione values were expressed as μg of GSH and GSSG per mg protein. The redox index was calculated as the GSSG/total glutathione ratio. Aliquots of the homogenate were collected and immediately tested for lipid peroxidation as thiobarbituric acid reactive substances (TBARS), according to the Mihara and Uchiyama method [17]. Liver TBARS concentrations were expressed as μmol/mg of protein.

Plasma Cholesterol

Plasma cholesterol levels were determined using the standard enzymatic colorimetric method of SPINREACT (Sant Esteve de Bas, Girona, Spain).

RT-PCR Analysis

Total RNA was extracted from frozen liver samples following the guanidinium thiocyanate/phenol reagent method [18]. β-actin cDNA was used as an internal control.

The sequences of the primers were as follows:

Catalase sense: 5′ GTGAGAACATTGCCAACCAC 3′; Catalase antisense: 5′ CTCGGGAAATGTCATCAAAAG 3′; Mn-SOD sense: 5′ GACAAACCTGAGCCCTAAGGG 3′; Mn-SOD antisense: 5′ CTTCTTGCAAACTATG 3′; Cu, Zn-SOD sense: 5′ GCCGTGTGCGTGCTGAA 3′; Cu,Zn-SOD antisense: 5′ TGACGATGCCGTGCTGCATG 3′; GPx sense: 5′CCAATCAGTTCGGACACCAG 3′; GPx antisense: 5′AAAGTTCCAGGCAATGTCGT 3′; GR sense: 5′TCACTGCTCCGCACATCC 3′; GR antisense: 5′ CTCAACACCGCCAGCGTTCTCC 3′; β-actin sense: 5′ TACAACCTCCTTGCAGCTCC 3′; β-actin antisense: 5′ GGATCTTCATGAGGTAGTCAGTC 3′.

Amplification products were resolved by electrophoresis on a 1.8% agarose gel containing 1 μg/ml ethidium bromide. The gel was then photographed under UV transillumination. The PCR bands on the photograph of the gel were scanned for quantification using a densitometer linked to a computer analysis system. Net band intensity (background-subtracted intensity) was normalized to β-actin values and plotted as arbitrary unit [19].

Statistical Analyses

Results are expressed as mean values and standard deviations. The present study was designed to have a power of 80% to detect a 20% relative difference between the two meat-diet consumptions in plasma cholesterol levels or Zn, Cu SOD expression. A pooled SD of 15% of data variability was assumed for this calculation. Two-way ANOVA (cholesterol and alga) followed by *post hoc* studies were performed to analyze the effect of each factor. The alga effect was checked by one-way ANOVA followed by the Bonferroni test, while the cholesterol effect was analyzed by the Student *t*-test. Results were considered significant at $P<0.05$. Statistical analyses were conducted using the SPSS version 15.0 statistical analysis packages (SPSS Inc., Chicago, IL, USA).

Results

Food Ingestion, Body Weight and Liver Weight

Table 2 shows the effect of alga and dietary cholesterol on food ingestion, body weight, and liver weight. Significant

Table 2 Effect of a cholesterol and non-cholesterol-enriched diets containing restructured meat with Wakame or Nori on food intake, body-weight gain, liver weight and hepatosomatic index of Wistar rats[a]

	Added cholesterol	Control meat diets (C, CC)	Wakame-meat diets (W, CW)	Nori-meat diets (N, CN)	ANOVA		
					Diet effect	Cholesterol effect	Diet × Cholesterol interaction
Food Intake (g/d)	No	19.09±1.31a	19.31±0.96b	17.90±1.19a	0.801	<0.001	<0.001
	Yes	21.21±1.22a ***	21.13±1.44a**	22.9±1.12b***			
Body-weight gain (g)	No	192.0±16.0a	185.1±18.9ab	172.8±13.5b	0.407	0.433	0.009
	Yes	184.3±19.2	170.2±15.5	190.2±14.1*			
Liver weight (g)	No	8.93±0.51	8.68±1.06	8.64±1.08	0.269	<0.001	0.161
	Yes	16.9±2.89 ***	16.47±2.05 ***	18.3±2.0***			
Hepatosomatic index[b]	No	2.87±0.24	2.85±0.26	2.96±0.32	0.482	<0.001	0.815
	Yes	5.51±0.65***	5.63±0.48 ***	5.77±0.63***			

[a] Values (mean ± SD, $n=10$) in the same row bearing different letters were significantly different ($P<0.05$). Values for the same diet but with different cholesterol contents bearing asterisks were significantly different (*$P<0.05$, **$P<0.01$, ***$P<0.001$). C, control group; W, Wakame group; N, Nori group. CC, CW, and CN consumed C, W, N basal diets enriched with cholesterol (2%) and cholic acid (0.4%)

[b] 100*liver weight (g)/bodyweight (g)

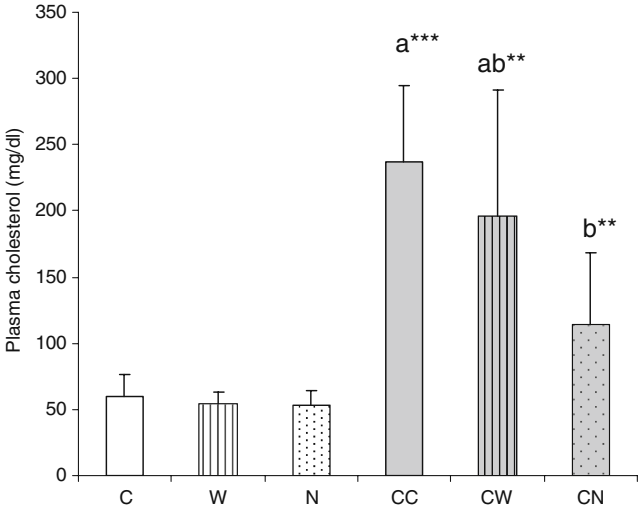

Fig. 1 Effect of cholesterol and non-cholesterol enriched diets containing restructured meat with Wakame or Nori on plasma cholesterol of Wistar rats. Values (mean ± SD, n=10) in the same dietary cholesterol level bearing different letters were significantly different (P<0.05). Values for the same diet but with different cholesterol content bearing asterisks were significantly different (*P<0.05, **P<0.01, ***P<0.001). C, control group; W, Wakame group; N, Nori group. CC, CW, and CN consumed C, W, N basal diets enriched with cholesterol (2%) and cholic acid (0.4%)

interactions were observed on food intake and body weight gain. Cholesterol in the diet significantly affected food intake, liver weight (P<0.001), and the hepatosomatic index (all, P<0.001). Body weight decreased in the N group (P<0.05) *vs*. C group.

Plasma Cholesterol Levels

Figure 1 shows the effect of alga and dietary cholesterol on plasma cholesterol levels. Significant alga × cholesterol interaction (P=0.001) was observed. Cholesterol in the diet significantly increased plasma cholesterol levels in CC (P< 0.001), CW, and CN (both P<0.01) animals. CN rats showed significantly (P<0.001) lower cholesterol levels than CC rats.

Liver Antioxidant Enzyme Activities and Expression

Table 3 shows the influence of alga and dietary cholesterol on the different enzyme activities and expression assayed. Non significant alga × dietary cholesterol in diet interactions was observed. However, dietary cholesterol significantly affected all parameters except catalase activity. Compared to the C diet, the W diet produced higher (P< 0.05) GR activity while the N diet increased total GPx, GR, and SOD activities (at least P<0.05). Supplementary cholesterol in the diet significantly decreased the activity of total GPx (P<0.001), Se-dependent GPx (P<0.001), and SOD (P=0.001) in the N group and Se-dependent GPx (P< 0.01), GR (P<0.01), and SOD (P<0.001) in the W group.

Gene expression of CAT, Mn-SOD, Cu,Zn-SOD GPx, and GR in liver homogenate, expressed in arbitrary units, is also included in Table 3. Representative gene expression profiles of the C, W, N, CC, CW, and CN groups assayed by RT-PCR analysis are presented in Fig. 2. The expression of all enzymes was significantly affected (P<0.001) by the alga × dietary cholesterol interaction. W diet significantly increased GPx and GR expression (at least P<0.001) and decreased that of catalase (P<0.01) while the N diet increased catalase and Mn-SOD expression (both P< 0.001) with respect to C diet. CC diet produced lower catalase, Cu,Zn-SOD, and GR expression (all P<0.001) but higher Mn-SOD (P<0.001) and GPx (P<0.05) expression than the C diet. CW diet produced lower GPx and GR expression (both P<0.001) and higher Mn-SOD (P<0.001) expression than W diet. Animals given the CN diet showed lower catalase, Mn-SOD and GPx, but higher GR expression than their CC counterparts while lower catalase, and higher Mn-SOD, Cu,Zn-SOD and GR expression (at least P<0.05) than their CW counterparts.

Glutathione Status and TBARS Concentrations

Table 4 shows the concentrations of GSH, GSSG, and total glutathione, the redox index, and TBARS levels. Non-significant alga × dietary cholesterol interaction was observed on these parameters. W diet produced higher total glutathione (P<0.05) and GSH (P<0.01) levels and lower redox index value (P<0.05) than C diet. N diet did not affect any of these parameters. Inclusion of cholesterol in the diet significantly decreased GSH (P<0.001) and increased GSSG concentrations, the redox index and TBARS levels (all P<0.001). CW rats presented higher total glutathione and GSH values and a lower redox ratio (P<0.05) than CC rats.

Discussion

Functional foods have been designed to improve lipoprotein profile [3, 4, 20] and antioxidant status [8, 21]. Present data suggest that consumption of alga-enriched RM offers protection against CHD, although the mechanisms involved vary depending on the kind of alga employed. While acceptance of the W and N diets compared favorably to that of the C diet, rats that consumed the N diet displayed a non-significant 9% lower body weight than those given the C diet. Wong et al. [22] reported that seaweed-fed rats and controls displayed similar food intake rates. Algae could be useful components of hypocaloric diets. Oben et al. [23] found that 1 floz/day of an oral infusion of ProAlgaZyme

Table 3 Effect of cholesterol and non cholesterol-enriched diets containing restructured meat with Wakame or Nori on enzymes antioxidant activities and expression in liver homogenates of Wistar rats[a]

	Added cholesterol	Control meat diets (C, CC)	Wakame-meat diets (W, CW)	Nori-meat diets (N, CN)	ANOVA		
					Diet effect	Cholesterol effect	Diet × Cholesterol interaction
Se-dependent GPx (nmol NADPH/min/mg protein)	No	2151±214	2380±373	2423±358	0.101	<0.001	0.279
	Yes	1666±201***	1893±274**	1654±396***			
Total GPx (nmol NADPH/min/mg protein)	No	2547±261a	2778±461ab	3160±500b	0.010	<0.001	0.148
	Yes	2079±384***	2452±486	2316±352***			
Se-dependent GPx/Total GPx	No	0.85±0.06a	0.86±0.06a	0.77±0.08b	0.016	0.042	0.763
	Yes	0.81±0.12a	0.78±0.09b*	0.72±0.13b			
GR (nmol NADPH/min/mg protein)	No	58.4±6.8a	70.4±11.6b	69.3±9.5a	<0.001	<0.001	0.986
	Yes	40.1±4.6a***	52.1±10.5b**	51.9±11.4b***			
Catalase (Units/mg protein)	No	348±128	396±106	389±119	0.905	0.809	0.797
	Yes	375±160	366±153	365±166			
SOD (Units/mg protein)	No	0.14±0.03a	0.14±0.03a	0.17±0.03b	0.059	<0.001	0.320
	Yes	0.10±0.05a	0.0 6±0.03b***	0.09±0.05ab***			
Catalase expression (AU)	No	81.7±6.2a	31.8±5.4b	99.8±6.2c	<0.001	<0.001	<0.001
	Yes	50.9±6. 8a ***	33.0±8.8b	33.3±5.7b***			
Mn-SOD expression (AU)	No	16.2±1.3a	14.0±1.2a	24.9±2.6b	<0.001	<0.001	<0.001
	Yes	40.5±2.5a ***	116.2±3.6b ***	31.8±4.0c***			
Cu,Zn-SOD expression (AU)	No	88.5±13.1	90.0±18.1	92.9±20.8	<0.001	0.003	0.002
	Yes	43.1±18.7a ***	95.7±24.0b	108.0±29.8b			
GPx expression (AU)	No	29.2±6.3a	55.3±3.4b	33.2±5.9a	0.001	<0.001	<0.001
	Yes	38.2±8.1a*	18.0±6.1b***	20.2±7.8b***			
GR expression (AU)	No	76.5±12.7a	136.3±8.4b	83.2±7.8a	<0.001	<0.001	<0.001
	Yes	24.3±9.4a ***	87.1±12.2b ***	59.0±10.0c***			

[a] Values (mean ± SD, n=10) in the same row bearing different letters were significantly different (P<0.05). Values for the same diet but with different cholesterol content bearing asterisks were significantly different (*P<0.05, **P<0.01, ***P<0.001)

[b] GSSG/Total glutathione. For diet details see text and footnote of Table 2

for 10-weeks reduces body weight and body fat. The final body weight of rats in the present study was higher than that of a previous study [8], although the average food intake of the present study was 10% lower. The inclusion of meat, together with the higher fat and energy content of the present experimental diets, at least partially explain the differences in food intake between both studies. According to Bocanegra et al. [8] and Murata et al. [24], body weight gain was negatively affected by supplementary dietary cholesterol. Animals in the CC, CW, and CN groups consumed more than their respective basal counterparts, partially because of the lower energy content of the cholesterol-enriched diets. Present results indicate that CC and CW rats had a tendency to gain less weight than C and W rats. This effect was not seen in CN rats respect to N counterparts, probably due to the higher food intake of CN rats. The lower feed efficiencies may explain the lower body weight gains despite a higher feed and energy intake on the cholesterol fed rats. Further, these lower feed efficiencies may suggest that the digestibility of the diet may be lower on the cholesterol fed diets, as the growth is determined by the amount of digestible energy.

The fact that W and N diets did not modify plasma cholesterol values was observed in studies using diets containing algae but not cholesterol [4, 8]. Concurring with other studies [4, 24, 25], cholesterol supplementation induced hypercholesterolemia in CC rats. Plasma cholesterol levels decreased in rats fed a cholesterol-enriched diet containing algal polysaccharides [26, 27]. While the CN diet partially inhibited dietary induction of hypercholesterolemia, the CW diet did not have the same effect. Ren et al. [28] described that some seaweed polysaccharides exert hypolipemic effects in rats fed a diet rich in sodium and cholesterol. Added to a casein-cholesterol diet, Nori induced lower plasma cholesterol levels in rats than Konbu [4]. Sodium alginate, funoran, porphyran, and carrageenan were found to interact with dietary cholesterol to facilitate its excretion, while dietary agar was almost inactive [26]. Cholesterol-enriched diets produced hepatomegaly and steatosis and increased the hepato-somatic index [8, 29].

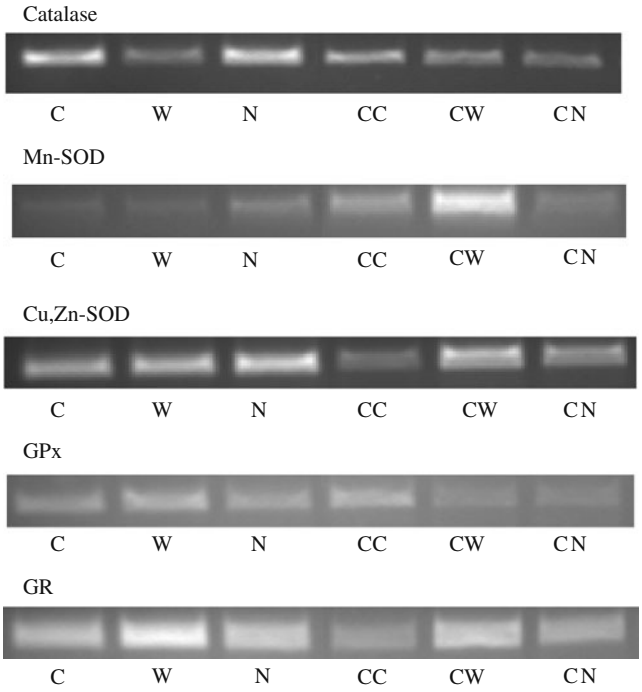

Fig. 2 Effect of a cholesterol-enriched diet containing restructured meat with Wakame or Nori on catalase, Mn-SOD, Cu,Zn-SOD, GPx, and GR mRNA expression, assayed by RT-PCR analysis, in liver homogenates of Wistar rats

Inclusion of Wakame or Nori did not decrease the hepatomegaly induced by cholesterol feeding. However, Bocanegra et al. [8] found that liver weight increased in rats fed diets enriched in cholesterol and algae. Wong et al. [22] did not find any significant differences in the liver weights of rats given various diets containing algae and cholesterol.

To the best of our knowledge this is the first study of the effect of seaweed-RM consumption on antioxidant enzyme gene expression and activities. Pork meat contains high levels of Fe and Cu [30]. Iron charge produces liver peroxidation and oxidative damage. Fe has been associated with oxidative stress but no evidence of the ability of the intestinal tract and/or the liver to decrease this oxidative stress exists. Furthermore, the inclusion of algae, rich in antioxidants, would counterbalance the pro-oxidant effect of Fe and/or Cu [3]. Study results suggest that the N diet affected the antioxidant status through a complex mechanism which involved total GPx, GR, and SOD activities. On the other hand, the W diet increased only GR values, suggesting that the increased glutathione recycling observed mainly as a result of this diet was responsible for the drop in the redox index. Only W-meat significantly decreased catalase mRNA levels. Catalase activity tended to increase in the liver of rats consuming W- meats or N-meats. Catalase protects the cells from hydrogen peroxide and plays an important role in adaptation to oxidant stress. Results of the W-diet are in accordance with those of Murata et al. [24] who found that a diet containing Wakame had no significant effect on catalase activity in rats.

The mechanisms by which N-diets and W-diets influence catalase, Mn-SOD, and GSH expression and activities in different ways have not yet been identified but must related to differences in their polyphenol, fiber, mineral, and fatty acid contents [3]. Brown seaweeds display high phlorotannin concentrations [31], and polymeric phlorotannins inhibit enzymes implicated in radical species production [32, 33]. According to Nikaido et al. [34], as blocks the production of GSH and total glutathione. Bocanegra et al. [8] suggested

Table 4 Effect of cholesterol and non cholesterol-enriched diets containing restructured meat with Wakame or Nori on glutathione status and TBARS concentrations in liver homogenates of Wistar rats[a]

	Added cholesterol	Control meat diets (C, CC)	Wakame-meat diets (W, CW)	Nori-meat diets (N, CN)	ANOVA		
					Diet effect	Cholesterol effect	Diet × Cholesterol interaction
Total glutathione (μg/mg protein)	No	9.48±0.78a	11.08±1.54b	9.80±1.34a	0.003	0.920	0.485
	Yes	8.74±1.92a	11.34±2.17b	10.42±2.20ab			
GSH (μg/mg protein)	No	6.97±0.78a	8.89±1.27b	7.62±0.98ab	<0.001	<0.001	0.984
	Yes	3.62±0.87a***	5.46±0.96b***	4.30±0.90a***			
GSSG (μg/mg protein)	No	2.51±0.58	2.20±0.32	2.18±0.60	0.650	<0.001	0.170
	Yes	5.12±1.30***	5.88±1.51***	6.12±1.55***			
Redox index[b]	No	0.27±0.06a	0.20±0.01b	0.22±0.04ab	0.001	<0.001	0.311
	Yes	0.58±0.06a***	0.52±0.05b***	0.59±0.06a***			
TBARS (μmol/mg protein)	No	9.34±2.63	10.4±2.63	9.72±3.75	0.551	<0.001	0.135
	Yes	62.3±11.1***	69.6±12.4 ***	68.5±8.1***			

[a] Values (mean ± SD, $n=10$) in the same row bearing different letters were significantly different ($P<0.05$). Values for the same diet but with different cholesterol content bearing asterisks were significantly different (*$P<0.05$, **$P<0.01$, ***$P<0.001$)

[b] GSSG/Total glutathione. For diet details see text and footnote of Table 2

negative effect of As on glutathione status after algae consumption. The As content of the restructured meat consumed in the present study was lower than that used by Bocanegra et al. [8] (data not shown) explaining, at least in part, the absence in the present study of a significant negative effect of W-meat and N-meat on GSH. W-meat actually increased total glutathione and GSH concentrations and decreased the redox index. Moreover, the increase observed in GR values could counterbalance the negative effect of As on GSH.

Present results on enzyme activities and expression seem contradictory; however, several studies suggest that these two parameters do not always follow a parallel course [19]. This lack of correlation may be due to the alteration of post-transcription events such as translation and transport of gene products. It could result in loss of functional molecules, minor post-transductional changes, and finally, no changes in GPx and GR activities. On the other hand, the cellular redox state and the GSSG/total glutathione ratio are involved in the activation of transcriptional factors such as API o NF-κB that participate in antioxidant enzyme expression.

Diets enriched in cholesterol induced oxidative stress, measured as TBARS, coinciding with the findings of Lin et al. [35]. Nonetheless, W-meat diet increased GSH levels and decreased the redox index in the presence of added dietary cholesterol. Cholesterol feeding decreased most enzyme activities in the CC, CW, and CN groups. However, supplementary cholesterol had different effects on CW and CN rats. While CW rats displayed 30% higher GR and 40% lower SOD levels than CC animals, rats in the CN group presented 30% higher GR values than their CC counterparts. These results contrast with those of Bocanegra et al. [8] who found that cholesterol feeding significantly increased Se dependent- and total GPx activities and GR levels (about 1.5-fold). As previously commented, meat contains high amounts of Fe, known to increase free radical stress [9]. In fact, present GSSG levels in C animals were much higher than those described by Bocanegra et al. [8]. Although W-meat was not able to completely block the decrease of GSH induced by dietary cholesterol, CW animals displayed higher expression of GR, Cu,Zn-SOD, and Mn-SOD, and lower expression of GPx, than their CC counterparts, suggesting that the CW diet affected anti-oxidant status positively by maintaining high GSH levels. GSH values and the redox index of CN rats indicate that their diet did not improve their antioxidant status, although increases in the expression of some antioxidant enzymes were observed. Ringseis and Eder [36] concluded that oxidized dietary cholesterol gives rise to significantly higher mRNA concentrations of GPx and SOD and GPx activity, but lower concentrations of total glutathione and GSH in the liver than rats fed diets containing non-oxidized cholesterol. Present study data concur with those findings.

In conclusion, a diet containing meat enriched in Wakame and Nori was well accepted by growing Wistar rats and induced adequate body weight gain. According to the present data, W-meat offers antioxidant properties while N-meat displays hypocholesterolemic effects. More studies are needed for a better comprehension of the differences observed in hypocholesterolemic and antioxidant effects of Nori-RM *vs*. Wakame-RM in order to permit extrapolation of present results to human.

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