Protein Insufficiency Aggravates the Enhanced Lipid Peroxidation and Reduced Activities of Antioxidative Enzymes in Rats Fed Diets High in Polyunsaturated Fat¹

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ABSTRACT To investigate the effects of dietary protein and polyunsaturated fat levels on tissue lipid peroxidation and antioxidative enzymes, Long-Evans male weanling rats were fed either an 8% lactalbumin diet containing 2% (L2), 5% (L5), 10% (L10), 15% (L15) or 20% (L20) soybean oil or a 20% lactalbumin diet containing 5% (N5) or 20% (N20) soybean oil for 8 weeks. The tissue thiobarbituric acid-reactive substances (TBARS) concentrations of the L2 group were similar to those of the N5 group except in plasma in which they were higher. The L5 group generally showed tissue TBARS concentrations comparable to the N20 group. Gradually increasing the dietary soybean oil level in the low protein diet further increased the tissue TBARS concentrations. The L20 group had significantly higher TBARS in RBC, liver, heart, kidney and muscle than the N20 group. The low protein-fed groups had lower activities of glutathione peroxidase (EC 1.11.1.9), superoxide dismutase (EC 1.15.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in liver and catalase (EC 1.11.1.6) in RBC than the N5 group. Compared with the N5 group, the N20 group also showed higher TBARS concentrations and lower activities of certain antioxidative enzymes in some tissues. The antioxidative enzyme activities decreased more drastically with the increasing dietary soybean oil level in the low protein-fed groups than in those fed a normal level of protein. Supplementation of 150 mg/kg of all-rac- α -tocopheryl acetate to the L15 diet slightly decreased the TBARS in plasma, heart and liver and restored the depressed activities of RBC superoxide dismutase and catalase. The results indicated that insufficiency of dietary protein aggravates the enhanced production of TBARS and the reduced activities of antioxidant enzyme in rats fed a high soybean oil diet. *J. Nutr.* 122: 1182-1189, 1992.

INDEXING KEY WORDS:

- rats • polyunsaturated fat
- protein • lipid peroxidation
- antioxidative enzymes

Free radical-mediated lipid peroxidation has been implicated in a variety of pathological processes (1-3). The susceptibility of a given organism to oxidative damage is influenced by the overall balance between the degree of oxidative stress and antioxidative capabilities. Nutrition plays an important role in determining the cellular antioxidative defense mechanism (4, 5). The status of many nutrients, such as vitamin E, selenium, sulfur-containing amino acids, copper and zinc, is known to be closely related to the antioxidative capability of the animal (4, 5). However, relatively little is known concerning an effect of dietary protein on the antioxidative defense mechanisms, despite speculation that protein malnutrition may result in the impairment of the antioxidative system (4, 5).

In exploring the effects of rancid frying oil and dietary protein levels on rat liver microsomal enzymes (6), we observed an unexpected elevation of in vitro hemolysis in erythrocytes of rats fed a low protein, fresh soybean oil diet, suggesting an enhanced tissue lipid peroxidation in these rats. This observation was further confirmed in later studies (7, 8). We speculate that feeding the high soybean oil (15% wt/wt) low protein (8% lactalbumin, wt/wt) diet might result in lowered antioxidative enzyme activities and enhanced lipid peroxidation in tissues such as RBC. Deneke et al. (9) reported a significant reduction in lung superoxide dismutase (SOD, EC 1.15.1.1)³ activity of rats fed a 3% casein diet and

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³Abbreviations used: G6PDH, glucose-6-phosphate dehydrogenase; GSHPx, glutathione peroxidase; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

TABLE 1
Composition of test diets

Ingredient ²	Diet code ¹							
	N5	N20	L2	L5	L10	L15	L15E ³	L20
	g/kg diet							
Lactalbumin	200	200	80	80	80	80	80	80
AIN-76 vitamin mixture ⁴	10	10	10	10	10	10	10	10
α -Cellulose	30	30	30	30	30	30	30	30
AIN-76 mineral mixture ⁴	35	35	35	35	35	35	35	35
Soybean oil	50	200	20	50	100	150	150	200
Choline chloride	3	3	3	3	3	3	3	3
Cornstarch	672	522	822	792	742	692	692	642

¹Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

²Lactalbumin, Sigma Co., St. Louis, MO; α -cellulose, ARBOCEL® Type BE 600/300, J. Bettenmaier & Sohne, Germany; soybean oil, Taiwan Sugar Company, Taipei, Taiwan; cornstarch, Roquette, France.

³L15E Diet: 150 mg/kg diet of all-*rac*- α -tocopheryl acetate was added to L15 diet.

⁴AIN-76 vitamin mixture and AIN-76 mineral mixture (11).

exposed to hyperoxic conditions and have also shown significantly lowered lung catalase (EC 1.11.1.6) activity in rats fed a protein-free diet and similarly exposed (10). In both studies, oxygen toxicity was potentiated by feeding a protein-deficient diet.

We hypothesize that inadequacy of dietary protein may result in lowered antioxidative enzyme activities and enhanced tissue lipid peroxidation. These effects of a low protein diet may not be significant at low polyunsaturated fat intake but may be manifested and aggravated when dietary polyunsaturated fat level is gradually increased. To test the hypothesis, we fed rats 8% lactalbumin diets containing various amounts of soybean oil ranging from 2 to 20 g/100 g, and measured the thiobarbituric acid-reactive substances (TBARS) in various tissues and antioxidative enzyme activities in liver and RBC. Control rats were fed the 20% lactalbumin diet containing either 5 or 20% soybean oil.

MATERIALS AND METHODS

Animals and diets. Fifty-six male Long-Evans weanling rats, weighing 42.8 ± 5.7 g, purchased from Laboratory Animal Center, College of Medicine, National Taiwan University, were housed individually in stainless-steel wire cages in a room maintained at $25 \pm 1^\circ\text{C}$ with a controlled 12-h light:dark cycle. The rats were randomly assigned to eight diet groups with seven rats in each group. Food and tap water were freely available. Body weight and food intake were recorded weekly. The NIH *Guide for the Care and Use of Laboratory Animals* was adhered to in the care and handling of the animals.

The composition of the eight test diets is shown in Table 1. The dietary protein was either supplied by 20% (normal protein) or 8% (low protein) lactalbumin. The soybean oil content of the diets was 2, 5, 10, 15 or 20% (wt/wt). The diet groups N5 and N20 denoted normal protein diets containing 5% and 20% soybean oil, respectively. Likewise, L2, L5, L10, L15 and L20 diets denoted low protein diets containing 2, 5, 10, 15 and 20% soybean oil (wt/wt), respectively. The L15E diet was the L15 diet supplemented with an excess amount of all-*rac*- α -tocopheryl acetate (150 mg/kg diet). Other ingredients were cellulose, starch, AIN-76 vitamin mixture and AIN-76 mineral mixture (11). In preparing the test diets the powdered ingredients were mixed in advance, and the oil portion was blended in weekly before feeding.

Tissue sampling and preparation. After 8 wk of feeding, rats were killed by carbon dioxide exposure between 0830 and 0930 h on five successive days. Blood was drawn by heart puncture using heparinized evacuated blood collecting tubes (Venoject, Terumo, Tokyo, Japan). Aliquots of blood samples were centrifuged at $1000 \times g$ for 10 min to separate plasma and RBC. Erythrocyte lysates (20%, v/v) were prepared by lysing aliquots of washed RBC with deionized water. Liver, kidney, heart, spleen, lung and gastrocnemius muscle were excised and weighed. Except for a portion of the liver, all the remaining tissues, as well as the plasma and part of the RBC lysates, were immediately frozen in liquid nitrogen and stored at -20°C until analysis.

The portion of the fresh liver sample was immediately homogenized in ice-cold 0.01 mol/L phosphate buffer (pH 7.4, containing 11.5 g/L KCl) with a Potter-Elvehjem-type homogenizer with a teflon pestle. Aliquots of the homogenate (100 g/L) were centrifuged at

TABLE 2

Final body weight, body weight gain, food intake and food efficiency of rats fed normal or low protein diets containing various levels of soybean oil¹

Diet group ²	Body weight gain/8 wk	Final body weight	Food intake	Food efficiency	Total protein intake	Total energy intake	Relative liver weight
	g	g	g/8 wk	g gain/g food	g/8 wk	MJ/8 wk	g/100 g body wt
N5	262.9 ± 15.1 ^d	305.7 ± 20.4 ^d	822.6 ± 53.2 ^c	0.320 ± 0.012 ^d	164.5 ± 10.6 ^d	13.54 ± 0.88 ^c	3.8 ± 0.3 ^{ab}
N20	275.7 ± 20.8 ^d	318.6 ± 25.7 ^d	716.3 ± 43.7 ^b	0.385 ± 0.024 ^c	143.3 ± 8.7 ^c	14.25 ± 0.79 ^c	3.9 ± 0.4 ^b
L2	178.4 ± 22.1 ^c	221.4 ± 25.5 ^c	754.4 ± 84.1 ^b	0.236 ± 0.008 ^a	59.4 ± 6.4 ^b	11.74 ± 1.25 ^b	4.2 ± 0.6 ^b
L5	167.4 ± 14.9 ^{bc}	210.3 ± 19.8 ^{bc}	696.7 ± 42.4 ^b	0.240 ± 0.009 ^{ab}	55.7 ± 3.4 ^b	11.45 ± 0.71 ^{ab}	3.7 ± 0.8 ^{ab}
L10	147.1 ± 17.5 ^a	190.0 ± 22.7 ^{ab}	608.5 ± 58.2 ^a	0.241 ± 0.009 ^{ab}	48.7 ± 4.7 ^a	10.65 ± 1.00 ^a	3.9 ± 0.4 ^b
L15	149.4 ± 13.6 ^{ab}	192.4 ± 15.6 ^{ab}	594.3 ± 32.1 ^a	0.251 ± 0.013 ^{bc}	47.5 ± 2.6 ^a	11.03 ± 0.59 ^{ab}	3.9 ± 0.4 ^b
L15E	159.1 ± 7.8 ^{ab}	202.2 ± 10.3 ^{abc}	608.2 ± 29.1 ^a	0.262 ± 0.007 ^c	48.7 ± 2.3 ^a	11.28 ± 0.54 ^{ab}	3.7 ± 0.3 ^{ab}
L20	142.1 ± 17.9 ^a	185.0 ± 20.7 ^a	547.4 ± 59.4 ^a	0.259 ± 0.011 ^c	43.8 ± 4.7 ^a	10.74 ± 1.17 ^a	3.3 ± 0.4 ^a

¹Values are means ± SD for seven rats in each group. Values within the same column not sharing a superscript are significantly different, $P < 0.05$.

²Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

12,000 × g for 20 min (20 PR-5 freeze centrifuge, RPR 18 rotor, Hitachi, Tokyo, Japan) and the post-mitochondria supernatants were retained.

Analytical methods. Blood samples were analyzed for hematocrit by centrifugation in capillary and for hemoglobin by the cyanomethemoglobin method (12). Freshly prepared RBC lysates were treated with chloroform-ethanol as described by McCord and Fridovich (13) and assayed for the activities of SOD (14) and catalase (15). Aliquots of the post-mitochondria supernatants were treated with Drabkin's reagent to remove hemoglobin as described by Paglia and Valentine (16) and assayed for the activity of glutathione peroxidase (GSHPx, EC 1.11.1.9) by coupling to the glutathione reductase reaction according to the method of Lawrence and Burk (17). The post-mitochondria supernatants, as well as the treated RBC lysates, were assayed for SOD activity by the inhibition of photo-oxidation of *o*-dianisidine as described by Misra and Fridovich (14). They were also assayed for the activity of catalase by the method of Luck (15). The glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity of liver post-mitochondria supernatants was assayed by the increase of absorbance at 340 nm as described by Deutsch (18). Protein contents of post-mitochondria supernatants were quantified by the method of Lowry et al. (19) using bovine serum albumin as the standard. All the enzyme activities were determined within the zero-order kinetic portion of reaction curves.

Frozen tissues, including liver, kidney, heart, spleen, lung and muscle, were homogenized and analyzed for TBARS according to the method of Ohkawa et al. (20). Frozen plasma and RBC lysates were also analyzed for TBARS by the methods of Yagi (21) and Brownlee et al. (22), respectively. 1,1,3,3-Tetraethox-

ypropane (Sigma Chemical, St. Louis, MO) was used as the standard for all thiobarbituric acid reactions and the results were calculated as nanomoles of malondialdehyde.

Statistical analysis. Data were expressed as means ± SD for seven rats in each group. The significance of difference among groups was analyzed statistically by ANOVA and Duncan's multiple range test using the "Oneway" program (23) in the SPSS Package. Differences were considered significant at $P < 0.05$.

RESULTS

Growth, food intake and food efficiency. Body weight gain, food intake and food efficiency values (g gain/g food) are shown in Table 2. As expected, rats fed the low protein diets showed significantly lower body weight gain, food intake and food efficiency compared with the N5 group ($P < 0.05$). The reduction in growth and food intake became greater when the soybean oil level in the low protein diet was increased stepwise. Due to the high dietary fat level, the N20 group consumed less food but had similar weight gains, showing higher food efficiency than the N5 group. However, when rats were fed low protein diets, increasing dietary soybean oil to 20% (L20 group) resulted in significantly lower body weight gain than in the L2 and L5 groups.

Blood analysis. In the normal protein diet groups, TBARS in RBC were not significantly different between the high soybean oil (N20) and the low soybean oil (N5) groups (Table 3). However, in rats fed the low protein diet, TBARS in RBC increased significantly with the increase of dietary soybean oil level. As shown in Table 3, the RBC TBARS concentration of

TABLE 3

Hematocrit, hemoglobin, thiobarbituric acid-reactive substances (TBARS) in plasma and RBC and the activities of superoxide dismutase (SOD) and catalase in RBC of rats fed normal or low protein diets containing various levels of soybean oil¹

Diet group ²	Hematocrit	Hemoglobin	TBARS			RBC SOD ³	RBC Catalase ⁴
			Plasma	RBC	RBC		
			$\mu\text{mol MDA}^5/\text{L}$	$\mu\text{mol MDA}/\text{L RBC}$	$\text{nmol MDA}/\text{g Hb}^6$		
	vol. fraction	g/L blood				U/g Hb	$\mu\text{U/g Hb}$
N5	0.44 ± 0.03 ^e	134 ± 21 ^{cd}	4.3 ± 0.1 ^a	3.0 ± 0.6 ^a	46.5 ± 5.7 ^a	250.5 ± 18.9 ^c	16.2 ± 4.3 ^c
N20	0.42 ± 0.01 ^d	157 ± 24 ^d	6.0 ± 0.6 ^{cde}	3.4 ± 0.4 ^{ab}	47.4 ± 4.7 ^a	202.0 ± 22.1 ^b	13.7 ± 4.1 ^b
L2	0.41 ± 0.01 ^{cd}	120 ± 9 ^{abc}	5.4 ± 0.4 ^b	3.7 ± 0.7 ^{ab}	58.3 ± 7.3 ^a	246.5 ± 25.0 ^c	13.7 ± 1.6 ^b
L5	0.40 ± 0.02 ^{bc}	134 ± 11 ^{bcd}	5.6 ± 0.3 ^{bc}	4.0 ± 0.8 ^b	61.2 ± 11.1 ^{ab}	241.8 ± 19.5 ^c	13.4 ± 1.1 ^b
L10	0.39 ± 0.01 ^{ab}	131 ± 17 ^{abc}	5.7 ± 0.4 ^{bcd}	4.8 ± 0.6 ^c	73.5 ± 13.0 ^{bc}	208.3 ± 28.2 ^b	11.2 ± 1.9 ^{ab}
L15	0.38 ± 0.04 ^a	115 ± 11 ^a	6.1 ± 0.5 ^{de}	5.3 ± 0.8 ^c	88.5 ± 19.5 ^{cd}	169.9 ± 34.2 ^a	9.3 ± 2.6 ^a
L15E	0.38 ± 0.01 ^a	117 ± 6 ^{ab}	5.5 ± 0.3 ^{bc}	5.0 ± 0.9 ^c	81.7 ± 16.5 ^c	245.9 ± 40.9 ^c	13.8 ± 3.1 ^b
L20	0.38 ± 0.04 ^a	125 ± 15 ^{abc}	6.6 ± 0.3 ^e	6.3 ± 1.2 ^d	99.2 ± 21.3 ^d	164.9 ± 27.5 ^a	9.0 ± 3.6 ^a

¹Values are means ± SD for seven rats in each group. Values within the same column not sharing a superscript are significantly different, $P < 0.05$.

²Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

³1 unit (U) of activity is equivalent to the activity of 1 μg purified bovine erythrocyte superoxide dismutase (Sigma Chemical, St. Louis, MO) in the riboflavin-o-dianisidine photo-oxidation system (14).

⁴Catalase, 1 microunit (μU) = 1 nmol H_2O_2 utilized/min.

⁵MDA = malondialdehyde.

⁶Hb = hemoglobin.

the L2 group was not significantly different from that of the N5 and N20 groups. However, TBARS concentration in RBC of the L20 group was twice that of the N5 and N20 groups. Similar trends existed among the plasma TBARS of various groups of rats except that plasma TBARS of the N20 group was 40% higher than that of the N5 group ($P < 0.05$). The results indicated that even at normal protein intake, high polyunsaturated fat intake may increase TBARS in plasma, but not in RBC. The L15E group had significantly lower plasma TBARS than in the L15 group, suggesting that supplementation of vitamin E effectively reduced plasma TBARS, but not RBC TBARS, in rats fed low protein-high soybean oil diet.

The RBC SOD activity of the L2 and L5 groups was not significantly different from that of the N5 group, indicating that inadequate protein intake did not change RBC SOD activity when the dietary fat level was low (Table 3). However, increased soybean oil intake, as in N20, L10, and especially L15 and L20 groups, resulted in significant reductions in RBC SOD activity.

Unlike the SOD activity, RBC catalase activity was lowered either by high soybean oil or low protein diet alone. The RBC catalase activity of the N5 group was significantly higher than in all the remaining groups. As in the case of RBC SOD, the activity of catalase was further depressed when diets contained both high soybean oil and low protein. The activity of this enzyme in RBC of L15 and L20 groups was only 56% that of the N5 groups ($P < 0.05$) and was also signifi-

cantly lower than the activities of the N20, L2 and L5 groups. Supplementation of vitamin E to the L15 diet significantly reversed the reduced RBC SOD and catalase activities to a level comparable to those of the L2 and L5 groups.

Tissue thiobarbituric acid-reactive substance concentrations. Rats fed high soybean oil diets generally showed higher levels of tissue TBARS (Table 4). Rats fed the normal protein and 20% soybean oil diet (N20) showed 17% (spleen), 50% (heart), 27% (kidney) and 74% (muscle) greater TBARS concentrations than those of the N5 group ($P < 0.05$). Tissue TBARS concentrations in the L2 group were not significantly different from those of the N5 group. When dietary soybean oil level was increased from 2% (L2) to 5% (L5) in low protein diet, we observed 29% (spleen), 71% (heart), 28% (lung), 44% (kidney) and 53% (muscle) increases in tissue TBARS concentration ($P < 0.05$). As the dietary soybean oil level was further increased in the low protein diet, rat tissue TBARS concentration also increased in parallel. As a result, the L20 group showed the highest TBARS concentrations, which were 56% (liver), 61% (spleen), 175% (heart), 49% (lung), 142% (kidney) and 104% (muscle) higher than in those of the N5 group. Furthermore, tissue TBARS levels of the L20 groups were also significantly higher in most tissues than in those of the N20 group, indicating that insufficiency of dietary protein aggravates the enhanced production of TBARS in rats fed a high soybean oil diet. Supplementation of vitamin E to the L15 diet significantly

TABLE 4

The concentration of thiobarbituric acid-reactive substances (TBARS) in various tissues of rats fed normal or low protein diets containing various levels of soybean oil¹

Diet group ²	TBARS					
	Liver	Spleen	Heart	Lung	Kidney	Muscle
	nmol MDA/g tissue ³					
N5	344.8 ± 40.5 ^a	307.9 ± 43.4 ^{ab}	156.6 ± 46.9 ^a	111.8 ± 16.6 ^{ab}	184.8 ± 30.9 ^a	207.5 ± 30.3 ^a
N20	405.7 ± 73.3 ^{ab}	360.0 ± 27.8 ^c	235.4 ± 46.2 ^{bc}	119.5 ± 28.3 ^{bc}	235.4 ± 30.3 ^b	361.3 ± 24.7 ^c
L2	333.4 ± 34.5 ^a	271.1 ± 34.1 ^a	131.1 ± 34.8 ^a	93.8 ± 13.1 ^a	177.9 ± 36.4 ^a	211.6 ± 25.6 ^a
L5	380.3 ± 34.1 ^{ab}	349.9 ± 14.6 ^{bc}	224.6 ± 76.4 ^b	120.4 ± 17.4 ^{bc}	256.6 ± 30.1 ^b	323.4 ± 50.4 ^b
L10	409.8 ± 62.5 ^b	398.7 ± 42.8 ^{bc}	316.1 ± 19.8 ^{de}	124.4 ± 30.5 ^{bc}	313.4 ± 38.9 ^c	375.5 ± 37.2 ^{cd}
L15	494.5 ± 82.8 ^c	427.6 ± 42.9 ^c	330.9 ± 24.7 ^e	130.8 ± 22.5 ^{bc}	364.6 ± 34.1 ^d	413.7 ± 33.8 ^{de}
L15E	397.2 ± 39.5 ^{ab}	365.1 ± 56.8 ^{bc}	273.9 ± 42.2 ^{cd}	123.9 ± 26.6 ^{bc}	334.0 ± 33.9 ^{cd}	388.5 ± 38.9 ^{cd}
L20	520.9 ± 96.5 ^c	436.7 ± 37.6 ^c	360.6 ± 30.5 ^e	140.1 ± 34.8 ^c	438.1 ± 24.8 ^e	431.7 ± 24.5 ^e

¹Values are means ± SD for seven rats in each group. Values within the same column not sharing a superscript are significantly different, $P < 0.05$.

²Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

³MDA = malondialdehyde.

reduced the elevated TBARS in liver and heart to a level comparable to those of the N20 group.

Liver antioxidative enzyme activities. The specific activity (U/mg protein) of liver GSHPx was significantly lower in L10, L15 and L20 groups than in the N5 group (Table 5). Neither raising dietary soybean oil (N20 vs. N5) nor reducing dietary protein (L2 and L5 vs. N5) alone altered this activity. The tissue GSHPx activity concentration (U/g liver) of the N5 group was significantly higher than that of all the

remaining groups, indicating that either high soybean oil or low protein alone could decrease the activity per gram of liver of this enzyme. When expressed as U/g body weight, the GSHPx activity was significantly lower in the N20 as well as in all the low protein diet groups except for the L2 group compared with the N5 group.

The specific activity of liver SOD was significantly higher in the N5 group than that of all the remaining groups of rats (Table 5). Tissue concentration and

TABLE 5

Hepatic glutathione peroxidase and superoxide dismutase activities of rats fed normal or low protein diets containing various levels of soybean oil¹

Diet group ²	Glutathione peroxidase ³			Superoxide dismutase ⁴		
	U/mg protein	U/g liver	U/g body weight	U/mg protein	U/g liver	U/g body weight
N5	0.46 ± 0.06 ^c	44.6 ± 3.1 ^e	1.64 ± 0.21 ^b	4.0 ± 1.3 ^b	325.5 ± 69.9 ^d	14 ± 4 ^b
N20	0.37 ± 0.05 ^{abc}	35.1 ± 4.2 ^{cd}	1.32 ± 0.21 ^a	2.9 ± 0.4 ^a	314.6 ± 72.4 ^{bcd}	10 ± 1 ^b
L2	0.42 ± 0.07 ^{bc}	39.9 ± 7.7 ^d	1.53 ± 0.29 ^b	3.3 ± 0.3 ^a	318.4 ± 83.0 ^{bcd}	13 ± 4 ^b
L5	0.35 ± 0.06 ^{abc}	31.9 ± 2.8 ^{abc}	1.30 ± 0.12 ^a	2.9 ± 0.2 ^a	282.4 ± 97.9 ^{abcd}	9 ± 1 ^{ab}
L10	0.34 ± 0.04 ^{ab}	30.9 ± 4.6 ^{abc}	1.15 ± 0.15 ^a	2.6 ± 0.4 ^a	226.0 ± 49.3 ^{abc}	8 ± 1 ^a
L15	0.32 ± 0.04 ^{ab}	26.3 ± 2.4 ^{ab}	0.97 ± 0.14 ^a	2.2 ± 1.0 ^a	201.0 ± 82.4 ^{ab}	8 ± 3 ^a
L15E	0.37 ± 0.03 ^{abc}	31.6 ± 3.0 ^{abc}	1.18 ± 0.12 ^a	2.8 ± 0.7 ^a	243.4 ± 86.4 ^{ab}	9 ± 3 ^{ab}
L20	0.28 ± 0.09 ^a	23.9 ± 7.1 ^a	0.87 ± 0.14 ^a	1.9 ± 0.1 ^a	171.7 ± 49.3 ^a	6 ± 2 ^a

¹Values are means ± SD for seven rats in each group. Values within the same column not sharing a superscript are significantly different, $P < 0.05$, Duncan's multiple range test.

²Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

³1 unit (U) = 1 μmole NADPH oxidized/min.

⁴1 unit (U) of activity is equivalent to the activity of 1 μg purified bovine erythrocyte superoxide dismutase (Sigma Chemical, St. Louis, MO) in the riboflavin-o-dianisidine photo-oxidation system (14).

TABLE 6

Hepatic catalase and glucose-6-phosphate dehydrogenase activities of rats fed normal or low protein diets containing various levels of soybean oil¹

Diet group ²	Catalase ³			Glucose-6-phosphate dehydrogenase ⁴		
	$\mu\text{U}/\text{mg protein}$	$\mu\text{U}/\text{g liver}$	$\mu\text{U}/\text{g body weight}$	$\text{U}/\text{mg protein}$	$\text{U}/\text{g liver}$	$\text{U}/\text{g body weight}$
N5	0.104 ± 0.038^a	10.6 ± 5.0^b	0.388 ± 0.192^b	0.026 ± 0.007^d	1.9 ± 0.3^d	0.081 ± 0.016^d
N20	0.081 ± 0.014^a	6.9 ± 1.6^a	0.248 ± 0.059^{ab}	0.017 ± 0.004^{ab}	1.4 ± 0.3^{cd}	0.060 ± 0.011^c
L2	0.081 ± 0.014^a	7.2 ± 1.3^{ab}	0.251 ± 0.100^{ab}	0.022 ± 0.006^c	1.9 ± 0.5^d	0.075 ± 0.015^d
L5	0.079 ± 0.004^a	6.7 ± 0.6^a	0.242 ± 0.011^a	0.015 ± 0.003^{ab}	1.3 ± 0.2^{bc}	0.046 ± 0.013^{bc}
L10	0.075 ± 0.010^a	6.1 ± 0.9^a	0.223 ± 0.027^a	0.014 ± 0.004^{ab}	1.1 ± 0.2^{ab}	0.039 ± 0.008^{ab}
L15	0.074 ± 0.003^a	5.9 ± 0.7^a	0.204 ± 0.015^a	0.010 ± 0.002^a	0.8 ± 0.2^a	0.028 ± 0.005^a
L15E	0.078 ± 0.009^a	6.5 ± 1.2^a	0.231 ± 0.043^a	0.015 ± 0.004^{ab}	1.1 ± 0.3^{ab}	0.044 ± 0.014^{bc}
L20	0.071 ± 0.016^a	5.2 ± 1.1^a	0.171 ± 0.017^a	0.010 ± 0.003^a	0.8 ± 0.2^a	0.023 ± 0.007^a

¹Values are means $\pm$ SD for seven rats in each group. Values within the same column not sharing a superscript are significantly different, $P < 0.05$.

²Diets are designated as N for normal protein (20 g lactalbumin/100 g diet) or L for low protein (8 g lactalbumin/100 g diet). The number following the L or N indicates the percentage (wt/wt) of soybean oil added to the diet.

³1 microunit (μU) = 1 nmole H_2O_2 utilized/min.

⁴1 unit (U) = 1 μmole NADPH oxidized/min.

activity/body weight of liver SOD of N20, L2 and L5 groups were not significantly different from those of the N5 group. However, L10, L15, L15E and L20 groups had significantly lower liver SOD activity concentration and activity/body weight than did the N5 group. The results indicated that SOD specific activity could be reduced by either a high soybean oil or a low protein diet alone, but only a low protein diet containing at least 10% soybean oil significantly lowered SOD activity per gram of liver and U/g body weight.

The hepatic catalase activity decreased in rats fed the high soybean oil or the low protein diets compared with the N5 group but the difference was not statistically significant when expressed as specific activity (Table 6). Except for the L2 group, all the remaining groups had significantly lower concentration of catalase activity in liver than the N5 group. The activity/body weight of liver catalase of L5, L10, L15, L15E and L20 groups were significantly lower than that of the N5 group.

The G6PDH specific activity in livers of N20 and L2 groups was slightly but significantly lower than that in liver of the N5 group ($P < 0.05$, Table 6). Moreover, the activity of liver G6PDH (U/mg protein, U/g liver and U/g body weight) of L5, L10, L15, L15E and L20 groups were further decreased compared with the N5 group ($P < 0.05$).

Although supplementation of dietary vitamin E to the L15 diet slightly increased the activities of liver GSHPx, SOD, catalase and G6PDH (Tables 5 and 6), the differences between L15E and L15 groups were statistically significant only for G6PDH when expressed relative to body weight.

DISCUSSION

Measurement of malondialdehyde by thiobarbituric acid reaction remains the most widely used method for assessing lipid peroxidation due to its simplicity and sensitivity, although it has long been criticized as lacking specificity. The present study demonstrates a significant role of dietary protein level in the peroxidative tissue damage. When rats are fed the low protein diet, the dietary soybean oil level must be as low as 2% to maintain most of their tissue TBARS at concentrations comparable to those in tissues of the N5 group. Increasing soybean oil to 5% in the low protein diet (L5) elevated the tissue TBARS to the level comparable to those of the N20 group, except for the muscle. Gradually increasing the soybean oil level in the low protein diet resulted in further augmentation of TBARS concentrations in all tissues measured. These data suggest that rats fed a low protein diet might be more susceptible to peroxidative tissue damage under the challenge of oxidative stress such as a high soybean oil intake.

In addition, the present study also showed that in response to the increased intake of soybean oil in rats fed the normal protein diet, there were significant increases in TBARS concentrations in plasma, spleen, heart, kidney and muscle, but not in RBC, liver and lung. Iritani et al. (24) have shown higher TBARS in the serum, liver and adipose tissue of rats fed a 10% corn oil diet compared with rats fed a 0.5% corn oil diet.

Polyunsaturated lipids are the principal chemical species vulnerable to free radical-initiated lipid peroxidation damage. Increasing dietary polyunsaturated fatty acid intake may raise the cell membrane

polyunsaturated fatty acid content and peroxidizability (25). Changing polyunsaturated fatty acid intake resulted in significant change in microsomal lipid peroxidation (26). Elevation of the dietary polyunsaturated fatty acid/saturated fatty acid ratio significantly increases RBC in vitro spontaneous hemolysis (27). Therefore, increased polyunsaturated fatty acid intake may partially contribute to the increased tissue TBARS concentrations in rats fed a high soybean oil diet.

Possessing the physiological functions of detoxifying hydroperoxides (GSHPx and catalase) and superoxide free radicals (SOD), these antioxidative enzymes have been considered to serve as important antioxidative defense mechanisms in the body (4). The NADPH generated by the G6PDH reaction supplies the reducing equivalents for the recycling of reduced glutathione, thus giving this enzyme an important role in the antioxidative defense system (4). Elevation of soybean oil level in the normal protein diet reduced the activities of these enzymes although not always significantly. Dietary polyunsaturated fats are known to be potent repressors of lipogenic enzyme gene expression, including G6PDH (28). The mechanism of the effect of dietary soybean oil on the activities of GSHPx, SOD and catalase is presently unknown.

Compared with rats fed a normal protein diet, the reduction in the activities of SOD in liver and RBC, GSHPx (U/g liver and U/g body weight) in liver, catalase in RBC and G6PDH (U/g liver and U/g body weight) in liver in response to the increase of dietary soybean oil was more severe when these animals were fed a low protein diet. For example, when expressed as U/g liver or U/g body weight, the activity of GSHPx of the N20 group was ~80% that of the N5 group; in contrast, these enzyme activities of the L20 group were only ~60% those of the L5 group. Moreover, low protein intake per se resulted in depression of the GSHPx (U/g and U/g body weight), SOD (U/mg protein), catalase (U/g liver and U/g body weight) and G6PDH (U/mg protein, U/g liver and U/g body weight) even when dietary soybean oil was as low as 5% (L5 vs. N5). These results suggest that the higher susceptibility to lipid peroxidation in the tissues of rats fed low protein diets may partially be attributable to the lowered antioxidative enzyme activities.

Protein malnutrition, resulting from low quality or quantity of dietary protein, not only retards animal growth but also changes the metabolism via modification of the hormonal profile. As a result, turnover of some enzyme protein could be specifically altered. For example, the liver microsomal detoxification enzyme activities and inducibility were shown to be lowered by low protein (29, 30) and low quality protein (31) diets. Iritani et al. (32) reported decreased lipogenic enzyme activities including G6PDH in rats fed soybean protein or gluten diets compared with

rats fed casein or fish protein diets. Further experiments are needed to clarify whether the transcription, translation, post-translation or degradation of the antioxidative enzyme proteins are affected by dietary protein inadequacy.

Although rats fed low protein diets consumed less food (grams per rats) than the N5 group, the food intake/body weight of these groups was, in fact, higher than that of rats fed the normal protein diet. It seems that inadequacy of dietary protein per se rather than lowered food intake is associated with higher susceptibility to tissue lipid peroxidation. Chipalkatti et al. (33) demonstrated that when mice were fed a normal diet, but their food intake was restricted to 50% of the ad libitum-diet group, lower heart lipofuscin content and lower liver lipid peroxidation were observed.

In conclusion, inadequate intake of dietary protein resulted in significantly higher tissue TBARS and lowered antioxidative enzyme activities in response to increasing dietary soybean oil level. This implies that the enhanced lipid peroxidation and reduced antioxidative enzyme activities resulting from feeding diets high in polyunsaturated fat were aggravated by protein insufficiency.

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