Degree of Protein Deficiency Affects the Extent of the Depression of the Antioxidative Enzyme Activities and the Enhancement of Tissue Lipid Peroxidation in Rats[1]

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ABSTRACT To investigate the effects of various dietary protein levels on tissue lipid peroxidation and antioxidative enzyme activities, four groups of Long-Evans male weanling rats were fed a 6, 8, 12 or 20% lactalbumin diet for 6 wk. The red blood cell in vitro spontaneous hemolysis values were 28.6, 24.9, 18.9 and 14.1% for the rats fed the 6, 8, 12 and 20% lactalbumin diets, respectively. The thiobarbituric acid–reactive substances (TBARS) in plasma, RBC, liver, kidney, muscle, lung, spleen and heart were lowest in the group fed the 20% lactalbumin diet. Rats fed the diet containing 12% lactalbumin had significantly greater TBARS in plasma, spleen, kidney and muscle compared with rats fed the 20% lactalbumin diet. Rats fed the 8% lactalbumin diet had markedly higher TBARS levels ($P < 0.05$) in all tissues examined than did those fed the 12% lactalbumin diet. The TBARS in all eight tissues of the rats fed the 6% lactalbumin diet were even higher than those of rats fed the 8% lactalbumin diet ($P < 0.05$). The activities of glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase in RBC were gradually lowered ($P < 0.05$) when the dietary protein level was decreased stepwise. The activities of these antioxidative enzymes in liver also were reduced significantly when dietary protein level decreased from 20 to 12% and then to 8%. These enzyme activities in the rats fed the diet containing 6% lactalbumin generally were not different from those of the rats fed 8% lactalbumin. Thus, the degree of protein deficiency affects the extent of the depression of the antioxidative enzyme activities and the enhancement of tissue lipid peroxidation. J. Nutr. 123: 803–810, 1993.

INDEXING KEY WORDS:

- *rats* • *lipid peroxidation*
- *dietary protein* • *antioxidative enzymes*

Free radical–mediated lipid peroxidation has been implicated in a variety of pathological processes (Halliwell 1987, Halliwell and Gutteridge 1989, McBrien and Slater 1989). The susceptibility of a given organism to oxidative damage is influenced by the overall balance between the degree of the oxidative stress and antioxidative capabilities. Nutrition plays an important role in determining the cellular antioxidative defense mechanism (Chow 1988). Dietary factors such as vitamin E, selenium, sulfur-containing amino acids, copper and zinc are known to affect the antioxidative ability of the organism (Capel 1988). However, there are few published reports concerning the role of dietary protein in the antioxidative defense mechanism, in spite of the presumption that protein malnutrition may impair the antioxidative defense system (Capel 1988, Chow 1988).

Golden and Ramdath (1986) proposed that free radical–mediated tissue damage is involved in the pathogenesis of kwashiorkor, mainly because of the inadequate protective and repair mechanism in protein-energy malnourished individuals. Deneke et al. (1983) reported a significant reduction in lung superoxide dismutase (SOD, EC 1.5.1.1)[3] activity of rats fed a 3% casein diet and exposed to hyperbaric oxygen. They (Deneke et al. 1985) further demonstrated significantly lowered lung catalase activity in rats fed a protein-free diet and subjected to similar exposure. In both of the studies, oxygen toxicity was potentiated by feeding the protein-deficient diet.

In exploring the effects of deteriorated frying oil and dietary protein levels on rat liver microsomal enzymes (Huang et al. 1988), we observed an unexpected elevation of erythrocyte in vitro hemolysis in

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[3]Abbreviations used: G6PDH, glucose-6-phosphate dehydrogenase; GSHPx, glutathione peroxidase; PMS, post-mitochondrial supernatants; SOD, superoxide dismutase; TBARS, thiobarbituric acid–reactive substances.

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rats fed a low protein fresh soybean oil diet, suggesting enhanced tissue lipid peroxidation in these rats. This result was further confirmed in later studies (Huang et al. 1989a and 1989b). The speculation that feeding the high polyunsaturated fat and low protein diet might result in lowered antioxidative enzyme activities and enhanced tissue lipid peroxidation was supported by a study using rats fed diets containing 8% lactalbumin with various soybean oil levels (Huang and Fwu 1992). Having observed these effects of a low protein diet, we sought in the present study to confirm the role of dietary protein in maintaining the balance between the antioxidative defense system and oxidative stress. If protein malnutrition causes the imbalance between the oxidative stress and antioxidative capability, this imbalance, like the growth retardation, can then be expected to be intensified when the dietary protein is gradually decreased. Based on this rationale, tissue lipid peroxidation and antioxidative enzyme activity in liver and RBC were measured in this study in which rats were fed diets gradationally reduced in protein content.

MATERIALS AND METHODS

Animals and diets. Twenty-four male Long-Evans weanling rats, weighing 54.0 ± 3.5 g, were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University. They were housed individually in stainless steel wire cages in a room maintained at 25 ± 2°C with a controlled 12-h light:dark cycle. The rats were randomly assigned to four diet groups (six rats per group), with each group fed one of the four test diets. Food and tap water were freely available. Body weight and food intake were recorded weekly. Care and handling of the animals conformed to the NIH *Guide for the Care and Use of Laboratory Animals* (NRC 1985).

The composition of the four test diets is shown in **Table 1**. The dietary protein was supplied by 6, 8, 12 or 20% lactalbumin. In preparing the test diets, the powdered ingredients were mixed in advance, and the oil portion was blended in each week before feeding.

Tissue sampling and preparation. After 6 wk of consuming the experimental diets, rats were killed by carbon dioxide exposure between 0830 and 0930 h on four successive days. Blood was drawn by heart puncture using heparinized evacuated blood collecting tubes (Venoject, Terumo Corp., Tokyo, Japan). Livers, kidneys, hearts, spleens, lungs and gastrocnemius muscles were excised and weighed. Aliquots of blood samples were centrifuged at 1000 × *g* for 10 min to separate plasma and RBC. Aliquots of the RBC were washed with five volumes of saline and lysed with four volumes of deionized water to prepare RBC lysate. Except for a portion of 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 they were analyzed for thiobarbituric acid–reactive substances (TBARS) within 2 wk. A 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 homogenizer with a Teflon pestle. Aliquots of the crude homogenate (100 g/L) were centrifuged at 12,000 × *g* for 20 min (20 PR-5 freeze centrifuge, RPR 18 rotor, Hitachi, Tokyo, Japan), and the post-mitochondrial supernatants (PMS) were retained.

TABLE 1

Composition of test diets

Ingredient[1]	Dietary lactalbumin level			
	6%	8%	12%	20%
	g/kg diet			
Lactalbumin	60	80	120	200
AIN-76 vitamin mixture[2]	10	10	10	10
AIN-76 mineral mixture[2]	35	35	35	35
Cellulose	30	30	30	30
Soybean oil	150	150	150	150
Cornstarch	712	692	652	572
Choline chloride	3	3	3	3

[1]Lactalbumin and choline chloride were from Sigma Chemical (St. Louis, MO), α-cellulose (ARBOCEL®, type BE 600/300) was from J. Bettenmaier & Söhne (Germany), soybean oil was from Taiwan Sugar Company (Taipei, Taiwan) and cornstarch was from Roquatte (France).

[2]American Institute of Nutrition (1977). Vitamin E was supplied by 50 mg/kg of all-*rac*-α-tocopheryl acetate.

Analysis. Blood samples were analyzed for hematocrit by centrifugation in capillary tubes and for hemoglobin by the cyanomethemoglobin method (Oser 1965). The in vitro hemolysis test was performed according to the method of Draper and Csallany (1969) as modified by Buckingham (1985). In this modification, washed RBC were suspended in PBS as a 0.5% (v/v) suspension and incubated at 37°C for 20 h. Aliquots of the RBC lysates were treated with chloroform-ethanol as described by McCord and Fridovich (1969) and assayed for the activities of SOD (Misra and Fridovich 1977). The freshly prepared RBC lysates and aliquots of the PMS were treated with Drabkin's reagent to remove hemoglobin, as described by Paglia and Valentine (1967), and assayed for the activity of glutathione peroxidase (GSHPx, EC 1.11.1.9) by coupling to glutathione reductase reaction according to the method of Lawrence and Burk (1976). Hydrogen peroxide (final concentration, 88.2 μmol/L) and cumene hydroperoxide (final concentration, 526 μmol/L) were used as substrates for RBC and liver, respectively. The liver PMS as well as the treated RBC lysates were assayed for SOD activity

TABLE 2

Final body weight, food intake, feed efficiency, and relative liver weight of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
Final body weight,[2] *g*	112.0 ± 16.4[a]	166.2 ± 19.0[b]	256.3 ± 23.6[c]	281.0 ± 20.4[d]
Food intake, *g/6 wk*	340.6 ± 49.2[a]	429.1 ± 45.4[b]	550.3 ± 52.2[c]	568.1 ± 52.1[c]
Feed efficiency, *g gain/g feed*	0.169 ± 0.022[a]	0.261 ± 0.017[b]	0.367 ± 0.013[c]	0.400 ± 0.009[d]
Liver weight, *g/100 g body wt*	4.2 ± 0.5[ab]	3.8 ± 0.2[a]	4.3 ± 0.4[b]	4.6 ± 0.4[b]

[1]Values are means ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$).

[2]Initial body weight of all the 24 rats was 54.0 ± 3.5 g. There were no significant differences in initial body weight among the four groups.

by the inhibition of photo-oxidation of *O*-dianisidine as described by Misra and Fridovich (1977). The glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity of liver PMS and RBC lysates was assayed by the increase of absorbance at 340 nm of NADPH as described by Deutsch (1983). Protein contents of PMS were analyzed by the method of Lowry et al. (1951) using bovine serum albumin as standard. In the measurement of enzyme activities, the amount of enzymes was adjusted to ensure the linearity of the reaction over the period of measurement and to ensure that the concentration of substrates was not rate-limiting.

Frozen tissues, including liver, kidney, heart, spleen, lung and muscle, were thawed, homogenized and analyzed for TBARS according to the method of Ohkawa et al. (1979). Frozen plasma and RBC lysates were also thawed and analyzed for TBARS by the method of Yagi (1982) and the method of Brownlee et al. (1977), respectively. 1,1,3,3-Tetraethoxypropane (Sigma Chemical, St. Louis, MO) was used as a standard for all thiobarbituric acid reactions, and the results were calculated as nanomoles of malondialdehyde.

Statistical analysis. Data are expressed as means ± SD for six 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 in the SPSS Package (Kim and Kohout 1975). Differences were considered significant at $P < 0.05$. Linear correlations between dietary protein level and TBARS in various tissues and between antioxidative enzyme activities and TBARS in liver and RBC were analyzed using SAS software.

RESULTS

General nutritional status and in vitro hemolysis. Final body weights, food intakes, feed efficiencies and relative liver weights are shown in **Table 2**. Growth and feed efficiency increased with the increase of dietary protein level and were significantly different among the four groups of rats. The increment of these measurements was highest when the dietary lactalbumin level was raised from 6 to 8% and was lowest when the dietary lactalbumin level was changed from 12 to 20%.

Rats fed the 20% lactalbumin diet had significantly higher hematocrits and hemoglobin concentrations than the other groups (**Table 3**). These blood measurements together with growth and feed efficiency data demonstrate the inadequate protein nutritional status of rats fed the 6, 8, and 12% lactalbumin diets.

The spontaneous RBC in vitro hemolysis values of the rats fed the diets containing 6, 8, 12 and 20% lactalbumin were 28.6, 24.9, 18.9 and 14.1%, respectively (Table 3). These values differed significantly from one another ($P < 0.05$).

Tissue thiobarbituric acid–reactive substances. Tissue TBARS were lowest in rats fed the 20% lactalbumin diet and increased with the decrease of dietary protein level (**Table 4**). When the dietary protein level was reduced to 12% lactalbumin, TBARS in plasma, spleen, kidney and muscle increased significantly ($P < 0.05$), in contrast to the nonsignificant increase in RBC, liver, lung, and heart. Further reduction of dietary protein to 8% and to 6% resulted in marked and significant increases of TBARS in all tissues measured ($P < 0.05$). As a result, the 6% lactalbumin–fed group showed the highest tissue TBARS concentrations, which were 2.4-fold (plasma), 3.0-fold (RBC), 1.5-fold (liver), 1.3-fold (spleen), 1.7-fold (heart), 1.6-fold (lung), 2.2-fold (kidney) and 1.6-fold (muscle) those of the 20% lactalbumin–fed group.

Antioxidative enzyme activities in RBC and liver. **Table 5** presents the GSHPx activity in RBC and liver. Rats fed the 20% lactalbumin diet had the highest activity of this enzyme in both RBC and liver. The GSHPx activity in RBC of rats fed the 12, 8 and 6% lactalbumin diets was 88, 72 and 57% that of rats fed the 20% lactalbumin diet. The decrease in dietary protein level also resulted in significant reduction in

TABLE 3

Hematocrit, hemoglobin concentration and in vitro hemolysis of RBC of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
Hematocrit	0.377 ± 0.01[a]	0.385 ± 0.024[a]	0.403 ± 0.016[a]	0.437 ± 0.030[b]
Hemoglobin, *g/L*	132 ± 6[a]	142 ± 8[ab]	149 ± 8[b]	165 ± 8[c]
RBC in vitro hemolysis,[2] %	28.6 ± 3.4[d]	24.9 ± 4.7[c]	18.9 ± 3.9[b]	14.1 ± 1.1[a]

[1]Values are means ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$).

[2]To measure spontaneous in vitro hemolysis, washed RBC were suspended in PBS as a 0.5% RBC suspension and incubated at 37°C for 20 h (Buckingham 1985). The spontaneous hemolysis was expressed as a percentage of total hemolysis, which was measured by completely hemolyzing the same concentration of RBC in distilled water.

liver GSHPx activity. When expressed as units per gram of liver, the enzyme activity of the animals fed the 12, 8 and 6% lactalbumin diets was 74, 43 and 32% that of rats fed the 20% lactalbumin diet. The differences among the four groups were all statistically significant ($P < 0.05$).

Similar to the changes observed for GSHPx activity, SOD activities in RBC and liver generally decreased with the decrease of dietary protein level (**Table 6**). Nevertheless, the difference in liver SOD activity between the groups fed 6 and 8% lactalbumin was not significant when expressed as units per milligram of protein and units per 100 g body wt.

The G6PDH activities were also lowered with the reduction in dietary protein level (**Table 7**). Rats fed the 20 and 12% lactalbumin diets had significantly higher G6PDH in RBC and liver than did rats fed the 6 and 8% lactalbumin diets. Nonetheless, the liver G6PDH activities of rats fed the 6 and 8% lactalbumin diets were not significantly different.

DISCUSSION

In the present study, 15% soybean oil was incorporated into the experimental diet because we observed very little difference in the tissue TBARS between the low protein diet group and normal protein group when the dietary polyunsaturated fat level was low (Huang and Fwu 1992).

Measurement of malondialdehyde by TBARS remains the most widely used method for the determination of lipid peroxidation due to its simplicity and sensitivity, although it has long been criticized as

TABLE 4

Thiobarbituric acid-reactive substances (TBARS) in plasma, RBC, liver, spleen, heart, lung, kidney and muscle of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
	μmol malondialdehyde/L			
Plasma	7.8 ± 0.9[d]	6.6 ± 0.4[c]	4.0 ± 0.8[b]	3.2 ± 0.5[a]
	nmol malondialdehyde/g hemoglobin			
RBC	107.2 ± 16.4[c]	78.7 ± 16.2[b]	42.6 ± 10.7[a]	35.9 ± 8.3[a]
	nmol malondialdehyde/g tissue			
Liver	500.6 ± 28.9[c]	455.8 ± 30.7[b]	364.5 ± 21.4[a]	334.0 ± 21.5[a]
Spleen	437.0 ± 32.0[d]	401.6 ± 25.9[c]	368.6 ± 16.6[b]	339.1 ± 25.6[a]
Heart	266.5 ± 24.7[c]	228.8 ± 36.1[b]	160.2 ± 13.4[a]	154.6 ± 19.2[a]
Lung	190.9 ± 21.4[c]	162.8 ± 15.8[b]	135.8 ± 16.2[a]	117.2 ± 13.5[a]
Kidney	426.8 ± 23.7[d]	314.1 ± 26.3[c]	229.7 ± 18.6[b]	191.1 ± 11.3[a]
Muscle	426.0 ± 33.4[d]	377.7 ± 16.1[c]	322.5 ± 24.5[b]	260.5 ± 18.5[a]

[1]Values are mean ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$).

TABLE 5

Activity of glutathione peroxidase in RBC and liver of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
RBC, *U/g hemoglobin*	19.1 ± 2.3[a]	23.9 ± 1.8[b]	29.6 ± 1.5[c]	33.3 ± 2.6[d]
Liver				
U/mg protein	0.26 ± 0.02[a]	0.28 ± 0.03[a]	0.35 ± 0.02[b]	0.41 ± 0.02[c]
U/g liver	11.1 ± 1.9[a]	15.2 ± 1.9[b]	26.0 ± 1.1[c]	35.2 ± 2.8[d]
U/100 g body wt	45.9 ± 8.2[a]	52.3 ± 7.6[a]	113.3 ± 11.3[b]	160.1 ± 19.5[c]

[1]Values are means ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$). One unit of glutathione peroxidase activity = 1 μmol NADPH consumed per minute under the assay condition.

lacking specificity. The present study clearly demonstrated that tissue TBARS increased significantly when dietary protein level was decreased, suggesting that inadequate intake of dietary protein might result in enhanced tissue lipid peroxidation. Moreover, the magnitude of this enhancement correlated with the extent of protein deficiency ($P < 0.0001$, **Table 8**).

Possessing the physiological functions of detoxifying hydroperoxides (GSHPx and catalase) and superoxide free radicals (SOD), the antioxidative enzymes (i.e., catalase, GSHPx and SOD) have been considered important antioxidative defense mechanisms in the body (Chow 1988). The NADPH generated by the G6PDH reaction supplies the reducing equivalents for the recycling of the oxidized glutathione, thus rendering this enzyme important in the antioxidative defense system (Chow 1988). Data reported in this study clearly indicate that inadequate intake of dietary protein resulted in depression of these enzyme activities. Moreover, the extent of the reduction in the antioxidative enzyme activities seemed to be related to the severity of protein deficiency, especially for RBC. A negative correlation ($P < 0.001$) was observed between the antioxidative enzyme activities and the tissue TBARS (Table 8). These results suggest that the enhanced lipid peroxidation in the tissues of rats fed a low protein diet may be attributable in part to the lowered antioxidative enzyme activities.

Protein malnutrition, resulting from low quality or quantity of dietary protein, not only retards animal growth but also affects 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 enzymes' activities and inducibility were shown to be lowered by low protein diets (Kato et al. 1980, Mgbodile and Campbell 1978) and low quality protein diets (Kato et al. 1981). Iritani et al. (1986) reported decreased lipogenic enzyme activities (including G6PDH) in rats fed soybean protein or gluten diets compared with rats fed casein or fish protein diets. Eisenstein and Harper (1991) concluded from their study on the relationship between protein intake and hepatic protein synthesis in rats that when protein intake is below the requirement level, the rate of liver protein synthesis may be limited by amino acid supply, by the capacity of the system for protein synthesis or by both. It is not clear why some cellular proteins (such as the

TABLE 6

Activity of superoxide dismutase in RBC and liver of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
RBC, *U/g hemoglobin*	147.9 ± 17.3[a]	186.1 ± 15.8[b]	234.1 ± 21.1[c]	277.1 ± 12.1[d]
Liver				
U/mg protein	2.5 ± 0.3[a]	2.8 ± 0.2[a]	3.4 ± 0.3[b]	3.6 ± 0.4[b]
U/g liver	259.8 ± 36.8[a]	311.9 ± 19.4[b]	354.3 ± 14.8[c]	392.3 ± 25.5[d]
U/100 g body wt	1083 ± 219[a]	1177 ± 58[a]	1542 ± 177[b]	1782 ± 149[c]

[1]Values are means ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$). One unit of superoxide dismutase activity = 1 μg purified bovine erythrocyte superoxide dismutase (Sigma Chemical, St. Louis, MO) in the riboflavin and *O*-dianisidine photoxidation assay system.

TABLE 7

Activity of glucose-6-phosphate dehydrogenase in RBC and liver of rats fed 6, 8, 12 and 20% lactalbumin diets for 6 wk[1]

	Dietary lactalbumin level			
	6%	8%	12%	20%
RBC, *U/g hemoglobin*	1.6 ± 0.2[a]	2.0 ± 0.2[b]	2.4 ± 0.1[c]	2.5 ± 0.2[c]
Liver				
U/g protein	14.3 ± 0.8[a]	16.3 ± 2.5[a]	22.9 ± 2.7[b]	26.4 ± 4.0[c]
U/g liver	1.4 ± 0.2[a]	1.7 ± 0.3[a]	2.4 ± 0.4[b]	2.7 ± 0.4[b]
U/100 g body wt	5.7 ± 1.0[a]	6.5 ± 1.1[a]	10.3 ± 1.9[b]	12.4 ± 1.1[c]

[1]Values are means ± SD for six rats in each group. Within the same row, values not sharing the same superscript are significantly different ($P < 0.05$). One unit of activity = 1 μmol NADPH oxidized per minute under the assay conditions.

antioxidative enzymes in the present study) are specifically altered by protein insufficiency.

The responses of rats to gradually lowered dietary protein levels were different for growth, hematocrit, hemoglobin and the antioxidative enzymes measured. When dietary lactalbumin level was varied from 6 to 12%, an approximately linear relationship between growth and dietary protein level was observed ($r = 0.96$, $P < 0.0001$). In contrast, much less difference in the body weight between the 12 and the 20% lactalbumin–fed groups was noticed, indicating 12% lactalbumin approached the requirement for maximum growth. Although rat growth was reduced to 40% when the dietary lactalbumin level was decreased from 20 to 6%, the reduction in hemoglobin value was only to 80%. On the other hand, the activities (u/mg protein) of GSHPx, SOD and G6PDH in liver were depressed to 63.4, 69 and 54.2%, respectively. Furthermore, these liver antioxidative enzyme activities were not significantly different in the groups fed the 6 and 8% lactalbumin diets, except when the activities of glutathione peroxidase and superoxide dismutase were expressed as units per gram of tissue. This implies that the reduction of antioxidative enzyme activities with the decrease of dietary protein level reached a plateau at the 8% lactalbumin level. It is not known at present if the plateau value of antioxidative enzyme activities represents a minimum requirement for maintenance.

Fatty liver is one of the known symptoms of protein-energy malnutrition. Enhanced lipid peroxidation can also be predicted from higher tissue lipid content. Although the liver lipid content was not measured in the present study, our previous report

TABLE 8

Correlations (r) between dietary protein levels (DPL) and tissue thiobarbituric acid-reactive substances (TBARS), and between TBARS and antioxidative enzyme activities in liver and RBC[1]

	Body weight gain	TBARS (nmol/g)					
		Liver	Muscle	Heart	Kidney	Spleen	Lung
DPL (%)	0.8723	–0.8554	–0.9088	–0.7807	–0.8617	–0.8254	–0.8128

	Liver (U/mg protein)		
	GSHPx	SOD	G6PDH
Liver TBARS (*nmol/g*)	–0.8734	–0.8059	–0.8385

	RBC (U/g hemoglobin)		
	GSHPx	SOD	G6PDH
RBC TBARS (*μmol/L RBC*)	–0.8663	–0.8179	–0.8355
Hemolysis (%)	–0.7910	–0.8748	–0.7462

[1]All the correlation coefficients are significant ($P < 0.0001$). GSHPx = glutathione peroxidase, SOD = superoxide dismutase, G6PDH = glucose-6-phosphate dehydrogenase.

(Huang et al. 1988) did not demonstrate any significant difference in liver triglyceride concentration between rats fed a 8% lactalbumin diet and rats fed a 27% lactalbumin diet. From these data, we speculate that protein content in a 8% lactalbumin diet, at least, is not low enough to cause fatty liver.

The RBC in vitro hemolysis test has long been used as a criterion for the assessment of vitamin E status. Rats fed a low protein diet showed elevated hemolysis in vitro even though their dietary vitamin E content (50 mg/kg) was similar to that of the normal protein diet control (20% lactalbumin group). Although the lowered antioxidative enzyme activity in RBC may partially contribute to the higher hemolysis, the possibility that feeding a low protein diet may result in inefficient utilization of vitamin E can not be excluded. Rajaram et al. (1977) reported lowered absorption, transport and distribution of all-*rac*- α-[5-CH_3-^{3}H]tocopherol in rats fed a 5% casein diet. Mouri et al. (1984 and 1986) showed that tissue vitamin E level is affected by the quantity and quality of dietary protein. When the dietary casein level was <10%, the RBC vitamin E level was significantly decreased compared with that in the control group (20% casein diet). They speculated that low protein intake may decrease the efficiency of tocopherol transport to tissue cells secondary to a reduction in circulating lipoprotein containing tocopherol. Furthermore, it may be difficult to maintain an adequate cellular tocopherol level due to a probable decrease in cellular tocopherol-binding protein. In our previous report, we found that supplementation of 150 mg/kg of all-*rac*-α-tocopheryl acetate to a low protein diet (containing 8% lactalbumin and 15% soybean oil) significantly decreased the TBARS in plasma, heart and liver and restored the depressed activities of RBC SOD and catalase, but not in the remaining tissues measured (Huang and Fwu 1992). That some tissues of rats fed a low protein diet did respond to the supplementation of vitamin E provides further evidence that vitamin E utilization may be impaired in protein malnutrition. The impaired vitamin E status may also partially be attributable to the enhanced lipid peroxidation in protein malnutrition.

The enhanced tissue lipid peroxidation may result from lowered antioxidative enzyme activities as well as impaired vitamin E utilization in protein deficiency. In rats with normal selenium status, the plasma GSHPx activity was not altered by vitamin E deficiency (Chow 1990). Currently we are investigating the vitamin E status of protein-deficient rats. Preliminary results showed that α-tocopherol concentrations in liver and RBC were not significantly changed by dietary protein deficiency, although α-tocopherol concentrations in such tissues as heart, lung, kidney, plasma, muscle and adrenal gland were significantly lowered. Because the antioxidative enzyme activities were measured in RBC and liver in the present study, the reduction in these enzyme activities seems, at least in part, to be a specific effect of protein deficiency. Nonetheless, it is still not known if the impaired vitamin E utilization may contribute as an indirect effect to the reduced antioxidative enzyme activities in protein-deficient rats.

Although rats fed low protein diets consumed less food than did rats fed the 20% lactalbumin control diet, the food intake:body weight ratio of these groups was, in fact, higher than that of rats fed the normal protein diet. Apparently, inadequacy of dietary protein per se rather than lowered food intake is associated with higher susceptibility to tissue lipid peroxidation. Chipalkatti et al. (1983) demonstrated that when mice were fed a normal diet but with food intake restricted to 50% of that of mice fed with ad libitum access, lower heart lipofuscin content and lower liver lipid peroxidation were observed.

Using graded levels of dietary protein, we confirmed the important role of adequate dietary protein in the maintenance of the balance between the antioxidative enzyme activities and tissue lipid peroxidation. The extent of the depression of the antioxidative enzyme activities and the enhancement of tissue lipid peroxidation were generally correlated with the degree of protein deficiency.

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