Effects of dietary sulfur-containing amino acids on oxidative damage in rat liver caused by *N*-nitrosodimethylamine administration

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Effects of dietary protein and S-containing amino acids on oxidative damage were investigated in rat liver. After feeding rats for 3 weeks from weaning, lower GSH levels and higher metallothionein (MT) levels were found in the liver of rats fed on a 10 % soyabean-protein-isolate (SPI)-based diet than those fed on a 10 % casein-based diet. After injection of *N*-nitrosodimethylamine (NDMA) at 20 mg/kg body weight, increases in lipid peroxide, determined as thiobarbituric-acid reactive substances (TBARS), and γ-glutamyltransferase (GGT) activity in the liver were observed in the 10 % SPI diet group. By supplementing the 10 % SPI diet with 0·3 % cystine or methionine, GSH levels were increased, while MT levels were decreased, and elevation in TBARS levels after NDMA injection was diminished. On the other hand, increase in GGT activity could be prevented only by methionine supplement. On a 20 % SPI or casein diet, TBARS concentration and GGT activity were not altered after NDMA injection with concomitant increase in GSH levels and decrease in MT levels. These results indicate that sufficient amounts of methionine and cystine in a diet are important to protect the liver from oxidative damage after NDMA administration, and GSH plays a primary role in the cellular protective function when compared with MT.

Dietary protein: Oxidative stress by *N*-nitrosodimethylamine: Glutathione: Metallothionein

N-Nitrosodimethylamine (NDMA), a potent carcinogenic and cytotoxic agent (Yahagi *et al*. 1977; Bartsch & Montesano, 1984; Nishikawa *et al*. 1997), was oxidized during metabolic activation with cytochrome P450 (Levin *et al*. 1986; Amelizad *et al*. 1988; Anundi & Lindros, 1992) and lipid peroxide concentration was raised in the organs of rats (Ahotupa *et al*. 1987; Taniguchi *et al*. 1999) and chicks (Taniguchi, 1997) after NDMA administration. Mutagenicity of NDMA was prevented by reducing substances such as ascorbic acid (Guttenplan, 1977), and thiol compounds, including GSH, to protect organisms against NDMA toxicity (Brambilla *et al*. 1992). GSH is the most abundant non-protein-thiol compound in living organisms and functions as a reducing agent in a variety of biochemical reactions (Meister & Anderson, 1983). Metallothionein (MT), another thiol-containing compound, plays essential roles in metabolism and detoxification of heavy metals (Bremner, 1987). In addition, there is increasing evidence that MT can act as a free radial scavenger (Thormalley & Vasak, 1985; Chubatsu & Meneghini, 1993; Sato & Bremner, 1993; Miura *et al*. 1997). Cd toxicity in GSH depletion was overcome by induction of MT (Chan & Cherian, 1992), and a compensatory mechanism of MT for GSH was suggested. Thus, MT was considered to play a role together with GSH in the protection against cytotoxicity induced by various chemicals. After administration of NDMA, GSH and MT levels were both elevated in rat liver (Taniguchi *et al*. 1999), and the increase in these thiol compounds was presumed to be an adaptive mechanism for toxicity of NDMA. GSH is known as a cystine reservoir in the liver, and reduction in hepatic GSH levels was reported when rats received diets deficient in S-containing amino acids (Tateishi *et al*. 1977). In contrast to GSH, MT synthesis was increased in rats maintained on a sulfhydryl-deficient diet (Sendelback *et al*. 1990).

In our previous study, hepatic γ-glutamyltransferase (GGT) activity was elevated and GSH levels were lowered when rats were fed on a soyabean-protein diet, but not on a casein diet (Taniguchi & Inoue, 1986). Elevation of GGT activity was reported in hepatoma (Fiala & Fiala, 1973; Yokosawa *et al*. 1981), and GGT activity in serum has been used as a marker for cancer diagnosis (Tsuji *et al*. 1990). The objectives of this present study were: (1) to demonstrate the changes in hepatic GSH and MT levels when rats were fed on a soyabean-protein-isolate (SPI)- or casein-based diet, which were relatively deficient or sufficient

Abbreviations: GGT, γ-glutamyltransferase; GSHPx, glutathione peroxidase; NDMA, *N*-nitrosodimethylamine; MT, metallothionein; SPI, soyabean protein isolate; TBARS, thiobarbituric-acid reactive substances.

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Table 1. Composition of 10 % and 20 % protein diets (g/100 g)*

Component	10 % protein diet	20 % protein diet
Soyabean protein or casein	10	20
Mineral mixture†	3·5	3·5
Vitamin mixture†	1	1
Choline bitartarate	0·25	0·25
Cellulose powder	5	5
Sucrose	10	10
Soyabean oil	5	5
Dextrin	15·5	15·5
Starch	49·75	39·75

* When a 10 % soyabean protein diet was supplemented with cystine or methionine, 0·3 g of each amino acid was added and the amount of starch was adjusted to 49·45 g.
† Composition of mineral and vitamin mixtures was that of the AIN-93 diet (Reeves *et al*. 1993) for growing rats; purchased from Oriental Yeast Co., Tokyo, Japan.

respectively, in S-containing amino acids; (2) to compare the relative importance of GSH and MT in protecting against NDMA-induced oxidative toxicity. The effect of diets differing in S-containing amino acid levels on hepatic GGT activity was also investigated, with and without administration of NDMA.

Materials and methods

Chemicals

NDMA (for GC) was purchased from Wako Chemical Ind., Ltd, Osaka, Japan). GSH (both reduced and oxidized), and glutathione reductase (from yeast) were purchased from Boehringer Mannheim Co. Ltd (Tokyo, Japan). γ-Glutamylglutamic acid, used as an internal standard in HPLC analysis, was purchased from Sigma Chemical Co. (St Louis, MI, USA). NADP and NADPH were purchased from Oriental Yeast Co., Ltd (Tokyo, Japan). 2-Thiobarbituric acid and tetramethoxypropane were purchased from Tokyo Kasei Kogyo Ltd (Tokyo, Japan). All other chemicals were of reagent grade.

Animals and diets

Weaning rats (Charles River, Shizuoka, Japan; male, Wistar, aged 21 d; body weight 52 (SD 3) g) were maintained for 3 weeks on semisynthetic diets which were made according to AIN-93 recommendations (Reeves *et al*. 1993). The composition of the diets is shown in Table 1. SPI (Fujipro R, Fuji Seiyu Co., Osaka, Japan) and casein (vitamin-free; ICN Biochemicals, Aurora, OH, USA) were used as protein sources. The crude protein content of SPI was 91·1 g/100 g, and the methionine and cystine contents of this crude protein were 1·4 g and 1·3 g/100 g respectively. The crude protein content of casein was 94·7 g/100 g, and the methionine and cystine contents of this crude protein were 3·1 g and 0·7 g/100 g respectively. The amino acid composition of the proteins was determined in Fuji Seiyu Research Institute, Osaka, Japan. The 20 % protein diet contained 20 g SPI or casein/100 g and other constituents were the same as in the 10 % protein diet. On a 20 % casein diet, containing 0·7 g total S-containing amino acids/100 g, the growth rate and hepatic GSH levels of the rats were nearly the same as those fed on a laboratory chow diet (CE-2, CLEA, Osaka, Japan) (data not shown). When a 10 % SPI diet was supplemented with S-containing amino acids, 0·3 g cystine or methionine was added to raise the level of amino acids to a level similar to that of a 20 % casein diet. Rats were given drinking water *ad libitum* and housed in individual cages in a temperature-controlled room at 20–22°C, with a controlled 12 h light–dark cycle. Food intake and body-weight gain of animals are shown in Table 2.

NDMA dissolved in saline solution (10 mg NDMA/ml) was injected intraperitoneally to rats prior to the experiment according to Ahotupa *et al*. (1987). In our previous work (Taniguchi *et al*. 1999) thiobarbituric-acid reactive substances (TBARS) was increased in the liver when more than 30 mg (399 μmol) NDMA/kg body weight was given to rats fed on a laboratory chow diet. To examine the effect of NDMA under milder treatment conditions, a dose of 20 mg NDMA/kg (0·1–0·2 ml NDMA solution) was employed throughout the present experiment.

Assay of glutathione and metallothionein

Rats were killed under anesthesia by inhalation of diethyl ether at 09.00–10.00 hours, 24 h after injection at NDMA. The liver was removed immediately for determinations of GSH and MT. For determination of GSH, a portion of

Table 2. Food intake, body-weight gain and liver weight of rats fed on different protein diets for 3 weeks*

(Mean values and standard deviations for six rats per group)

	Food intake (g)		Body-weight gain (g/3 weeks)		Liver weight (g/100 g body weight)	
Diet	Mean	SD	Mean	SD	Mean	SD
10 % soyabean protein	274·9[a]	45·8	61·8[a]	12·6	4·3[a]	0·2
10 % soyabean protein +0·3 % cystine	214·8[b]	27·7	42·4[b]	8·8	4·5[a]	0·4
10 % soyabean protein +0·3 % methionine	360·8[c]	26·7	93·2[c]	11·9	5·2[b]	0·4
10 % casein	330·4[d]	22·3	90·8[c]	11·9	4·4[a]	0·4

[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different: $P < 0·05$.
* For details of diets see Table 1.

the liver (0·2–0·5 g) was homogenized in ice-cold 5 % perchloric acid. Using the supernatant after centrifugation at 2 500 ***g*** for 10 min, GSH (reduced and oxidized forms) was determined after derivatization with dinitrobenzene fluoride according to the method of Reed *et al*. (1980), using γ-glutamylglutamic acid as an internal standard. For MT analysis, portions of liver (0·5 g) were kept at −80°C until use. The liver samples were homogenized in ice-cold 1·15 % KCl under N_2 atmosphere, and MT levels were assayed using the Hg^{2+} saturation method (Naganuma *et al*. 1987). Hg levels in the final preparations were determined by O_2 combustion–Au amalgamation method (Jacobs *et al*. 1960), using a Rigaku Hg analyser SP-3 (Nippon Instruments Co., Osaka, Japan). MT levels were expressed as the amount of Hg bound to thionein molecule.

Lipid peroxide analyses

Tissues (about 0·5 g) were promptly excised and homogenized in nine times tissue volume of 1·15 % KCl solution containing 10 mM-butylhydroxytoluene as an antioxidant. Using 100 μl homogenate, the amount of lipid peroxide in the liver was determined as TBARS according to Ohkawa *et al*. (1979), and TBARS concentration was calculated from the absorbance at 530 nm as malondialdehyde with tetramethoxypropane as an external standard. The lipid peroxide level was expressed as nmol malondialdehyde.

Enzyme assays

For enzyme assays, liver (about 1·0 g) was homogenized in five times tissue volume of 0·25 M-sucrose solution. After solubilizing with 1 % Triton X-100 in 0·1 M-Tris-HCl buffer, pH 8·0, GGT activity was determined (Szasz, 1969) with γ-glutamyl *p*-nitroanilide as a substrate. One unit of enzyme activity was defined as the amount of enzyme required for the formation of 1 μmol *p*-nitroaniline/min.

The homogenates in 0·25 M-sucrose solution were centrifuged at 104 000 ***g*** for 60 min to obtain a cytosol fraction, and glutathione peroxidase (GSHPx) and superoxide dismutase were then determined. GSHPx activity was measured using cumene hydroperoxide as a substrate (Tappel, 1978), and one unit of the enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol NADPH/min at 30°C. Superoxide dismutase activity was determined with a commercial kit (SOD Test-Wako, Wako Pure Chemical Ind., Ltd, Osaka, Japan), principally based on the xanthine oxidase method (McCord & Fridovich, 1969). The enzyme activity was expressed as mU/mg protein.

Catalase activity was determined using the supernatant prepared following solubilization of 0·25 M-sucrose homogenates with sodium cholate. Catalase activity was calculated from the decrease in absorption of perborate (220 nm) at 30°C (Thomson *et al*. 1978).

Protein was determined using bovine serum albumin as a standard (Lowry *et al*. 1951).

Statistical analyses

Each experiment was performed with six rats, and results are presented as means and standard deviations. Statistical analyses were performed with ANOVA. Post hoc comparisons of means were performed with Fisher's PLSD test. A probability of $p < 0{\cdot}05$ was considered significant.

Results

Changes in glutathione and metallothionein levels

GSH levels in the liver were lower in the SPI group than in the casein group (Table 3). After injection of NDMA, GSH levels were elevated in both 10 %-protein-diet groups, but the increase was modest in the SPI group as compared to the casein group. On supplementing a 10 % SPI diet with cystine or methionine, GSH levels were elevated to 5·9–7·0 μmol/g liver, almost the same levels as those in rats fed a 20 % casein or laboratory chow diet (5–8 μmol/g liver; Taniguchi *et al*. 1999). However, GSH levels after NDMA

Table 3. Glutathione and metallothionein levels, and lipid peroxide in rats fed on different protein diets for 3 weeks and in response to administration of *N*-nitrosodimethylamine*

(Mean values and standard deviations for six rats per group)

Diet	NDMA (mg/kg BW)†	GSH (μmol/g liver)		MT (nmol Hg/g liver)		TBARS			
						Liver (μmol/g)		Serum (nmol/ml)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 % soyabean protein	0	$1{\cdot}2^{a}$	0·3	244^{a}	70	$1{\cdot}35^{a}$	0·14	$6{\cdot}2^{a}$	1·0
	20	$2{\cdot}7^{b}$	0·5	301^{b}	51	$4{\cdot}06^{b}$	0·92	$13{\cdot}0^{b}$	2·7
10 % soyabean protein +0·3 % cystine	0	$5{\cdot}9^{cd}$	0·4	66^{c}	14	$0{\cdot}98^{ac}$	0·17	$5{\cdot}0^{ac}$	0·5
	20	$6{\cdot}4^{de}$	1·6	206^{a}	46	$1{\cdot}27^{a}$	0·15	$7{\cdot}0^{d}$	1·3
10 % soyabean protein +0·3 % methionine	0	$7{\cdot}0^{e}$	0·6	104^{dc}	31	$0{\cdot}89^{c}$	0·11	$3{\cdot}6^{c}$	0·4
	20	$5{\cdot}9^{c}$	0·8	211^{a}	21	$1{\cdot}26^{ac}$	0·14	$5{\cdot}2^{ac}$	0·6
10 % casein	0	$1{\cdot}9^{b}$	0·3	124^{d}	44	$0{\cdot}87^{c}$	0·11	$4{\cdot}7^{ac}$	1·5
	20	$5{\cdot}1^{c}$	1·0	205^{a}	55	$1{\cdot}07^{c}$	0·10	$4{\cdot}5^{c}$	1·0

NDMA, *N*-nitrosodimethylamine; BW, body weight; GSH, glutathione; MT, metallothionein, TBARS, thiobarbituric-acid reactive substances.

[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different: $P < 0{\cdot}05$.

* For details of diets see Table 1.

† NDMA at a dose of 20 mg/kg body weight or saline only (control), was administered by intraperitoneal injection to six rats from each dietary regimen. Samples for determination of GSH and MT in the liver, and TBARS in the liver and serum were prepared 24 h after injection.

Table 4. Superoxide dismutase, glutathione peroxidase, catalase and γ-glutamyltransferase activities in rats fed on different protein diets for 3 weeks and in response to administration of *N*-nitrosodimethylamine*

(Mean values and standard deviations for six rats per group)

Diet	NDMA (mg/kg BW)†	SOD (U/mg protein) Mean	SD	GSHPx (U/mg protein) Mean	SD	Catalase (U/mg protein) Mean	SD	GGT (mU/mg protein) Mean	SD
10 % soyabean protein	0	9·6[a]	1·4	0·29[ac]	0·03	219[a]	25	1·30[a]	0·25
	20	7·6[b]	0·8	0·24[a]	0·05	134[bd]	10	2·62[b]	0·46
10 % soyabean protein	0	7·5[b]	0·4	0·33[bc]	0·06	156[cd]	19	1·39[a]	0·25
+0·3 % cystine	20	6·1[c]	0·6	0·25[a]	0·04	128[b]	7	2·52[b]	0·65
10 % soyabean protein	0	9·6[a]	0·4	0·36[b]	0·03	167[c]	10	0·84[c]	0·22
+0·3 % methionine	20	8·2[bd]	1·1	0·29[ac]	0·03	140[bd]	18	1·19[ac]	0·21
10 % casein	0	9·1[ad]	1·0	0·44[d]	0·04	230[a]	24	0·81[c]	0·30
	20	7·5[b]	0·6	0·35[b]	0·03	177[c]	15	1·01[ac]	0·20

NDMA, *N*-nitrosodimethylamine; BW, body weight; SOD, superoxide dismutase; GSHPx, glutathione peroxidase; GGT, γ-glutamyltransferase.
[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different: $P < 0{\cdot}05$.
* For details of diets see Table 1.
† NDMA at a dose of 20 mg/kg body weight, or saline only (control), was administered by intraperitoneal injection to six rats from each dietary regimen. Samples for enzyme determination were prepared 24 h after injection.

injection were not elevated in cystine- or methionine-supplemented-diet groups. These results indicated that hepatic GSH levels in rats fed on diets supplemented with S-containing amino acids reached a maximum level, or that protein levels in the diet were insufficient to increase GSH levels further, as observed with a 20 % casein diet. Hepatic MT levels were higher in the SPI group than in the casein group. By supplementing a 10 % SPI diet with cystine or methionine, MT levels were lowered to almost those found in rats fed on a 10 % casein or 20 % SPI diet. In contrast to GSH, MT levels were negatively correlated with the total amount of S-containing amino acids in the diets. When GSH levels were low, MT levels were high, and a reverse relationship was shown between these two sulfhydryl compounds. After injection of NDMA, MT levels were elevated in all diet groups.

Increase in lipid peroxide

The concentration of lipid peroxide determined as TBARS was high in the liver of 10 % SPI- and cystine-supplemented groups (Table 3). After injection of NDMA, TBARS was increased greatly in the liver and serum of a 10 %-SPI group, but this response was diminished by supplementation of methionine or cystine. When rats were fed on the 20 % SPI diet, TBARS in the liver was not increased by NDMA treatment.

Antioxidant enzyme activities

Superoxide dismutase activity was low in the cystine-supplemented group, but not different between all other groups (Table 4). GSHPx activity was significantly lower in the 10 % SPI group than in the 10 %-casein group, while catalase activity was not different between these two groups. After NDMA injection, activities of superoxide dismutase and catalase were decreased in all groups. In the SPI group, GSHPx activity was significantly lower than that in the methionine-supplemented or the casein group. After NDMA injection the activity was not decreased significantly in the SPI group, but decreased in all other groups.

γ-Glutamyltransferase activity

Hepatic GGT activity was high in the 10 %-SPI and cystine-supplemented groups compared with methionine-supplemented and 10 %-casein groups (Table 3). After

Table 5. Effects of 20 % protein diets (fed for 3 weeks) on glutathione, metallothionein and lipid peroxide levels, and γ-glutamyltransferase activity in the liver and in response to administration of *N*-nitrosodimethylamine*

(Mean values and standard deviations for six rats per group)

Diet	NDMA (mg/kg BW)†	GSH (μmol/g) Mean	SD	MT (nmol Hg/g) Mean	SD	GGT (mU/mg protein) Mean	SD	TBARS (μmol/g) Mean	SD
20 % soyabean protein	0	3·0[a]	0·4	77[a]	13	0·86[a]	0·39	1·42	0·34
	20	5·9[b]	0·9	159[b]	76	1·07[ab]	0·38	1·51	0·43
20 % casein	0	8·0[c]	1·1	14[c]	1	0·53[ac]	0·22	1·57	0·13
	20	9·5[d]	0·8	110[ab]	24	0·53[ac]	0·11	1·75	0·19

NDMA, *N*-nitrosodimethylamine; BW, body weight; GSH, glutathione; MT, metallothionein; GGT, γ-glutamyltransferase; TBARS, thiobarbituric-acid reactive substances.
[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different: $P < 0{\cdot}05$.
* For details of diets see Table 1.
† NDMA at a dose of 20 mg/kg body weight or saline only (control), was administered by intraperitoneal injection to six rats from each dietary regimen. GSH and MT levels, GGT activity and TBARS in the liver were determined 24 h after injection.

NDMA injection, GGT activity was increased significantly in the former groups. Elevation of the activity after NDMA injection was prevented by methionine supplementation of the 10 % SPI diet, but cystine had no suppressing effect. GGT activity in the 20 %-SPI and 20 %-casein groups was 0·86 (SD 0·39) and 0·53 (SD 0·22) mU/mg protein respectively, and was not elevated after NDMA administration.

Discussion

Intracellular concentration of reducing substances is important as well as the amount of antioxidant enzymes in protecting organisms against oxidative stress. When rats were maintained on a 10 % SPI diet, TBARS were greatly increased in the liver and serum after injection of NDMA at 20 mg/kg. However, this was not observed in rats maintained on a 20 % casein diet in this study or on a laboratory chow diet (Taniguchi *et al*. 1999). The increase of TBARS in a 10 %-SPI group may be caused by low GSH concentration as well as low GSHPx activity. Using isolated hepatocytes, toxicity and an increase in lipid peroxidation were not observed when cells were incubated with 0·5–5 mM NDMA for up to 18 h, and GSH-dependent cytotoxicity did not appear, as shown by lack of potentiation of toxicity in the presence of buthionine-sulfoximine, an inhibitor for GSH synthesis (Anundi & Lindros, 1992). Inconsistent results of GSH-dependent protection between isolated hepatocytes and intact liver in the present study might be partly due to the time to elicit toxicity. In isolated cells, a longer incubation time might be required or antioxidant enzyme activity might be not affected. By feeding a 10 % SPI diet, GSHPx activity was decreased, and oxidative toxicity could be intensified under severe depletion of GSH. By supplementing a 10 % SPI diet with methionine or cystine, MT levels were lowered, while GSH levels and GSHPx activity were both elevated. MT synthesis in the liver is stimulated by various chemicals that induce oxidative stress (Ochi, 1988; Bauman *et al*. 1991; Sato & Bremner, 1993; Iszard *et al*. 1995; Nakagawa *et al*. 1995; Nakagawa *et al*. 1998), and also by decrease of food consumption (Bremner & Davies, 1975; Sendelback *et al*. 1990). In the present experiment, hepatic MT levels were not controlled by food intake. Food consumption of the cystine-supplemented group was the lowest, as were the MT levels. GSH levels correlated with S-containing amino acid levels in the diets, while MT levels were negatively correlated with both dietary S-containing amino acids and with GSH levels in the liver. An increase in MT levels may be explained as a biological defence mechanism against oxidative stress when GSH levels were depleted. Methionine was shown to be a direct source for MT synthesis (Houghton & Cherian, 1991). In this experiment, MT levels of the methionine-supplemented group were not significantly different from the cystine-supplemented group. Feeding on a diet with a sufficient amount of cystine, methionine conversion to cysteine could be restrained, resulting in MT levels of a cystine-supplemented group similar to those of a methioine-supplemented group. SPI contains 1–2 g phytate/100 g (Honig & Wolf, 1987), and mineral–phytate interactions may prevent absorption of metals, such as Zn, which induces MT synthesis. MT levels were higher in a 10 %- or 20 %-SPI-diet group than in the corresponding casein-diet groups, which contained no phytate. MT levels of a 10 %-SPI group were lowered at the same phytate level by supplementing with S-containing amino acids. These results indicated that phytate interaction was not implicated in the regulation of MT levels by feeding a 10 % SPI diet. MT synthesis was stimulated when GSH was depleted (Nakagawa *et al*. 1995), however MT levels were elevated regardless of GSH levels after administration of NDMA. In contrast, the increase of GSH was limited to high GSH levels. GSH and MT are both thought to protect against menadione toxicity, but the role of MT was presumed to be secondary to that of GSH (Chan *et al*. 1992). In the present work, it is suggested that GSH plays a primary role in the defence against the oxidative toxicity of NDMA, even at high MT levels.

Hepatic GGT activity was high in the 10 % SPI- and cystine-supplemented-diet groups, and was increased by NDMA treatment in both diet groups. In contrast, this response was not observed in methionine-supplemented or casein-diet groups. The suppressing effect on GGT activity in methionine-sufficient diets could be explained by a role of methionine in methylation of the GGT gene. During the early stage of carcinogenesis, GGT-positive foci in rat liver decreased with *S*-adenosyl-L-methionine administration in a dose-dependent manner (Pascale *et al*. 1991), and high GGT activity was demonstrated in fetal liver in which the GGT gene was hypomethylated (el Yaagoubi *et al*. 1995). As a defence mechanism against NDMA toxicity, cystine and methionine were both important in elevating levels of GSH to help suppress lipid peroxidation, and methionine was particularly effective in controlling GGT activity and probably in carcinogenesis. The 10 % SPI diet contained 1·3 g methionine, 1·2 g cystine, and 91·1 g crude protein/kg, and the 10 % casein diet contained 94·7 g crude protein, 2·9 g methionine and 0·6 g cystine/kg. This difference in S-containing amino acids in the two protein diets could effect different protection mechanisms against the oxidative and carcinogenic toxicity of NDMA. Since elevation of GGT activity was found in hepatoma (Fiala & Fiala, 1973; Yokosawa *et al*. 1981), susceptibility to carcinogenic agents may be promoted by a methionine-restricted diet.

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