Long-Term Consumption of a Methionine-Supplemented Diet Increases Iron and Lipid Peroxide Levels in Rat Liver

Nobuko Mori1 and Kimiko Hirayama

College of Medical Science, Kumamoto University, 4–24–1 Kuhonji, Kumamoto 862-0976, Japan

ABSTRACT  Methionine is a protective factor against various types of liver damage, but excessive dietary methionine is hepatotoxic. Because the mechanisms of L-methionine–related hepatotoxicity are poorly understood, the effect of long-term excessive L-methionine intake on the metabolism of iron and antioxidants was studied in rat liver to determine whether oxidative stress is involved. Wistar male rats were fed either an L-methionine–supplemented (16.0 g/kg) diet or a control diet for 1, 3, 6, and 9 mo. The growth rate of L-methionine–supplemented rats was significantly slower than that of controls. Iron, ferritin, and thiobarbituric acid-reactive substances (TBARS) levels in the liver were greater in supplemented rats than in controls. Serum iron and transferrin levels were significantly lower in L-methionine–treated rats compared with controls. Serum ferritin did not differ between the two groups. Hepatic glutathione peroxidase activity, catalase activity and total glutathione concentrations were higher in rats fed the L-methionine-supplemented diet at 1 and 3 mo, but not at 6 and 9 mo. These results indicate that long-term consumption of excess L-methionine by rats may affect primarily iron metabolism rather than the antioxidant defense system and, consequently, induce an accumulation of iron. J. Nutr. 130: 2349–2355, 2000.

KEY WORDS: • methionine • lipid peroxidation • antioxidant • iron • liver • rats

Methionine is a protective factor against various types of liver damage. For example, methionine has been shown to prevent lipid accumulation in the liver (Feo et al. 1986). Moreover, S-adenosyl-L-methionine, an activated form of L-methionine, was reported to show highly beneficial effects in the treatment of ethanol-mediated liver injury and intrahepatic cholestasis (Almasio et al. 1990, Lieber et al. 1990). On the other hand, the intake of excess L-methionine by rats markedly suppresses voluntary food intake and causes growth retardation (Harper et al. 1970). It also induces tissue damage including liver enlargement, fatty liver and erythrocyte membrane damage (Klavins et al. 1963). Moreover, liver damage was observed in patients with inborn errors of metabolism associated with hypermethioninemia (Labrune et al. 1990). Lynch and Strain (1989) found that L-methionine supplementation increased hepatic lipid peroxidation and iron levels with increased activities of catalase (EC.1.11.1.6) and glutathione peroxidase (GPX, EC.1.11.1.9), and reduced that of copper-zinc superoxide dismutase (CuZnSOD, EC.1.15.1.1). They proposed that excess L-methionine intake induced the production of oxidative metabolites resulting from methionine metabolism and caused defects in the enzymatic antioxidant defense system with iron accumulation, increasing lipid peroxidation. Toborek et al. (1996) proposed that methionine might induce lipid peroxidation by increasing free radical formation during thiol autoxidation and generated by activated neutrophils. Although methionine itself does not participate in autoxidation, its metabolites, such as homocysteine, cysteine, reduced glutathione and protein-bound thiols, are susceptible to autoxidation. Free radicals generated during the oxidation of homocysteine to homocystine were documented as a mechanism of homocysteine toxicity (Strakebaum and Harlan 1986). Moreover, free radicals produced during autoxidation of cysteine, another product of methionine catabolism, have been proposed to play the main role in cysteine-mediated injury of hepatocytes (Biaglow et al. 1984). However, the detailed mechanisms of methionine hepatotoxicity are still poorly understood.

Recently, many investigators have studied the relationship between reactive oxygen species and various diseases, and many of the reactions that involve reactive oxygen species often require the presence of a transition metal such as iron. Transfusional iron overload and pathologically increased iron uptake in genetic hemochromatosis exceed the extracellular iron-binding capacity of transferrin as well as the intracellular iron storage capacity of ferritin, leading to permanent cell and tissue damage. Excess iron deposition in hepatocytes is most likely related to enhancement of oxidative stress and free radical reactions stimulated by iron. Major pathophysiologic consequences of iron accumulation in liver include the development of hepatocellular carcinoma. Approximately one third of patients with hemochromatosis and cirrhosis develop hepatocellular carcinoma (Bonkovsky 1991, Niederau et al. 1985).

As described above, nonprotein-bound “free” iron is thought to catalyze the formation of highly reactive OH rad-
icals that damage membranes and DNA. Therefore, it is of utmost importance for both the cells and the organism to maintain iron homeostasis to ensure iron supply while preventing the accumulation of excess iron. The key proteins in iron metabolism are transferrin, transferrin receptor (TfR) and ferritin. The expression of transferrin is regulated mainly transcriptionally. On the other hand, those of TfR and ferritin are cotranslational, controlled by intracellular iron level via specific mRNA-protein interactions in the cytoplasm (Eisenstein et al. 1997, Henzke and Kühn 1996, Klausner et al. 1993). Particular hairpin structures, called iron-responsive elements (IRE) in the respective mRNAs, are recognized by trans-acting proteins known as iron-regulatory proteins (IRP), and this controls the rate of mRNA translation or stability.

In the last few years, several investigators have demonstrated that IRP (especially IRP-1) is activated rapidly by oxidative stress to bind IRE (Eisenstein and Blemings 1998, Henzke and Kühn 1996, Pantopoulos and Henzke 1998). This finding has established a distinct regulatory connection between the control of the iron metabolism and the response to oxidative stress. This regulatory link is particularly important to understand, considering that iron toxicity is based largely on Fenton chemistry, i.e., the generation of highly reactive hydroxyl radicals from the reaction of H2O2 with Fe2+ (Halliwel and Guttridge 1990).

We speculate that accumulation of these methionine metabolites by excess methionine intake causes oxidative stress in the liver and may subsequently affect iron regulation. The objective of this study was to investigate the effect of excessive dietary methionine on the iron metabolism including iron-binding proteins, and on the antioxidant defense system in rat liver.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats, 7 wk old, weighing 180–200 g were purchased from Clea Japan (Tokyo, Japan). The rats were divided into groups of 3 or 4 and housed in hanging stainless steel wire cages with free access to the standard diet (CE2; Clea Japan) and water in a room at a controlled temperature (22 ± 1°C). Lights were maintained on a 12-h light:dark cycle (lights on from 700 to 1900 h). Rats were acclimated to the facility for 5 d. On d 6, they were divided into two groups (n = 5 or 6/group) as follows: a control diet and a methionine group containing 1% methionine-supplemented diets (MSD). They were given free access to the experimental diets and tap water. Body weight and food intake were recorded throughout this study. Care and treatment of the animals complied with the requirements of the Laboratory Animal Research Center, Kumamoto University School of Medicine.

The compositions of the experimental diets (control diets and MSD) are shown in Table 1. Control diets were based on the AIN Rodent Diets, AIN-93M; they contained 14% casein, and were obtained from Dyets (Bethlehem, PA). Reeves et al. (1993) proposed that for long-term studies using nonpregnant animals, and after completion of the rapid growth phase, the animals should be provided with the AIN-93M diet, which we adopted for our study.

Sample collection. After 1, 3, 6 and 9 mo of consuming the experimental diets, the rats were anesthetized with diethyl ether, and perfused with ice-cold saline, and the liver was quickly removed, rinsed in ice-cold saline and weighed. In addition, the livers were cut into several portions, weighed again, immediately frozen using dry ice/isopropylalcohol and stored at −80°C until analysis for several components.

### TABLE 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>MSD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>466</td>
<td>450</td>
</tr>
<tr>
<td>Dehydrated cornstarch</td>
<td>155</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mineral mixture3</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>—</td>
<td>1.6</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>t-Butylhydroquinone5</td>
<td>0.008</td>
<td>0.008</td>
</tr>
</tbody>
</table>

1 Based on AIN-93M Rodent diets (Reeves et al. 1993).

2 MSD, methionine-supplemented diet.

3 Mineral mix (AIN-93M-MX, per kg diet): CaCO3, 12.5 g; KH2PO4, 8.75 g; NaCl, 2.59 g; K2SO4, 1.631 g; CaH2K2O7 · H2O, 0.98 g; MgO, 0.84 g; Fe₂₃H₆O₂7 · nH₂O, 0.21 g; ZnCO3, 58 mg; MnCO3, 22 mg; CuCO3, 11 mg; KIO3, 0.35 mg; Na2SeO3, 0.36 mg; (NH₄)₆Mo7O24 · 4H₂O, 0.28 mg; Na₂SiO₃ · 9H₂O, 50.8 mg; Cr₂(SO4)3 · 12H₂O, 9.63 mg; H₂BO₃, 2.85 mg; NaF, 2.22 mg; Na₂CO₃, 1.11 mg; LiCl, 0.61 mg; Na₂MoO₄ · 2H₂O, 0.23 mg.

4 Vitamin mix (AIN-93-VX, per kg diet): nicotinic acid, 30 mg; pantothenate, 16 mg; pyridoxine-HCl, 7 mg; thiamin-HCl, 6 mg; riboflavin, 6 mg; folic acid, 2 mg; biotin, 0.2 mg; cyanocobalamin, 25 mg; all-rac-a-tocopherol acetate, 150 mg; all-trans-retinyl palmitate, 5 mg; cholecalciferol, 2.5 mg; phylloquinone, 0.75 mg.

5 Added as an antioxidant.

Determination of iron and ferritin. Liver samples were homogenized in 4 volumes of an ice-cold 50 mmol/L potassium phosphate buffer (pH 7.0), 1 mmol/L (±)-dithiothreitol, 5 mL/L 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride and 1% BHT-ethanol. An aliquot of this liquid was heated at 80°C for 15 min and centrifuged at 10000 × g for 15 min at 4°C (high speed micro refrigerated centrifuge MRX-150, TOMY); the supernatants obtained were used as the ferritin fraction.

Hepatic iron concentration was determined by flame atomic absorption spectrometry (AAS). The liver homogenate (1 mL) and ferritin fraction (2 mL) were digested by wet ashing using a nitric perchloric acid mixture in a borosilicate test tube placed in a hot bath. The digested sample was then diluted to 10 mL with deionized water before analysis. Serum iron and total iron-binding capacity (TIBC) were measured with Fe C-Test and UIBC-Test kits, respectively (Wako Pure Chemical, Osaka, Japan).

Hepatic and serum ferritin concentration were determined by ELISA using two types of polyclonal antibodies against rat liver ferritin.

Determination of thiobarbituric acid-reactive substances (TBARS). For the assessment of lipid peroxidation, TBARS in liver and blood were determined according to the method of Yagi (1982). Although the level of TBARS is nonspecific and can produce large artifacts, it is very commonly used as an indicator of lipid peroxide levels. The lipid peroxide level in blood was measured with a Lipid Peroxidation-Test Kit (Wako Pure Chemical). For the measurement of liver triglyceride level, liver tissue was extracted with methanol/chloroform (1:2) according to the method of Folch et al. (1957). Triglyceride levels in the liver extract and serum were determined using a Triglyceride-Test (Wako Pure Chemical) and a TG-EN Kainos Kit (Kainos Laboratory, Tokyo, Japan), respectively.

Determination of antioxidants. For the determination of total glutathione concentration, ~0.5 g of fresh liver was homogenized in ice-cold 0.46 mol/L perchloric acid containing 1 mmol/L EDTA, then centrifuged at 10,000 × g for 5 min at 4°C (MRX-150, TOMY). The resulting supernatants were used. Hepatic total glutathione con-
centration was determined by the method of Tietze (1969). For the measurement of individual antioxidative enzyme activities, ~0.5 g of fresh liver was homogenized in ice-cold 50 mmol/L potassium phosphate buffer (pH 7.0). An aliquot of the crude homogenate was mixed with an equal volume of 10 mmol/L potassium phosphate buffer (pH 7.0), 0.2 mol/L KCl, 2 mmol/L EDTA, 0.1% Triton X-100, then centrifuged at 10000 × g for 5 min at 4°C (MRX-150, TOMY). The resulting supernatants were used to determine CuZnSOD activity, and total and selenium-dependent GPX (T-GPX and Se-GPX) activity, according to the method of Spitz and Oberley (1989) and Lawrence and Bürk (1976), respectively. In addition, an aliquot of the crude homogenate was mixed with an equal volume of 50 mmol/L potassium phosphate buffer (pH 8.0) and 12 mmol/L sodium cholate, then centrifuged at 10,000 × g for 5 min at 4°C (MRX-150, TOMY). The resulting supernatants were used for the determination of catalase activity according to Thomson et al. (1978).

**Determination of other serum components.** Lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum, the serum concentrations of total protein, albumin and blood urea nitrogen (BUN), were measured with the following kits: LDH CII-Test, Transaminase CII-Test, A/G B-Test and BUN B-Test, respectively. These kits were purchased from Wako Pure Chemical.

The protein concentration was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

**Statistical analysis.** Data are expressed as means ± SD. The significance of difference between the two groups at each feeding period was analyzed statistically by the Mann-Whitney U test using StatView software (Abacus Concepts, Berkeley, CA). Differences were considered significant at P < 0.05.

**RESULTS**

**Growth and food intakes.** At the end of the feeding periods (1, 3, 6 and 9 mo), body weights of the rats fed MSD were significantly lower than those of rats fed the control diet (Fig. 1). Ratios of liver weight to body weight did not differ at any time. Voluntary food intake tended to be lower (P < 0.01 at 1 and 3 mo, and P = 0.11 at 6 mo) in rats fed MSD feeding (data not shown).

**Iron and ferritin concentration in liver and serum.** Hepatic iron concentrations were significantly higher in MSD-fed rats than in control rats at 3, 6 and 9 mo (Fig. 2A). In particular, iron levels of the nonferritin fraction in MSD-fed rats were significantly elevated at 3 mo; thereafter, those in the

**FIGURE 1** Body weights of rats fed control or methionine-supplemented (MSD) diets. Each point represents the mean ± SD, n = 5 or 6. *Significant difference (P < 0.05) between the groups at the end of feeding.

**FIGURE 2** Iron (panel A) and ferritin (panel B) concentrations in liver of rats fed control or Met-supplemented diets. Vertical bars are means ± SD, n = 5 or 6. *Significantly different (P < 0.05) from control group at each feeding period.

**FIGURE 3** Iron concentrations in serum of rats fed control or Met-supplemented diets. Value are means ± SD, n = 5 or 6. *Significantly different (P < 0.05) from control group at each feeding period. Abbreviation: TIBC, total iron-binding capacity.
ferritin fraction also were significantly elevated. Hepatic ferritin concentrations were higher in MSD-fed rats than in controls at 3 and 6 mo (Fig. 2B). Serum TIBC, which corresponds to serum transferrin, at 1, 3 and 9 mo and serum iron levels at 6 and 9 mo were lower in MSD-fed rats than in controls (Fig. 3). No significant difference was found in serum ferritin concentrations between the two groups throughout the experiment (data not shown).

**FIGURE 4** Lipid peroxidation (panel A), triglyceride (TG) (panel B) and the ratio of thiobarbituric acid reactive substances (TBARS) to TG (panel C) in liver of rats fed control or Met-supplemented diets. TBARS, an indicator of lipid peroxide levels, are expressed as nmol malondialdehyde (MDA)/mg protein, and TG levels are converted as triolein. For all panels, values are means ± SD, n = 5 or 6. *Significantly different (P < 0.05) from control group at each feeding period.

**FIGURE 5** Lipid peroxidation (panel A), triglyceride (TG) (panel B) and the ratio of thiobarbituric acid reactive substances (TBARS) to TG (panel C) in serum. TBARS, an indicator of lipid peroxide levels, are expressed as nmol malondialdehyde (MDA)/mg protein, and TG levels are converted as triolein. For all panels, data are means ± SD, n = 5 or 6. *Significantly different (P < 0.05) from control group at each feeding period.
**Lipid peroxidation in liver and serum.** Hepatic TBARS were higher in MSD-fed rats than in control rats at 6 and 9 mo (Fig. 4A), whereas serum TBARS were lower than in controls at these time points (Fig. 5A). Because hepatic and serum triglyceride concentrations were depressed significantly by MSD feeding (Fig. 4B and 5B), the ratio of TBARS to triglycerides in liver and serum of MSD-fed rats was higher than that of controls at 3 and 6 mo (Figs. 4C and 5C).

**Hepatic antioxidants levels.** Compared with control rats, MSD-fed rats had higher T-GPX and Se-GPX activities at 1 mo and higher glutathione concentrations and catalase activity at 3 mo (Table 2). No other differences were detected. MSD feeding did not affect LDH, AST and ALT activities in serum, for which they are used commonly as a marker of liver damage, and the other serum component levels tested in this study (data not shown).

**DISCUSSION**

Long-term intake of excessive L-methionine in rats elevated iron level and lipid peroxidation in the liver, but depressed iron and transferrin levels in serum. This treatment temporarily elevated GPX activity, catalase activity and total glutathione concentration in the liver at the early stages (1 and 3 mo) but not at the later stages (6 and 9 mo). Hepatic SOD activity was not affected by diet. These data suggest that the accumulation of iron by loading of excess L-methionine might be due to a disorder of the iron metabolism, probably caused by methionine metabolites, rather than to an alteration in antioxidant defense systems. It has been reported that excess iron in cells stimulates OH radical formation and exerts potent toxic effects (Bokovsky 1991, Halliwell and Gutteridge 1990). In living systems, iron is normally bound to proteins, which prevents the iron from participating in reactions that could lead to cellular damage. Iron is associated with transferrin in serum, is incorporated into cells via TfR and is stored as ferritin (van Eijk and De Jong 1992). Hence, in the cells, ferritin would act as a critical defense system for oxidative stress by sequestering nonprotein-bound iron, which catalyzes the production of OH radicals (Ball et al. 1992). When iron levels increase in the cells, ferritin synthesis is induced, and the synthesis of transferrin and its receptor is inhibited simultaneously, resulting in the inhibition of iron uptake by cells. When iron levels decrease, the opposite phenomenon occurs. Thus, cellular iron levels are maintained. In this study, serum transferrin levels were lowered at 1 and 3 mo, whereas hepatic iron levels were elevated with only a slight elevation of ferritin synthesis at 3 mo in rats fed MSD. Consequently, the iron levels in the nonferritin fraction were higher in the MSD feeding group at 3 mo. At 6 mo, ferritin synthesis was activated further by MSD feeding, corresponding to changes in the iron levels; thus, a large percentage of iron was incorporated into ferritin. At 9 mo, serum iron levels were depressed significantly with lower levels of transferrin, but the hepatic iron levels remained higher than those of controls for up to 9 mo. These results suggest that the regulatory system of tissue iron levels might be disrupted by excess L-methionine intake.

The critical proteins that control the availability of iron within the cell are TfR and ferritin; their expression is modulated post-transcriptionally by intracellular iron via IRE-IRP interaction (Eisenstein et al. 1997, Hentze and Kühn 1996, Klausner et al. 1993). In the presence of excess ferrous iron, the 3Fe-4S clusters in IRP are converted to 4Fe-4S clusters, which are inactive in IRE-binding. Accordingly, degradation of IRE mRNA and translation of ferritin mRNA are stimulated. In iron deficiency, however, 3Fe-4S clusters in IRP remain intact and bind to IRE. Thus, TfR mRNA is stabilized, its synthesis is stimulated and ferritin synthesis is depressed (Basilion et al. 1994, Haile et al. 1992, Tang et al. 1992).

Recently, it was revealed that signals other than iron levels could regulate IRP and modulate cellular iron metabolism (Eisenstein and Blemings 1998, Hentze and Kühn 1996). Binding capacity of IRP to IRE is also affected by nitric oxide (Botou et al. 1998, Kennedy et al. 1997, Pantopoulos et al. 1996), phosphorylation by protein kinase C, hypoxia/reoxygenation (Hanson and Leibold 1998) and changes in cell proliferation or differentiation. Moreover, its capacity is activated rapidly by oxidative stress, leading to increased TfR mRNA levels and suppression of ferritin synthesis (Martins et al. 1995, Pantopoulos and Hentze 1995 and 1998). This relationship between iron metabolism and oxidative stress provides a new point in understanding of the mechanism of methionine-related hepatotoxicity via iron accumulation, which probably participates in the highly reactive and toxic hydroxyl radical formation from the reaction of Fe3+ with H2O2. Toborek et al. (1996) reported that excess methionine supplementation increased hepatic lipid peroxidation in rabbits; they assumed that excess methionine feeding might pro-

| TABLE 2 |

**Hepatic antioxidant enzyme activities and glutathione (GSH) concentration in rats fed control (C) and methionine-supplemented diets (MSD)**

<table>
<thead>
<tr>
<th></th>
<th>1 mo</th>
<th></th>
<th>3 mo</th>
<th></th>
<th>6 mo</th>
<th></th>
<th>9 mo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>MSD</td>
<td>C</td>
<td>MSD</td>
<td>C</td>
<td>MSD</td>
<td>C</td>
<td>MSD</td>
</tr>
<tr>
<td>T-GPX, U/mg protein</td>
<td>476.2 ± 86.4</td>
<td>655.1 ± 74.8*</td>
<td>490.9 ± 34.5</td>
<td>444.4 ± 74.8</td>
<td>552.0 ± 139.1</td>
<td>578.4 ± 120.5</td>
<td>457.9 ± 76.7</td>
<td>393.4 ± 102.2</td>
</tr>
<tr>
<td>Se-GPX, U/mg protein</td>
<td>427.1 ± 70.8</td>
<td>500.8 ± 69.6*</td>
<td>396.2 ± 67.8</td>
<td>340.2 ± 92.8</td>
<td>370.6 ± 105.2</td>
<td>336.4 ± 75.0</td>
<td>321.8 ± 58.3</td>
<td>266.7 ± 54.4</td>
</tr>
<tr>
<td>Catalase, U/mg protein</td>
<td>367.5 ± 93.8</td>
<td>436.0 ± 12.3</td>
<td>378.5 ± 19.7</td>
<td>441.8 ± 54.3*</td>
<td>373.8 ± 52.1</td>
<td>355.9 ± 39.0</td>
<td>332.1 ± 25.7</td>
<td>343.6 ± 48.6</td>
</tr>
<tr>
<td>CuZnSOD, U/mg protein</td>
<td>37.2 ± 6.5</td>
<td>35.2 ± 6.1</td>
<td>44.8 ± 7.8</td>
<td>41.7 ± 7.0</td>
<td>53.2 ± 3.4</td>
<td>49.3 ± 4.1</td>
<td>46.6 ± 8.4</td>
<td>43.7 ± 11.5</td>
</tr>
<tr>
<td>GSH, μmol/g wet liver</td>
<td>5.25 ± 0.65</td>
<td>5.73 ± 1.36</td>
<td>4.35 ± 1.02</td>
<td>5.96 ± 0.69*</td>
<td>5.42 ± 1.49</td>
<td>5.51 ± 1.91</td>
<td>4.64 ± 1.45</td>
<td>5.44 ± 0.81</td>
</tr>
</tbody>
</table>

1 Results are means ± so, n = 5 or 6; * significantly different (P < 0.05) from control value at each feeding period.
2 Abbreviations used: T-GPX, total-glutathione peroxidase; Se-GPX, selenium-dependent glutathione peroxidase; CuZnSOD, copper-zinc superoxide dismutase.
more an accumulation of toxic metabolites, such as homocysteine and cysteine, which are known to be susceptible to autoxidation. In our study, l-methionine feeding elevated TBARS but depressed hepatic triglycerides (Fig. 4). Elevated TBARS may reflect lipid peroxidation in phospholipids rather than in triglycerides. These oxidative stresses caused by toxic methionine metabolites might activate the binding capacity of IRP to IRE, then stimulate TR synthesis and inhibit ferritin synthesis. The higher levels of nonferritin-bound iron at 3 mo in our study might be caused by enhancement of iron uptake via stimulation of TR synthesis. In spite of continuous oxidative stress by long-term feeding, these elevated iron levels might cause the dissociation of the IRP/IRE complex into IRP, which could have resulted in stimulation of ferritin synthesis and reduction of nonferritin-bound iron at 6 mo. However, further oxidative stress by long-term excess l-methionine feeding again activated IRP/IRE binding, leading to suppression of ferritin synthesis and stimulation of TR synthesis. Consequently, hepatic iron levels were kept higher during the experimental period in the MSD group. Thus, excess methionine intake may disturb iron metabolism, leading to iron accumulation in the liver. Subsequently, the excess iron might accelerate the production of reactive oxygen species, which may contribute to the development of cancer and to brain aging, such as in Alzheimer’s and Parkinson’s diseases (Lan and Jiang 1997, Loeffler et al. 1995).

Reactive oxygen species have been implicated in the pathogenic process of a number of diseases such as atherosclerosis (Wittum and Steinberg 1991). Various nutritional risk factors for these diseases have been found. Fau et al. (1988) reported that oxidative damage caused by excess methionine feeding for 2 y might have been responsible for the accelerated aging effects in the aortas of rats. Toborek et al. (1995) also indicated that excess methionine diets might induce the development of atherosclerosis due to increasing lipid peroxidation. Therefore, oxidative damage resulting from the methionine metabolites may be a causal factor in the development of the atherosclerotic-type lesions associated with homocystinuria and homocystinemia. Moreover, men with mild methionine intolerance and provable homocystinemia are at increased risk of coronary artery disease. Thus, exposure to the high methionine content of the Western diet may predispose individuals to this disease.

Lynch and Strain (1989) reported that hepatic iron, TBARS levels, catalase activity and GPX activity in rats fed a 2.0% l-methionine-supplemented diet were significantly elevated at 49 d compared with control rats fed a diet containing 18% casein and 0.2% l-methionine. However, hepatic SOD activity was reduced remarkably by treatment with excess methionine. Toborek et al. (1996) showed a significant elevation in liver TBARS and antioxidant enzyme (catalase, SOD and GPX) activities in rabbits fed a 0.3% methionine-enriched diet compared with those fed a diet containing 24% protein. Some differences between their findings and ours might result from the differences in dose of methionine, feeding duration and protein concentrations of the control diet.

In summary, excess l-methionine intake may induce oxidative stress in the liver, then disrupt the iron metabolism via the IRE/IRP system and subsequently result in the accumulation of iron in liver.


