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Induction of increased phosphatidylcholine hydroperoxide by an iron-deficient diet in rats

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The effect of iron-deficiency on lipid peroxidation in male and female rats was investigated and an attempt was made to understand the underlying mechanisms based on the changes in lipid hydroperoxide formation and relevant hepatic enzymes. Forty 4-week-old male and female rats were fed either an iron-deficient diet or a control diet for 4 weeks. The amount of iron in the microsome and cytosol of liver cells decreased 84 to 55% in the iron-deficient rats, and the amount of copper increased 222 to 1206% in the subcellular fraction of the males, but it only increased 14 to 43% in the microsome and cytosol fraction in the females. Serum and liver triglycerides, phospholipid, and cholesterol increased in iron-deficient male rats, but in female, only cholesterol slightly increased. Serum and liver phosphatidylcholine hydroperoxide (PCOOH) increased 55% and 61%, respectively in the iron-deficient males, and increased less in the females (26% and 10%, respectively). Increased xanthine oxidase (XOD) activity, decreased superoxide dismutase (SOD) activity, and markedly increased glutathione peroxidase (GSH-Px) activity were observed in the liver of the males. The lower XOD and SOD activities and slightly GSH-Px in the females may explain the difference in production of PCOOH according to sex. The role of copper accumulation in the production of lipid peroxides in iron-deficient rats is discussed.
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Introduction

Dietary iron-deficiency is a common nutritional problem in infants, children, and women of reproductive age. Secondary nutritional effects, such as changes in enzyme activity, hyperlipidemia, increased hepatic copper accumulation, and impaired cellular growth, are important, in addition to the anemia and resultant health effects.[1–8]

Lipid peroxidation has received a great deal of attention in connection with its pathological effects and possible contributions to diseases such as atherosclerosis, cancer, and aging. Production of lipid peroxides and other free radicals partly depends on the catalyzing function of iron. Excess intake and storage of iron induces increased production of free radicals and is thought to cause various diseases. In regard to lipid peroxidation, iron-deficiency has reported to decrease serum thiobarbiturate reactive substances (TBARS) in rats,[9,10] and a resultant decrease in DNA adducts has been reported in addition to the decreased production of TBARS.[10] The TBA method, however, measures various radicals and active oxygen, and is influenced by the presence of iron and copper ions.[11,12]

Iron intake influences the composition of essential fatty acid and lipids in rat plasma and erythrocytes.[2,13] These difference may reflect on the production of lipid peroxides. Different iron storage capacity according to sex also influences the reactions that product lipid peroxides.

The purpose of this study is to clarify the effect of iron-deficiency on lipid peroxidation in male and female rats, and to discuss the mechanism of lipid hydroperoxide production based on changes in various enzymes.

Methods and materials

Animals

Forty 4-week-old male and female Wistar-strain rats were purchased from the Clea Japan, Inc, Tokyo. The rats were divided to

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Table 1 Composition of the iron-deficient and control diets (%)

	Fe-deficiency	Control	Source
Casein, milk	22	22	New Zealand Daily Board, Wellington, NZ.
Starch, corn	32	32	Nippon Shokuhin Kakou Co., Ltd., Aichi, Japan
Sucrose	30	30	Wako Pure Chemical Co., Ltd., Osaka, Japan
Oil, corn	5	5	Ajinomoto, Co. Ltd., Tokyo, Japan
Cellulose	5	5	Tokyo Roshi Co. Ltd., Tokyo, Japan
Mineral mix	4*	4	According to Harper AE.: J Nutr 68:408, 1959
Vitamin mix	2	2	Takeda Chemical Co., Ltd., Osaka, Japan

*The mineral mix did not contain Fe $(C_6H_5O_7)/6H_2O$.

four groups of 10 each and housed in stainless steel wire cages in a room maintained at 20°C and 50 to 60% relative humidity with a 12-hr light:dark cycle. The rats were fed either an iron-deficient or a control diet for 4 weeks. The diet and distilled water were made freely available. Tail blood was taken for the hemoglobin assay[14] 1 day before sacrifice. All rats were sacrificed by exsanguination from the carotid artery. This study was approved by the Animal Care and Use Committee of the Tokyo University of Agriculture.

Diets

The composition of the iron-deficient diet and the control diet is shown in *Table 1*. The iron-deficient diet was prepared from basal diet by adding iron-free Harper mineral mix.[15] Mineral contents were determined by atomic absorption spectrophotometry. Final composition of minerals in the control and iron-deficient diet was as follows; iron (53 vs. 8 mg/kg), copper (20 vs. 19 mg/kg), and zinc (14 vs. 15 mg/kg), respectively.

Blood

Blood was collected into tubes from the carotid artery at sacrifice, and centrifuged at 600 × g for 15 min to obtain serum. The liver was perfused with cold 0.9% NaCl solution via the portal vein until the blood color of the liver disappeared.

Subcellular fractionation

The liver was removed rapidly and frozen in liquid nitrogen. To obtain the subcellular fractions, centrifugation was performed according to the method of Sherman et al.[5] After thawing the liver, it was mixed with Tris-HCl buffer containing 0.25 M sucrose, pH 7.4. Nine volumes of the buffered sucrose were added to one volume of chopped liver, and the mixture was homogenized in a Teflon-glass homogenizer ice cooled with ice. The homogenates were centrifuged at 600 × g for 15 min, and the sediment was considered the nuclear fraction. It was composed mainly of nuclei, but it was contaminated with plasma membranes and broken cells. The supernatant was further centrifuged at 9,000 × g for 20 min, and the sediment was composed mainly of mitochondria and lysosomes (mitochondria fraction). The supernatant was then centrifuged at 105,000 × g for 60 min to separate the microsomal fraction (endoplasmic reticulum, Golgi apparatus, and ribosomes) from the cytosol. This cytosol was used for determination of xanthine oxidase, superoxide dismutase, Se-dependent glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidase activities. Iron and copper content of each fraction was measured by atomic absorption spectrophotometry.

Measurement of serum and hepatic lipids

Whole serum was used to measure serum lipids. Serum triglycerides,[16] phospholipids,[17] total-cholesterol[18] and HDL-cholesterol[18] were determined with reagent kits from Kyowa Medex Co., Ltd. or Wako Pure Chemical Industries, Ltd.

Liver lipids were extracted by the method of Folch et al.[19] Total liver lipids were determined by the method of weight measurement,[20] and the above lipid fractions were measured with the same kits.

Lipid peroxide extraction

Total lipids were extracted from the liver homogenate with a chloroform-methanol (2:1, v/v) solution for chemiluminescence (CL)-HPLC. First, 2 ml of 0.15 M NaCl containing 0.002% butylated hydroxytoluene (BHT), as an antioxidant was added to 200 mg of liver homogenates, then 5 mL chloroform/methanol (2:1, v/v) solution was added, and mixed vigorously for 1 min. The mixture was centrifuged at 3,000 rpm for 10 min, and the chloroform layer at the bottom containing liver total lipid was collected. After dehydration with anhydrous sodium sulfate, the chloroform layer was evaporated in a rotary evaporator and dried under a nitrogen gas stream. The extracted total lipid obtained was weighed gravimetrically and diluted with 200 μL of chloroform/methanol (2:1, v/v), and a 20 μL sample was subjected to hydroperoxide assay by CL-HPLC.

CL-HPLC

Phospholipid (phosphatidylcholine) hydroperoxides, PCOOHs, in the total lipid fraction from the liver were determined by the CL-HPLC method.[21,22] This analytical system consists of normal-phase HPLC and a hydroperoxide-specific chemiluminescence detector. The HPLC column, Shodex SIL-5B, was placed in a column oven (Shodex AD-30C). The column mobile phase was chloroform/methanol/1-propanol/water (1:9:2:0.1, V/V) and a flow rate of 1.1 ml/min was maintained with a Shodex DS-4 pump. After being passed through a Shodex UV-41 detector set at 234 nm (conjugated diene absorption), the column elute was mixed with a chemiluminescence reagent at the post-column mixing joint. The chemiluminescence reagent was prepared by dissolving 10 μg/mL of cytochrome c (from horse heart) and 1 μg/mL of luminol (3-aminophthaloyl hydrazine) in 50 mM borate buffer (pH 9.3). The chemiluminescence flow rate was 1.0 mL/min (Shodex DS-4). The chemiluminescence generated was measured with the Shodex CL-2 detector. A calibration curve was prepared by using authentic PCOOH prepared by photooxidation of egg yolk PC. The hydroperoxide concentrations are expressed as pmol of hydroperoxide.

Enzyme activities

Xanthine oxidase (XOD) activity was measured as the rate of uric acid formation from xanthine either in the presence of NAD+ (xanthine dehydrogenase: XDH) or absence of NAD+ (XOD) at 25°C by 295 nm UV absorbance and expressed as μg/mg protein/60 min.[23] Superoxide dismutase (SOD) activity was measured

by the inhibition of nitrite reduction (50%) mediated via superoxide anion generated by xanthine/xanthine oxidase system at 37°C. It was monitored at 550 nm light absorbance. One activity unit (50% inhibition) was expressed as NU (nitrite unit)/mg protein/30 min.[24] Se-dependent glutathione peroxidase activity (GSH-Px) was determined by measuring oxidized NADPH in the presence of glutathione reductase after addition of H_2O_2 substrate at 25°C for 1 min,[25] and is expressed as nmol of NADPH oxidized/min/mg protein. Phospholipid hydroperoxide glutathione peroxidase (PHGPX) activity was determined with the similar procedure of GSH-Px substituting H_2O_2 to PCOOH, and measured at 340 nm.[24] The ceruloplasmin level was measured by the modified method of paraphenylenediamine calorimetrical method.[26] The amount of protein was determined by the procedure of Lowry et al.[27] Bovine serum albumin was used as a standard for protein level.

Statistical analysis

The data are expressed as mean values and standard deviation, and were analyzed by Levene's test. After examining equality of variance by Levene's test, the data were assessed using the nonparametric Kruskal-Wallis test when differences were detected. When there were no differences (parametric), the data were analyzed by two-way ANOVA. Bonferroni's test was used for multiple range tests. Differences were considered significant at $P < 0.05$. All the statistical analyses were performed with the SPSS package program Ver 6.1J on Windows 95.[28]

Results

Growth and iron-storage in rats fed an iron-deficient diet: Growth was suppressed by the iron-deficient diet from the 8th day in the males and from the 12th day in the females (*Figure 1*). After 28 days of being fed the iron-deficient diet, the body weight of the iron-deficient rats was almost 25% lower. This difference was a result of decreased consumption. However, there were no clear differences in appearance or movement throughout experiment. The blood hemoglobin value was markedly lower in the iron-deficient rats (*Figure 2*): 70 g/L in the males (control: 168 g/L) and 86 g/L in the females (control: 195 g/L).

The iron level in the subcellular fractions of liver cells were reduced markedly in the microsome and cytosol fractions of the iron-deficient rats (*Table 2*). The decreases in iron in the nucleus and mitochondria fractions were more prominent in the females. The amount of copper, on the other hand, markedly increased in all fractions in the males, but was less in the females.

Changes in serum and liver of lipids

In the control rats, all serum lipids except triglycerides were higher in the females (*Table 3*). In the iron-deficient rats, serum lipids changed in different way. Triglycerides increased 327% in the males, but decreased 28% in the females. Phospholipids were increased 31% in the males, but were almost unchanged in the females. Total cholesterol increased 18% in the males but was unchanged in the females. HDL cholesterol decreased in both males and females, by 30% and 16%, respectively. LDL+VLDL cholesterol, on the other hand, increased in both iron-deficient males and females.

Liver triglycerides increased 69% in iron-deficient male rats, but it increased 27% in iron-deficient female rats

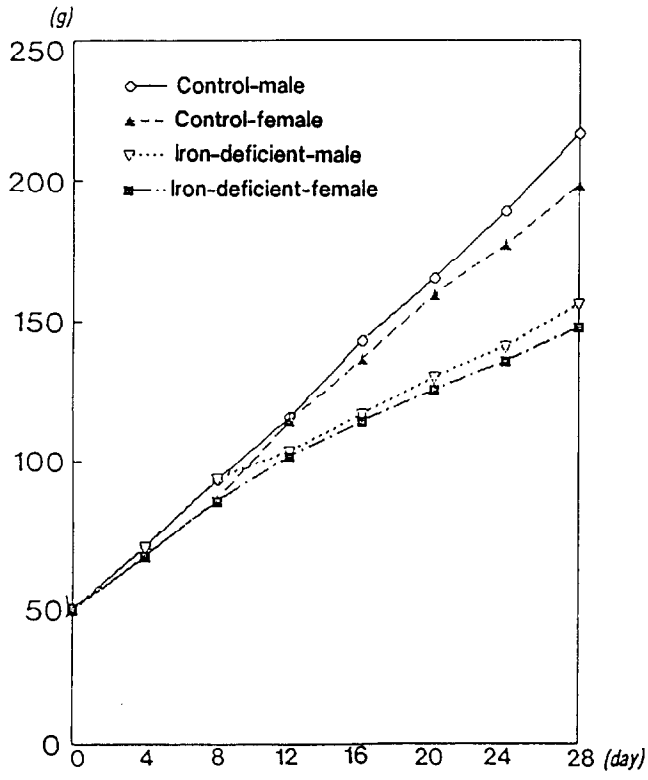

Figure 1 Growth curve of control and iron-deficient rats. Growth retardation was present in both male and female rats.

(*Table 4*). The cholesterol level in females was slightly increased by iron-deficiency.

Changes in PCOOH levels

The amount of PCOOH level in control rats was 375.2 nmol/L in serum and 449.4 pmol/g in the liver in males. The PCOOH level in the serum of control females was 307.5 nmol/L versus 535.6 pmol/g in the liver (*Table 5*). PCOOHs increased 55% and 61% in male serum and liver as a result of iron-deficiency, but only 26% and 10% in female serum and liver, respectively.

Changes of enzymes

There was marked increase in XOD+XDH activity in the liver of both iron-deficient male and female rats (*Table 6*). It approximately doubled in the males, but only increased about 50% in females. XOD activity only increased in the males. GSH-PX showed marked a sex difference in both the control and iron deficient rats. The females had three times higher activity initially, and it was still more than double after iron-deficient feeding was completed. SOD tended to decrease slightly in the males and increase in the females as a result of iron deficiency, although the difference was not statistically significant. PHGPX tended to decrease in both males and females, −33% and −15%, respectively. Ceruloplasmin level decreased, despite the marked accumulation of copper in the liver.

A significant positive correlation was found between serum PCOOH and XOD, and between liver PCOOH and

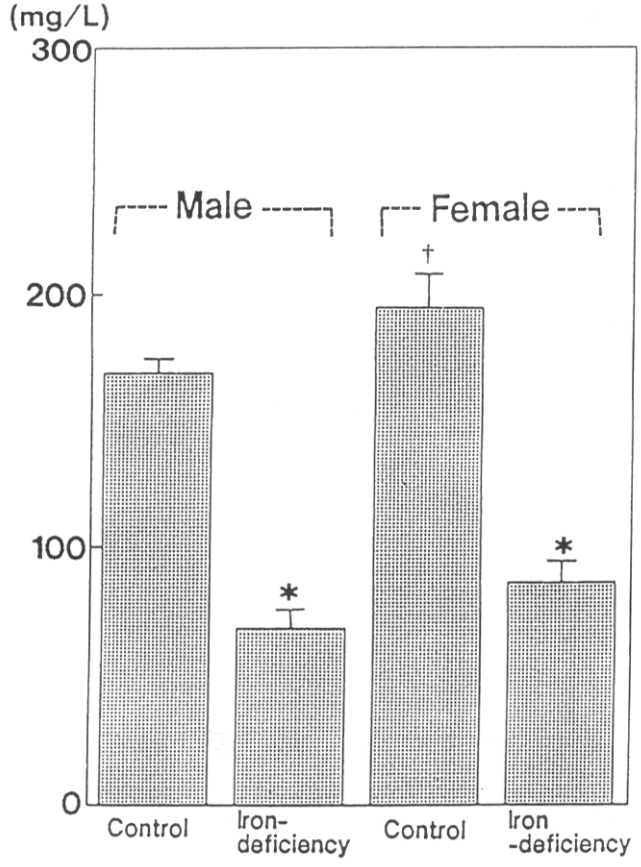

Figure 2 Value of hemoglobin in control and iron-deficient rats. In both male and females, marked reduction in hemoglobin was noticed. Height of columns and bars indicate means ± SD. *Significantly different from sex-:matched control $P < 0.05$. †Significantly different from treatment-matched male $P < 0.05$.

GSH-Px and a significant negative correlation between serum PCOOH and PHGPX, or XOD and SOD (*Table 7*).

Discussion

It is well known that the amount of iron stored differs greatly according to sex, because is partly regulated by estrogen.[29] The amount of iron in the subcellular fractions of hepatic cells in the control rats was higher in the females than in males. In the iron-deficient rats, decrease in iron was more marked in the female rats than in the male rats, especially in the mitochondria and cytosol fraction.

Iron deficiency causes various changes in the level of plasma lipids. Sherman et al.[3] reported elevated plasma lipid levels, such as triglycerides, cholesterol, and phospholipids, in rat pups born of anemic dams. The mechanism of the hypertriglyceridemia is considered to be a rate of increased conversion from glucose, which was converted from accumulated lactic acid under the low oxygen concentration associated with iron-deficiency.[3,30,31] The increase in phospholipid was influenced by the increased amount of triacylglycerol. Although the level of cholesterol did not change much, there was a marked in decrease in HDL-cholesterol that seemed attributable to decreased lecithin

Table 2 Amount of iron and copper in the subcellular fractions of liver cells

Fractions	Iron (nmol/g wet tissue)						Copper (nmol/g wet tissue)					
	Male			Female			Male			Female		
	Control	Iron-deficient	%	Control	Iron-deficient	%	Control	Iron-deficient	%	Control	Iron-deficient	%
Nucleus	430.1 ± 87.8	250.9 ± 59.1*	−42	758.0 ± 170.2†	270.6 ± 64.5*	−64	45.5 ± 14.8	443.7 ± 145.8*	+875	23.6 ± 1.7	75.2 ± 32.4†	+218
Mitochondria	327.9 ± 48.4	207.9 ± 53.8*	−37	639.7 ± 134.4†	250.9 ± 82.4*	−61	9.9 ± 1.6	129.5 ± 49.9*	+1206	27.1 ± 4.4	46.6 ± 12.7†	+72
Microsome	412.5 ± 98.6	82.4 ± 26.9*	−80	444.4 ± 157.7	82.4 ± 39.4*	−81	4.6 ± 1.7	31.0 ± 17.2*	+579	10.2 ± 2.0	8.8 ± 1.7†	+14
Cytosol	528.6 ± 102.1	240.1 ± 150.5*	−55	668.4 ± 150.5†	173.8 ± 64.5*	−74	50.2 ± 5.2	161.8 ± 97.6*	+222	31.0 ± 5.3	44.4 ± 6.0†	+43

Values are means ± SD.
*Significantly different from sex-matched control $P < 0.05$.
†Significantly different from treatment-matched male $P < 0.05$.
Two-way ANOVA showed that iron content was significantly decreased by the iron-deficient diet. There was no interaction between iron intake and sex.

Table 3 Changes in serum lipids in iron-deficiency

	Male			Female		
	Control	Iron-deficient	%	Control	Iron-deficient	%
Triglycerides (mmol/L) (mg/L)	17.3 ± 3.4 (1532 ± 299)	73.9 ± 9.5* (6543 ± 842)	+327	11.8 ± 4.44 (1047 ± 393)	8.51 ± 2.17† (754 ± 192)	−28
Phospholipids (mg/L)	1555 ± 149	2043 ± 416*	+31	1986 ± 161†	1902 ± 401	−4
Total cholesterol (mmol/L)	1.91 ± 0.35	2.26 ± 0.39	+18	2.58 ± 0.46†	2.55 ± 0.61	−1
LDL + VLDL-cholesterol (mmol/L)	0.99 ± 0.30	1.62 ± 0.46*	+63	1.40 ± 0.38	1.57 ± 0.61	+12
HDL-cholesterol (mmol/L)	0.92 ± 0.12	0.64 ± 0.24	−30	1.16 ± 0.18	0.98 ± 0.36†	−16

Values are means ± SD.
*Significantly different from sex-matched control $P < 0.05$.
†Significantly different from treatment-matched male $P < 0.05$.
Two-way ANOVA showed significant differences in phospholipid and LDL/VLDL cholesterol levels according to iron intake, and that total cholesterol and HDL-cholesterol were influenced by sex. There was no interaction between iron intake and sex.

Table 4 Changes of lipids in the liver in iron-deficiency (mg/g wet tissue)

	Male			Female		
	Control	Iron-deficient	%	Control	Iron-deficient	%
Total Lipids	48.2 ± 10.4	65.9 ± 15.9*	+37	59.4 ± 10.3	51.3 ± 12.8	−14
Triglycerides	21.3 ± 5.0	36.1 ± 11.8*	+69	38.9 ± 15.4†	28.4 ± 8.4	−27
Phospholipids	16.5 ± 1.5	20.3 ± 1.4*	+23	27.0 ± 3.6†	26.5 ± 2.0†	−2
Total Cholesterol	1.47 ± 0.33	1.72 ± 0.29	+17	1.30 ± 0.20	1.43 ± 0.22	+10

Values are means ± SD.
*Significantly different from sex-matched control $P < 0.05$.
†Significantly different from treatment-matched male $P < 0.05$.
Two-way ANOVA showed that phospholipid was significantly influenced by both iron intake and sex, although interaction was not significant. Interaction between sex and iron intake was present in regard to total lipids (Fe, NS; Sex, NS; Fe × Sex: 0.04).

Table 5 Changes in PCOOHs in the serum and liver in iron-deficiency

	Male			Female		
	Control	Iron-deficient	%	Control	Iron-deficient	%
Serum (nmol/L)	375.2 ± 69.1	581.0 ± 198.3*	+55	307.5 ± 40.9	387.2 ± 115.4	+26
Liver (pmol/g wet tissue)	449.4 ± 64.0	790.5 ± 249.0*	+61	535.6 ± 159.1	588.2 ± 30.1	+10

Values are means ± SD.
*Significantly different from sex-matched control $P < 0.05$.
†Significantly different from treatment-matched male $P < 0.05$.
Two-way ANOVA showed that liver PCOOH was significantly influenced by iron intake, and interaction of iron intake and sex was present (Fe, 0.01: Sex, NS: Fe × Sex, 0.04).
Two-way ANOVA showed that serum PCOOH in rats was significantly influenced by both iron intake and sex, although interaction between them was not significant (Fe, 0.01: Sex, 0.01: Fe × Sex, NS).

Table 6 Changes in enzyme activity in iron-deficiency

	Male			Female		
	Control	Iron-deficient	%	Control	Iron-deficient	%
XOD + XDH[1)]	8.65 ± 3.42	15.54 ± 4.09*	+80	7.52 ± 3.18	11.20 ± 1.97†	+49
XOD[1)]	3.09 ± 1.57	5.80 ± 2.73	+88	3.39 ± 1.17	2.57 ± 1.82	−24
SOD[2)]	577.9 ± 151.5	496.3 ± 35.2	−14	397.5 ± 30.0	448.8 ± 128.2	+13
GSH-Px[3)]	281.8 ± 23.0	1172.7 ± 195.5*	+316	937.1 ± 218.5†	2394.6 ± 527.8*†	+155
PHGPX[3)]	2.85 ± 0.90	1.90 ± 0.58	−33	2.44 ± 2.01	2.08 ± 0.68	−15
Ceruloplasmin						
(mg/L)	411.1 ± 32.2	313.0 ± 37.9*	−31	493.4 ± 48.8†	371.9 ± 40.6*†	−25
(U/L)[#]	360.8 ± 28.3	247.4 ± 43.2*	−31	433.1 ± 42.8†	326.4 ± 35.6*†	−25

Values are means ± SD. [1)]μg/60 min/mg protein, [2)] NU/30 min/mg protein, [3)] nmol/min/mg protein.
*Significantly different from sex-matched control $P < 0.05$.
†Significantly different from treatment-matched male $P < 0.05$.
XOD + XDH, mixed activity of xanthine oxidase and xanthine dehydrogenase; XOD, xanthine oxidase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; PHGPX, phospholipid hydroperoxide glutathione peroxidase. Enzymes were extracted from liver. Ceruloplasmin levels were of plasma.
[#], oxidase activity.

cholesterol acyltransferase activity.[32] These lipid changes were not as prominent in the female rats as in males. Estrogen is involved in lipid metabolism,[33–35] so the lipid levels were rather stable. Lipids in the liver exhibited similar changes as lipids in plasma, except for the low level of phospholipids and cholesterol.

Yoshiji et al.[9] showed that the choline-deficient-diet-induced accumulation of TBARS was antagonized by combination with dietary iron deficiency. They also observed lower production of 8-OHdG, which was probably caused by the decreased production of TBARS. TBARS, however, was measured by different reactions and dose not directly indicate the level of free radicals.[10]

The Fe^{2+}-dependent decomposition of hydrogen peroxide is controlled by the Fenton reaction. This reaction is closely related to the production of free radicals and member of active oxygen family. Decreases in iron (40 to 80% in our experiment) appear to decrease the activity of the Fenton reaction. Measurement of PCOOH is the most accurate method of assessing the lipid peroxide formation in biological membranes.

Contrary to the Yoshiji's results, we found the elevated PCOOH levels in both the serum and liver of male iron-deficient rats. The increase in PCOOH levels in iron deficiency is thought to be attributable to accumulation of copper in the liver.[5,35,36] The lesser increase in female rats, is probably due to less accumulation of copper. Transition metals, such as iron and copper, easily activate O_2 to active oxygen. Oxidation by various chemicals is also known to requires very small amount of transition metals, such as either iron or copper.[37] Unstable copper ion may promote the Fenton reaction in place of ferrous ion. In Wilson's disease, the level of radicals increases as copper accumulation in the liver.[38] Iron-deficiency induced copper accumulation in the present experiment, and serum ceruloplasmin level reflected this. Low ceruloplasmin in the serum suggested the improper release of copper from the liver, which resulted in the copper accumulation in the liver. Thus, pathologically low iron intake is not beneficial for reducing free radicals in the body, instead, it increased the amount of radicals because of excess catalytic activity of copper ion.

Table 7 Correlation between PCOOH and relevant enzyme activities in the liver (males)

	LPCOOH	SPCOOH	XOD	SOD	GSH-Px
SPCOOH	.1470				
	(.728)				
XOD	.3633	.6759*			
	(.302)	(.046)			
SOD	−.4696	−.5769	−.7009		
	(.202)	(.081)	(.024)*		
GSH-Px	.6636*	.4164	.4479	−.1487	
	(.036)	(.231)	(.167)	(.556)	
PHGPX	.0832	−.6838*	−.2970	.5146	−.4779
	(.819)	(.042)	(.375)	(.105)	(.116)

(Coefficient/two-tailed significance).
L, liver; S, serum; *$P < 0.05$.

PCOOH levels must also be influenced by radical-forming and scavenging enzymes. SOD is an O_2^- scavenger, and GSH-Px, which acts on glutathione reduction and catalysis, is a radical scavenger. Ceruloplasmin and bilirubin are examples of endogenously produced nonenzymatic radical quenching antioxidants. Chief component analysis of these enzymes showed that XOD was significantly positively correlated with PCOOH and that SOD was negatively correlated with PCOOH. The PCOOH level in iron-deficient female rats did not increase as in males. A complex interaction between estrogen and these enzymes was suspected, but the antioxidant activity of estrogen itself also may prevent the production of PCOOH. GSH-Px activity was higher in the females among both the control and the iron-deficient rats. This also decreased the production of $\cdot OH$ from H_2O_2.

Our results indicated that free radicals could not be scavenged if this increased XOD activity caused O_2-dependent mobilization of ferritin iron. The increase of XOD activity was not caused by the release of Fe^{2+} from ferritin because ferritin should have been already consumed in the

liver of iron-deficient male rats during the 4 weeks of feeding. The mechanism of the increased XOD activity under these circumstances is unknown, and the resulting O_2^- was insufficiently scavenged by SOD in the iron-deficient male. Contrary to this result, GSH-Px also showed higher activity in the iron-deficient male than in the control male. GSH-Px seemed to be induced in iron-deficient male without stimulation by H_2O_2, which is produced by SOD. These findings suggested that an imbalance between prooxidant and antioxidant enzymes that was resulted in an interaction inadequate to scavenge lipid peroxides. Further study is necessary in this regard.

In conclusion, PCOOHs are increased in male iron-deficient rats by hepatic copper accumulation, and by unbalanced interaction between prooxidant and antioxidant enzymes, but in females, the various effects of estrogen itself strongly prevented the formation of lipid peroxides by iron-deficiency. Diet can significantly affect the antioxidant status of an individual, and an adequate amount of iron is required to maintain lipid peroxides at a safe level.

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