Trace Nutrients Research 32 : 27－33 (2015)

Original Article

Time effects of a high-fat diet on hepatic iron concentrations in rats

Katsuko TAKAI, Masayuki FUNABA and Tohru MATSUI
*Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University**

Summary

Nonalcoholic fatty liver disease (NAFLD) is caused by the excessive accumulation of fat in the liver, and includes simple fatty liver and nonalcoholic steatohepatitis (NASH). Iron overload in the liver has been implicated in a pathogenesis of NASH because it induces oxidative stress. Although high-fat (HF) diets have been reported to increase hepatic iron concentrations, this remains controversial. We hypothesized that a HF diet may increase hepatic iron concentrations in the short term, but that this effect disappears when the duration of the dietary treatment is extended. In order to determine the time effects of a HF diet on hepatic iron concentrations, male Sprague-Dawley rats were fed a control (CT) or HF diet for 4, 8, or 12 weeks. The HF diet increased hepatic triacylglycerol concentrations at 4 weeks, and these levels remained stable until the end of the experimental period. Hepatocyte ballooning was observed at each week in the livers of rats fed the HF diet; however, it may have been immature at 4 weeks. Furthermore, many large and small vesicles were detected in the livers of the HF group at 8 and 12 weeks, but not at 4 weeks. Oxidative stress markers, such as the concentration of thiobarbituric acid reactive substances (TBARS) and the mRNA expression of heme oxygenase-1, in the liver were higher in the HF group than in the CT group at 8 weeks. A higher concentration of TBARS was also observed at 4 weeks. However, these differences disappeared by 12 weeks. The HF diet did not affect the mRNA expression of inflammation markers such as interleukin-6 and cluster of differentiation 45 during the experimental period. A histological examination revealed the absence of inflammation in the livers of HF rats. Hepatic iron concentrations were not affected by the HF diet throughout the experimental period. These results demonstrated that the HF diet did not affect iron concentrations in the liver.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is caused by the excessive accumulation of fat in the liver, and its pathogenesis does not involve significant alcohol consumption or viral infection. NAFLD includes simple fatty liver and nonalcoholic steatohepatitis (NASH), the progressed form of NAFLD[1).

Oxidative stress and inflammatory cytokines have been identified as causative factors of inflammation and fibrosis in simple fatty liver[2). Some NASH patients have high hepatic iron concentrations, and iron overload has been suggested to induce NASH through the production of oxidative stress[3). Therefore, phlebotomy is sometimes used to treat NASH[4). Hepatic iron concentrations were previously shown to be increased by the consumption of a high-fat (HF) diet for 2 weeks following a 3-week iron depletion period in rats[5). Furthermore, the consumption of a HF diet for 4 weeks increased hepatic iron concentrations when dietary zinc concentrations were marginally low[6).

Obesity has been proposed as a risk factor for an iron deficiency[7). Diet-induced obese rats were previously reported to have higher iron requirements[8). Furthermore, hepatic iron concentrations were decreased in mice by the consumption of a HF diet for 16 weeks[9).

Our previous study showed that the consumption of a high-fat and high-sucrose diet for 12 weeks induced oxidative stress and inflammation, increased the mRNA expression of hepcidin, a hormone that suppresses iron absorption[10), and decreased iron concentrations in the rat liver[11). These findings suggested that the expression of hepcidin was up-regulated by inflammation, resulting in reductions in hepatic iron concentrations because the expression of hepcidin is known as an inflammatory reaction in the liver[10).

Therefore, the relationship between HF diets and hepatic iron concentrations remains controversial. We

* Address : Kitashirakawa Oiwakecho, Kyoto (〒606-8502)

hypothesized that hepatic iron concentrations may be increased by the short-term consumption of a HF diet, but then decrease in the long term. We herein examined the effects of a HF diet on hepatic iron concentrations over time and investigated the relationship between hepatic iron concentrations, oxidative stress, and inflammation.

Materials and Methods

1. Animals and diets

Thirty-six male, pathogen-free Sprague-Dawley rats aged 4 weeks were purchased from Japan SLC (Shizuoka, Japan). Rats were divided into a control diet group (CT) or HF diet group and fed one of the experimental diets (Table 1) for 4, 8, or 12 weeks after a 1-week adaptation period. The American Institute of Nutrition-93G (AIN-93G) diet[12] was provided for all rats in the adaptation period and CT rats in the experimental periods. HF rats were fed a HF diet in the experimental periods. The energy concentrations of the diets were estimated by the calorie factor of Atwater[13] for the calculation of energy intake (Table 1).

All rats were individually housed in stainless steel cages in a temperature-, humidity-, and lighting-controlled room (24°C, 50%, and 5:00-19:00, respectively) with free access to the respective diets and distilled water. Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Animal Care Committee, Kyoto University), and the experimental protocol was approved by the ethics committee (no. 26-43).

2. Sample collection and biochemical analyses

Body weight and food intake were individually measured once a week and every day, respectively, during the experimental period. At the end of the experimental period, a blood sample was obtained from the abdominal aorta under isoflurane anesthesia after overnight fasting. Livers and fat pads (inguinal, epididymal, mesenteric, and perinephric) were excised and weighed. Plasma was separated by centrifugation and frozen at -80°C until analyzed. The weight of the fat pads was used to calculate the adiposity index (Adiposity index = the summed weight of fat pads/body weight × 100)[14].

The concentration of triacylglycerol (TG) in the liver was measured using the Triglyceride E-test kit (Wako Pure Chemical, Osaka, Japan) after chloroform-methanol extraction.

The hepatic concentration of thiobarbituric acid reactive substances (TBARS) was measured using a commercial kit (OXItek TBARS Assay Kit, Enzo Life Sciences Inc., NY, USA). The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using a commercial kit (Transaminase CII-test kit, Wako Pure Chemical).

Total RNA was extracted from liver samples and quantitative reverse transcription PCR (qRT-PCR) was performed (Roter-Gene 6000, Corbett Research, Sydney, Australia) as described previously[15]. The mRNA expression of hepcidin, heme oxygenase-1 (*Ho-1*), interleukin-6 (*Il-6*), and cluster of differentiation 45 (*Cd45*) was determined by the qRT-PCR method. The PCR primers used are shown in Table 2. The mRNA of glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) was used as an internal control. Amplification was performed with 40 cycles. The threshold cycle (Ct) value was determined, and the abundance of gene transcripts was calculated from the Ct value using *Gapdh*. When a transcript was not detected by the qRT-PCR analysis, the relative expression level was calculated as 40 of the Ct value for the transcript.

The concentration of plasma iron was measured using the Fe C-test kit (Wako Pure Chemical). Iron concentrations in the diets and livers were determined using an atomic absorption spectrophotometer (AA-6600F; Shimadzu, Kyoto, Japan) after wet-digestion with nitric acid and hydrochloric acid. Protein concentrations in livers were determined using the BCA method[16] to calculate hepatic iron concentrations.

Formalin-fixed, paraffin-embedded liver tissue sections were stained with hematoxylin and eosin for histological

Table 1 Composition of experimental diets (g/kg air dry matter)

	CT	HF
Cornstarch	397.5	115.5
Dextrinized cornstarch	132	132
Sucrose	100	100
Casein	200	200
Lard	0	312
Soybean oil	70	40
Cellulose	50	50
Mineral premix[1]	35	35
Vitamin premix[2]	10	10
L-Cystine	3	3
Choline bitartrate	2.5	2.5
TBHQ	0.014	0.014
Energy concentration (kcal/kg)[3]	3868	5280
Iron concentration (mg/kg diet)	40.12	39.89

[1] AIN-93G-MX.
[2] AIN-93-VX.
TBHQ, tert-butylhydroquinone.
[3] Energy concentrations were estimated by the calorie factor of Atwater[13].

Table 2 Primer pairs used for quantitative RT-PCR

Genes	Forward primer	Reverse primer
Gapdh	5'-ACAACTTTGGCATCGTGGA-3'	5'-CTTCTGAGTGGCAGTGATGG-3'
Hamp	5'-GCTGCCTGTCTCCTGCTT-3'	5'-AGCCGTAGTCTGTCTCGTCTG-3'
Ho-1	5'-TTTCCGCCTCCAACCAGCGA-3'	5'-TGGGACATGCTGTCGAGCTGTG-3'
Il-6	5'-AGCCCACCAGGAACGAAAGTCAAC-3'	5'-ACAACATCAGTCCCAAGAAGGCAAC-3'
Cd45	5'-ACAAGACAGAAGGGTGCAGAC-3'	5'-CATTGAACATGGGGAAGCAT-3'

Gapdh, Glyceraldehyde 3-phosphate dehydrogenase; *Hamp*, Hepcidin gene; *Ho-1*, heme oxygenase-1; *Il-6*, interleukin-6; *Cd45*, cluster of differentiation 45.

observations.

3. Statistical analysis

Data are presented as means ± SEM (n = 6). Data on mRNA expression as well as AST and ALT activities were log-transformed to provide an approximation of a normal distribution before analyses. Significant differences among groups for all data, except for mRNA, were calculated by a two-way ANOVA followed by Tukey's test as a post hoc test (SAS Institute Inc., NC, USA). Differences in mRNA data between the CT and HF groups in the same feeding period were evaluated by the 2-tailed Student's *t*-test. A difference of $P < 0.05$ was considered significant.

Results

Feed intake was significantly lower in the HF group than in the CT group at 4 and 8 weeks (Table 3). No significant difference was observed in body weight gain between the CT and HF groups during the experimental period. Thus, feed efficiency was significantly higher in the HF group than in the CT group. Since dietary nutrient concentrations other than fat and carbohydrate were designed to be almost constant in the diets (Table 1), iron intake was significantly lower in the HF group than in the CT group because of the lower feed intake by the HF group.

Energy intake was significantly higher in the HF group than in the CT group at 12 weeks, whereas no significant difference was observed at 4 or 8 weeks (Table 4). Liver weights were significantly higher in the HF group than in the CT group at 8 weeks. The adiposity index was significantly higher in the HF group than in the CT group at 12 weeks. Hepatic TG concentrations were significantly higher in the HF group than in the CT group during the experimental period, whereas the duration of the experimental period did not affect hepatic TG concentrations in either group.

TBARS is an oxidative stress marker[17]. In the present study, hepatic TBARS concentrations were significantly higher in the HF group than in the CT group at 4 and 8 weeks; however, this difference disappeared by 12 weeks (Table 5). Plasma AST and ALT activities are markers of liver injury. In the present study, no significant differences were observed in plasma AST activity between the dietary groups, whereas plasma ALT activity was significantly higher in the HF group than in the CT group at 8 and 12 weeks.

Hamp is a gene of the iron regulatory hormone, hepcidin[10]. Ho-1 has been identified as an oxidative stress marker[17], and Il-6 and Cd45 are used as markers of inflammation[18, 19]. In some rats in each group, *Ho-1* and *Il-6* transcripts were not detected by qRT-PCR analyses. Therefore, relative expression levels were calculated as 40 of the Ct value for the transcript. No significant differences were observed in the hepatic expression of *Hamp*, *Il-6*, or *Cd45* between the dietary groups and these also remained unchanged in each dietary group (Table 6). The expression of *Ho-1* in the liver was slightly higher ($P = 0.090$) in the HF group than in the CT group at 8 weeks only.

Iron concentrations in plasma were not affected by the dietary treatment or experimental period (Table 7). Hepatic iron concentrations based on wet weights and protein concentrations were not affected by the dietary treatment.

Hepatocyte ballooning, a typical change observed in hepatocytes with NAFLD[20], was observed in the HF group at each period (Fig. 1). However, it may have been immature at 4 weeks. Many large and small vesicles were observed in the livers of the HF group at 8 and 12 weeks. On the other hand, this abnormal histology was not observed in the livers of the CT group throughout the experimental period. Inflammatory cell infiltration was not detected in the livers of either group throughout the experimental period.

Discussion

The HF diet decreased feed intake and increased feed

Table 3 Effects of a high-fat diet and feeding period on feed intake, body weight gain, feed efficiency, and iron intake

	Feed intake (g/day)		Body weight gain (g/day)		Feed efficiency (g/g)		Iron intake (mg/day)	
	CT	HF	CT	HF	CT	HF	CT	HF
4 weeks	21.25 ± 0.69^{a}	15.96 ± 0.74^{c}	8.55 ± 0.36^{a}	8.79 ± 0.58^{a}	0.40 ± 0.01^{b}	0.55 ± 0.01^{a}	0.85 ± 0.03^{a}	0.64 ± 0.03^{b}
8 weeks	21.40 ± 0.65^{a}	17.60 ± 0.38^{bc}	6.50 ± 0.24^{b}	7.18 ± 0.18^{b}	0.19 ± 0.00^{f}	0.27 ± 0.00^{d}	0.86 ± 0.03^{a}	0.70 ± 0.02^{b}
12 weeks	20.14 ± 0.58^{ab}	17.66 ± 0.62^{bc}	4.76 ± 0.16^{c}	5.85 ± 0.18^{bc}	0.24 ± 0.00^{e}	0.33 ± 0.01^{c}	0.81 ± 0.02^{a}	0.70 ± 0.02^{b}
ANOVA Effect (P)								
Diet	< 0.001		< 0.05		< 0.001		< 0.001	
Feeding period	NS		< 0.001		< 0.001		NS	
Diet × Feeding period	NS		NS		< 0.001		NS	

Values are the mean ± SEM (n = 6). Mean values with different superscript letters were significantly different (P < 0.05).
Feed efficiency = Body weight gain / Feed intake.
Iron intake = Iron concentration in the diet × Feed intake.

Table 4 Effects of a high-fat diet and feeding period on energy intake, liver weight, the adiposity index, and liver TG

	Energy intake (kcal/day)		Liver weight (g)		Adiposity index		Liver TG (mg/g liver)	
	CT	HF	CT	HF	CT	HF	CT	HF
4 weeks	79.27 ± 2.57^{b}	81.25 ± 3.76^{ab}	11.36 ± 0.53^{b}	12.53 ± 0.79^{b}	5.15 ± 0.25^{c}	6.80 ± 0.40^{bc}	14.07 ± 1.84^{b}	80.84 ± 8.74^{a}
8 weeks	81.31 ± 2.46^{ab}	91.28 ± 1.98^{a}	13.44 ± 0.68^{b}	18.56 ± 1.96^{a}	7.95 ± 0.33^{b}	8.97 ± 0.53^{ab}	19.26 ± 4.10^{b}	81.33 ± 10.48^{a}
12 weeks	76.98 ± 2.23^{b}	92.15 ± 3.24^{a}	14.33 ± 0.89^{ab}	18.68 ± 1.29^{a}	7.33 ± 0.64^{bc}	10.44 ± 0.87^{a}	21.93 ± 3.45^{b}	78.35 ± 9.45^{a}
ANOVA Effect (P)								
Diet	< 0.001		< 0.001		< 0.001		< 0.001	
Feeding period	NS		< 0.001		< 0.001		NS	
Diet × Feeding period	NS		NS		NS		NS	

Values are the mean ± SEM (n = 6). Mean values with different superscript letters were significantly different (P < 0.05).
Energy intake = Energy concentration of the control or high-fat diet × Feed intake.
Adiposity index = the sum of adipose tissue mass (inguinal, epididymal, mesenteric, and perinephric fat) / body weight × 100.
TG, triacylglycerol.

Table 5 Effects of a high-fat diet and feeding period on liver TBARS and plasma ALT and AST activities

	Liver TBARS (nmol/mg protein)		ALT IU/L		AST IU/L	
	CT	HF	CT	HF	CT	HF
4 weeks	21.71 ± 2.26^{c}	70.42 ± 16.10^{ab}	1.09 ± 0.03^{b}	1.11 ± 0.05^{b}	1.80 ± 0.02	1.89 ± 0.03
8 weeks	28.62 ± 2.41^{bc}	78.94 ± 13.78^{a}	0.93 ± 0.09^{b}	1.59 ± 0.14^{a}	1.78 ± 0.02	2.08 ± 0.12
12 weeks	30.15 ± 6.09^{bc}	58.46 ± 15.82^{abc}	1.00 ± 0.07^{b}	1.64 ± 0.19^{a}	1.82 ± 0.02	2.08 ± 0.14
ANOVA Effect (P)						
Diet	< 0.001		< 0.0001		< 0.01	
Feeding period	NS		NS		NS	
Diet × Feeding period	NS		< 0.01		NS	

Values are the mean ± SEM (n = 6). Mean values with different superscript letters were significantly different (P < 0.05).
Data on AST and ALT were log-transformed to provide an approximation of a normal distribution before analyses.
TBARS, thiobarbituric acid reactive substances; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

efficiency, but did not affect body weight gain. Rats are known to consume food to satisfy their energy requirements; energy intake was previously shown to remain constant in rats even if dietary fat content ranged between 1 and 30%[21]. Similar results were observed in the present study; the HF diet decreased feed intake, whereas energy intake did not change or slightly increased.

The HF diet increased hepatic TG concentrations at 4 weeks, and these levels remained stable until the end of the experiment. Histological observations revealed hepatocyte ballooning in the livers of the HF group at each period, but this appeared to be immature at 4 weeks. Many large and small vesicles were detected in the livers of the HF group at 8 and 12 weeks, but not at 4 weeks. These results clearly demonstrated that the HF diet induced fatty liver within 4 weeks. On the other hand, the liver histologically changed thereafter. The adiposity index was increased in the HF group at 12 weeks, indicating that HF rats became obese. These results suggested that the HF diet induced fatty liver prior to the induction of obesity in rats.

TBARS and Ho-1 are well-known markers of oxidative stress[17]. The HF diet increased TBARS concentrations in the liver at 4 and 8 weeks, but this increase disappeared at 12 weeks. The hepatic expression of *Ho-1* was slightly increased in the HF group at 8 weeks only. These results demonstrated that the HF diet

Table 6 Effects of a high-fat diet on hepatic mRNA expression at different feeding periods

	4 weeks		8 weeks		12 weeks	
	CT	HF	CT	HF	CT	HF
Hamp	1.00 ± 0.90	0.60 ± 0.39	1.00 ± 0.71	0.70 ± 0.34	1.00 ± 0.44	0.37 ± 0.14
Ho-1	1.00 ± 0.83	0.85 ± 0.48	1.00 ± 0.89	6.47 ± 3.32	1.00 ± 0.64	0.53 ± 0.25
Il-6	1.00 ± 0.96	0.03 ± 0.03	1.00 ± 0.43	1.08 ± 0.58	1.00 ± 1.00	2.08 ± 1.19
Cd45	1.00 ± 0.39	0.94 ± 0.56	1.00 ± 0.37	1.71 ± 0.91	1.00 ± 0.31	0.95 ± 0.22

Values are the mean ± SEM (n = 6). Data on mRNA were log-transformed to provide an approximation of a normal distribution before analyses.
The expression of mRNA was expressed as a relative value to the respective control group. Differences between the control group and high-fat group were evaluated by a *t*-test. In some rats, *Ho-1* and *Il-6* transcripts were not be detected by qRT-PCR analyses; therefore, the relative expression level was calculated as 40 of the Ct value for the transcript.
Hamp, Hepcidin gene; *Ho-1*, heme oxygenase-1; *Il-6*, interleukin-6; *Cd45*, cluster of differentiation 45.

Table 7 Effects of a high-fat diet and feeding period on iron concentrations

	Plasma iron (μg/dL)		Liver iron (μg/g)		Liver iron (μg/mg protein)	
	CT	HF	CT	HF	CT	HF
4 weeks	73.58 ± 5.98	97.50 ± 16.76	82.14 ± 4.55	92.78 ± 6.87	5.48 ± 0.32	7.17 ± 0.82
8 weeks	71.86 ± 5.06	82.89 ± 1.92	114.18 ± 7.54	98.11 ± 9.17	7.27 ± 0.56	7.13 ± 0.54
12 weeks	81.42 ± 8.33	84.28 ± 4.95	111.38 ± 10.12	113.22 ± 8.50	6.58 ± 0.54	7.57 ± 0.46
ANOVA Effect (P)						
Diet	NS		NS		NS	
Feeding period	NS		< 0.05		NS	
Diet × Feeding period	NS		NS		NS	

Values are the mean ± SEM (n = 6). Mean values with different superscript letters were significantly different (P < 0.05).

induced oxidative stress, but only temporarily. It currently remains unclear why oxidative stress disappeared with the long-term consumption of the HF diet.

Inflammation is a major characteristic of NASH[1]. No significant differences were observed in the hepatic expression of *Il-6* or *Cd45* between the HF and CT groups during the experimental period. Further inflammation was not histologically observed, even in the HF group, during the experimental period. On the other hand, ALT, but not AST activity in plasma was higher in the HF group than in the CT group at 8 and 12 weeks, suggesting that hepatic lesions were induced by the HF diet. Therefore, slight inflammation may have been induced by the HF diet that was not detected by gene expression or histological analyses.

Bowering et al.[5] previously reported that iron concentrations in the rat liver were increased by feeding a HF diet high in lard for 2 weeks following a 3-week iron depletion period. Boesch-Saadatmandi et al.[6] found that the consumption of a HF diet high in beef tallow for 4 weeks increased hepatic iron concentrations when dietary zinc concentrations were marginally low. On the other hand, the present results showed that the HF diet high in lard did not affect hepatic iron concentrations during the experimental period. The reason for this inconsistency currently remains unknown; however, the response of hepatic iron concentrations to HF diets may be induced by a disturbance in iron and zinc metabolism, as reported by Bowering et al.[5] and Boesch-Saadatmandi et al.[6] We previously demonstrated that a high-lard and high-sucrose diet increased the expression of hepcidin in the liver by inducing inflammation, which then reduced iron concentrations[11]. The HF diet may have induced inflammation that was insufficient to enhance the expression of hepcidin in the present experiment. The HF diet did not affect the expression of hepcidin and, thus, did not decrease hepatic iron concentrations.

It is important to note that animals had free access to the diets in all cited studies using HF diets. Since a decrease in feed intake was observed in rats given HF diets, the intakes of iron and other nutrients were less in animals fed these diets than in their respective controls. Therefore, the effects of a HF diet include reduced intakes of iron and other nutrients, which may have, in turn, affected hepatic iron concentrations.

NASH patients with mild to moderate iron deposition in the liver and high serum ferritin levels are now regarded as having dysmetabolic iron overload syndrome (DIOS)[22]. An increase in hepatic iron levels was previously reported in mice fed a diet high in corn oil for 5 weeks[23] and in rats given a diet high in coconut fat for 8 weeks[24] or 12 weeks[25], and these animals are regarded as models of DIOS. Therefore, the type of fat may affect the response of hepatic iron concentrations to the consumption of a HF diet. Further studies are

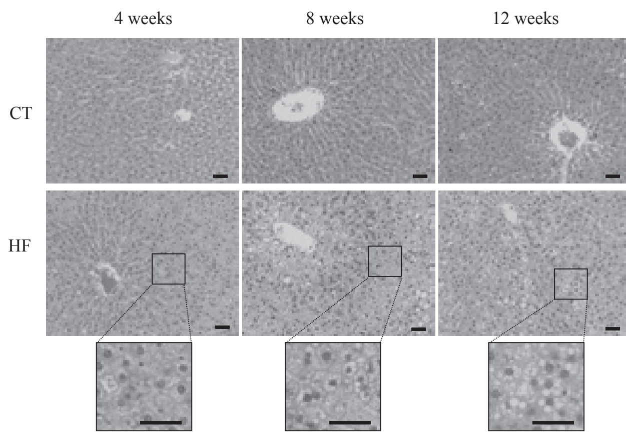

Fig. 1 Representative histology of the liver of rats fed control (CT) and high-fat (HF) diets (hematoxylin and eosin staining).
Ballooned hepatocytes are shown in enlarged figures. Scale bars, 40 μm.

needed in order to clarify the effects of different types of fat on hepatic iron concentrations.

References

1) Armutcu F, Coskun O, Gürel A, Kanter M, Can M, Ucar F, Unalacak M (2005) Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. Clin Biochem 38: 540-547.
2) Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114: 842-845.
3) George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW (1998) Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 114: 311-318.
4) Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K, Nakashima T, Okanoue T (2006) Effect of iron reduction by phlebotomy in Japanese patients with nonalcoholic steatohepatitis: A pilot study. Hepatol Res 36: 315-321.
5) Bowering J, Masch GA, Lewis AR (1977) Enhancement of iron absorption in iron depleted rats by increasing dietary fat. J Nutr 107: 1687-1693.
6) Boesch-Saadatmandi C, Most E, Weigand E (2007) Influence of dietary fat and zinc supplementation on the iron utilization in growing rats. Ann Nutr Metab 51: 395-401.
7) Micozzi MS, Albanes D, Stevens RG (1989) Relation of body size and composition to clinical biochemical and hematologic indices in US men and women. Am J Clin Nutr 50: 1276-1281.
8) Bertinato J, Aroche C, Plouffe LJ, Lee M, Murtaza Z, Kenney L, Lavergne C, Aziz A (2013) Diet-induced obese rats have higher iron requirements and are more vulnerable to iron deficiency. Eur J Nutr 53: 885-895.
9) Chung J, Kim MS, Han SN (2011) Diet-induced obesity leads to decreased hepatic iron storage in mice. Nutr Res 31: 915-921.
10) Ganz T, Nemeth E (2012) Hepcidin and iron homeostasis. Biochim Biophys Acta 1823: 1434-1443.
11) Takai K, Funaba M, Matsui T (2015) Steatohepatitis is developed by a diet high in fat, sucrose, and cholesterol without increasing iron concentration in rat liver. Biol Trace Elem Res (in press).
12) Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127: 838-841.
13) Atwater WO, Rosa EB (1899) A new respiration calorimeter and experiments on the conservation of energy in the human body. I. Phys Rev Ser I 9: 129-163.
14) Taylor BA, Phillips SJ (1996) Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics 34: 389-398.
15) Takemoto S, Yamamoto A, Tomonaga S, Funaba M, Matsui T (2013) Magnesium deficiency induces the emergence of mast cells in the liver of rats. J Nutr Sci Vitaminol 59: 560-563.
16) Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150: 76-85.
17) Gonzales S, Perez MJ, Perazzo JC, Tomaro ML (2006) Antioxidant role of heme oxygenase-1 in prehepatic portal hypertensive rats. World J Gastroenterol 12: 4149-4155.
18) Elsharkawy AM, Mann DA (2007) Nuclear factor-κB and the hepatic inflammation-fibrosis-cancer axis. Hepatology 46: 590-597.
19) van der Hoeven JA, Ploeg RJ, Postema F, Molema I, de Vos P, Girbes AR, van Suylichem PT, van Schilfgaarde R, Ter Horst GJ (1999) Induction of organ dysfunction and up-regulation of inflammatory markers in the liver and kidneys of hypotensive brain dead rats: a model to study marginal organ donors. Transplantation 68: 1884-1890.
20) Yeh MM, Brunt EM (2007) Pathology of nonalcoholic fatty liver disease. Am J Clin Pathol 128: 837-847.

21) Yoshida A, Harper AE, Elvehjem CA (1958) Effect of dietary level of fat and type of carbohydrate on growth and food intake. J Nutr 66: 217-228.
22) Dongiovanni P, Fracanzani AL, Fargion S, Valenti L (2011) Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. J Hepatol 55: 920-932.
23) Ahmed U, Oates PS (2013) Dietary fat level affects tissue iron levels but not the iron regulatory gene HAMP in rats. Nutr Res 33: 126-135.
24) Meli R, Mattace Raso G, Irace C, Simeoli R, Di Pascale A, Paciello O, Pagano TB, Calignano A, Colonna A, Santamaria R (2013) High fat diet induces liver steatosis and early dysregulation of iron metabolism in rats. PLoS One 8: e66570.
25) Dongiovanni P, Lanti C, Gatti S, Rametta R, Recalcati S, Maggioni M, Fracanzani AL, Riso P, Cairo G, Fargion S, Valenti L (2015) High fat diet subverts hepatocellular iron uptake determining dysmetabolic iron overload. PLoS One 10: e0116855.