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Genetic Variation of Basal Iron Status, Ferritin and Iron Regulatory Protein in Mice: Potential for Modulation of Oxidative Stress

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ABSTRACT. Toxic and carcinogenic free radical processes induced by drugs and other chemicals are probably modulated by the participation of available iron. To see whether endogenous iron was genetically variable in normal mice, the common strains C57BL/10ScSn, C57BL/6J, BALB/c, DBA/2, and SWR were examined for major differences in their hepatic non-heme iron contents. Levels in SWR mice were 3- to 5-fold higher than in the two C57BL strains, with intermediate levels in DBA/2 and BALB/c mice. Concentrations in kidney, lung, and especially spleen of SWR mice were also greater than those in C57BL mice. Non-denaturing PAGE of hepatic ferritin from all strains showed a major holoferritin band at approximately 600 kDa, with SWR mice having >3-fold higher levels than C57BL strains. SDS PAGE showed a band of 22 kDa, mainly representing L-ferritin subunits. A trace of a subunit at 18 kDa was also detected in ferritin from SWR mice. The 18 kDa subunit and a 500 kDa holoferritin from which it originates were observed in all strains after parenteral iron overload, and there was no major variation in ferritin patterns. Although iron uptake studies showed no evidence for differential duodenal absorption between strains to explain the variation in basal iron levels, acquisition of absorbed iron by the liver was significantly higher in SWR mice than C57BL/6J. As with iron and ferritin contents, total iron regulatory protein (IRP-1) binding capacity for mRNA iron responsive element (IRE) and actual IRE/IRP binding in the liver were significantly greater in SWR than C57BL/6J mice. Cytosolic aconitase activity, representing unbound IRP-1, tended to be lower in the former strain. SWR mice were more susceptible than C57BL/10ScSn mice to the toxic action of diquat, which is thought to involve iron catalysis. If extrapolated to humans, the findings could suggest that some people might have the propensity for greater basal hepatic iron stores than others, which might make them more susceptible to iron-catalysed toxicity caused by oxidants. BIOCHEM PHARMACOL **59**;2:115–122, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. iron; ferritin; liver; mice; genetic variation; diquat

Fundamental processes such as DNA synthesis, energy production, oxygen transport, nitric oxide synthesis and action, and drug metabolism are dependent on iron [1, 2]. On the other hand, it is well recognised that 'free' iron may catalyse a variety of radical reactions leading to oxidative stress [2, 3]. To minimise the pool of such potentially damaging iron, the absorption and metabolism of iron in cells are rigorously controlled by the synthesis of iron-binding proteins such as transferrin and ferritin [4]. Despite the tight control mechanisms in normal people, many pathological processes are thought to be associated with uncontrolled pools of 'free' iron acting either as a proliferative agent or as a catalyst of free radical oxidative damage. In prospective epidemiological studies, increased risks of heart disease and cancer are associated with moderately elevated levels of iron storage [5, 6]. In addition, there is considerable evidence to support the proposals that the mechanism of action of many toxic and carcinogenic drugs, herbicides, and other chemicals such as diquat, adriamycin, and asbestos are associated with free radical mechanisms catalysed by iron [2]. Chemical-induced colorectal, renal, hepatic, and mammary cancers are enhanced by increased iron status [7–10]. Thus, in some circumstances iron levels and availability may be crucial factors in free radical mechanisms of toxicity and cancer. Human genetic diseases causing massive iron accumulation and pathology are well studied (e.g. idiopathic haemochromatosis) and may even be widely carried as a result of the mutation of the *HFE* gene [11, 12]. It is also possible that other genetic variations in aspects of iron metabolism and storage exist which have yet to be found. These would not need to be detrimental or even apparent in normal life, but might make individuals more or less susceptible to pathological or chemical processes that involve iron through generation of oxygen radicals or by stimulation of proliferation.

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In mice, a gene defect of transferrin production has been observed, leading to hypotransferrinemia and hepatic siderosis [13, 14]. Mild to moderate siderosis has also been observed in mouse models of thalassemias [15, 16] and in a β2-microglobulin knockout mouse [17]. Recently, a mouse line null for *hfe* has been constructed in which accumulation of hepatic iron occurs [18]. In studying some strains of inbred mice, Leboeuf *et al.* found that hepatic and serum iron levels and transferrin percent saturation varied up to 2-fold between females of strains examined, but these two variables were not interdependent [19]. We and others have reported that untreated male SWR mice develop uroporphyria following chronic consumption of 5-aminolaevulinic acid, a precursor of heme synthesis. The DBA/2 and C57BL/6J strains were more resistant [20, 21]. The mechanism of this porphyria is thought to involve an iron-mediated oxidative process with resulting accumulation of uroporphyrin, the product of uroporphyrinogen oxidation [22]. In contrast, SWR mice were much more resistant than C57BL/10ScSn mice to hepatic protoporphyria caused by the orally active iron chelator 1,2-diethyl-3-hydroxypyridin-4-one (CP94), which was developed for clinical use in both iron overload and non-overload situations [23]. In this case, the mechanism of toxicity in the C57BL strain seemed to be the consequence of depletion to critical levels of hepatic iron available for heme synthesis, with the resulting accumulation of protoporphyrin. These findings were consistent with the preliminary observation that hepatic iron concentrations in SWR mice were significantly higher than in the C57BL/10ScSn strain. In this work, we have investigated this observation in more detail.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Sigma-Aldrich Co. except for diquat dibromide, which was purchased from Greyhound Chemicals.

Animals and Treatments

The five strains of mice were bred under identical conditions at the MRC Toxicology Unit (1991–1993) and fed RM3 diet (SDS, U.K.) or were supplied by Harlan Ltd., the National Institute of Medical Research (London), or Jackson Laboratories. Unless otherwise indicated mice were male and 7–10 weeks of age. Mice received iron–dextran (600 mg Fe/kg) by i.p. injection. Diquat dibromide was administered in saline by a single i.p. injection (20 mg/kg). Mice were killed by cervical dislocation or by CO_2 asphyxiation. Blood was obtained by cardiac puncture and ALT* activity measured with a Sigma kit. Liver fixed in 10% neutral buffered formalin was stained for iron by the Perls' reaction.

* *Abbreviations:* ALT, alanine aminotransferase; IRE, iron responsive element; and IRP, iron regulatory protein.

Non-heme Iron Levels and Extraction of Ferritin

Tissue levels of non-heme iron were determined by adaptation of the method of Bothwell [24]. To extract ferritin, control and iron-treated livers were homogenised by the methods described in Massover [25] and Dean *et al.* [26]. Livers were homogenised in water (1:9 w/v) and centrifuged at 3000 *g* for 30 min. The supernatant was heated at 70° for 10 min, cooled, recentrifuged, and the supernatant acidified to pH 4.8 with 0.1 M acetic acid for 1 hr at 40°. The insoluble material was removed and the pH readjusted to 6.5. The pellet from 50% NH_4SO_4 precipitation of this preparation was redissolved in water and either quantitated by immunoblotting or further purified through Sephadex G150 prior to PAGE.

Electrophoresis and Immunodetection of Ferritins

Polyclonal sera against ferritin were raised in guinea-pigs using hepatic ferritin isolated from iron-loaded C57BL/10ScSn mice. Both the major (600 kDa) and minor (500 kDa) holoferritins after non-denaturing PAGE were cut out of the gels, homogenised, and used directly for injection combined with Freund's adjuvant. Non-denaturing PAGE and SDS-PAGE were carried out using 6% and 15% gels, respectively, as described [25, 26] and stained with Coomassie blue. After PAGE, samples were transferred to nitrocellulose by semi-dry blotting. Ferritin bound to nitrocellulose was incubated with antiferritin antibody and then with goat anti-guinea-pig immunoglobulin G linked to alkaline phosphatase. Immunoreactivity was visualised after incubation with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium. Quantitation was by densitometry against purified 600 kDa ferritin. Antibodies prepared against the major and minor holoferritins gave similar results, and the findings reported are those using the antibody to the former. Separate antibodies against H- and L-ferritins can be prepared, but the polyclonal antibodies prepared here against the holoferritins and which detected the subunits on SDS-PAGE probably detected both ferritin forms, although formed against preparations from iron-loaded liver, and may have preferentially detected the L-chain more strongly. Two bands were observed with mouse spleen preparations, but the relative antigenicities are not known. Ferritin in the diquat experiment was determined by direct application of extract (2–5 μL) to nitrocellulose without PAGE.

Iron Metabolism

Uptake of ^{59}Fe from tied-off duodenol segments of anaesthetised mice was performed as described previously, using a mixture of $^{59}FeCl_3$ in unlabelled 250 μM ferric nitrilotriacetate [27, 28]. Duodenal radioactivity was a measure of mucosal retention, and radioactivity in the rest of the body gave a measure of transfer to the plasma and tissues. The sum represents total uptake. Liver uptake was measured

separately in these experiments to give a value of clearance from plasma. Non-heme iron for these studies was determined as described in Ref. 28. Estimation of cytosolic aconitase activity and total IRP measurements by a gel shift assay with labelled IRE oligonucleotide were determined by methods described previously [29–31]. A ^{32}P-labelled RNA probe specific to the IRE sequence was transcribed from 2 oligonucleotides, the first containing the IRE sequence in the 5′ to 3′ direction followed by the T7 promoter sequence in the 3′ to 5′ direction, and the second containing the T7 promoter sequence in the 5′ to 3′ direction. The sequences and further details can be found in the references [29–31]. The RNA binding assay was then carried out by incubating 0.1 μg cytosol (isolated from liver samples as previously reported [31]) with 10,000 cpm of ^{32}P-labelled RNA. To assess total IRP capacity, reactions were carried out in duplicate in the presence and absence of 2% 2-mercaptoethanol. Samples were then analysed on a non-denaturing acrylamide gel and quantitated by phosphorimaging [31], which has a much greater range than densitometry. Actual binding of IRE with IRP in the liver is expressed as a percent of total binding determined in the presence of 2-mercaptoethanol.

Statistics

Difference between groups was assessed by analysis of variance and Dunnett's test, and *t*-tests with significance set as $P < 0.05$.

RESULTS

Tissue Levels of Non-heme Iron

The observation of a difference between SWR and C57BL/10ScSn mice in hepatic iron levels was first confirmed in a study of five commonly used strains bred in the MRC Toxicology Unit from 1991–1993. Non-heme iron concentrations were measured in the livers of 7- to 10-week-old male mice that were bred at the same time, under identical environmental conditions, and fed the same diet. Levels in SWR were significantly higher than in the C57BL/10ScSn or C57BL/6J strains (Table 1). Values for the DBA/2 and BALB/c strains lay between the extremes observed for SWR and C57BL mice. Histological examination of liver with Perls' reaction for iron showed distinct, but patchy, periportal distribution in SWR mice. The significant difference in iron levels between the SWR strain and the C57BL strains was not a reflection of breeding source, since a similar difference was obtained when mice were investigated over a six-year period and obtained from other breeding establishments (Table 1). However, absolute values did vary and may have reflected local dietary sources and conditions. A similar difference between strains was also observed for females (results not shown). The difference occurred mainly after weaning (Table 1) and levels of iron reached a plateau after a few months. The C57BL/10ScSn and SWR strains were still significantly different at 8 months of age, but the variation was less marked (C57BL/10ScSn 82 ± 7, SWR 135 ± 17 μg/g tissue, N = 5). Analyses of other tissues from C57BL/10ScSn and SWR mice showed that the difference in non-heme iron levels was not restricted to the liver. Significant variations in levels of splenic iron as well as those of lung and kidney were also observed (Table 2). In a separate analysis, there was a small but significant difference in serum iron (C57BL/10ScSn 390 ± 35, SWR 545 ± 15 μg/dL, N = 3).

TABLE 1. Strain variation of hepatic non-heme iron contents in mice from various sources

Strain	Source	Non-heme iron (μg/g tissue) Adult	Weanling
C57BL/6J	a	25 ± 3 (6)	46 ± 8 (4)
C57BL/6J	d	45 ± 5 (5)	
C57BL/10ScSn	a	26 ± 2 (4)	37 ± 3 (7)
C57BL/10ScSn	b	34 ± 3 (4)	
BALB/c	a	80 ± 12(4)	54 ± 11(4)
DBA/2	a	111 ± 11(4)	47 ± 11(3)
DBA/2	b	103 ± 8 (4)	
SWR	a	179 ± 10(7)	78 ± 4 (4)
SWR	b	204 ± 9 (4)	
SWR	c	227 ± 18(6)	
SWR	d	142 ± 33(5)	

Mice were obtained from the following sources: a—bred on site in the same positive pressure isolator (1991–1993), b—Harlan Ltd., U.K., c—National Institute for Medical Research, U.K., d—Jackson Laboratories, U.S.A. All mice from any one source were bred and fed under identical conditions (1991–1997). Results are means ±SEM, with numbers per group in parentheses.

Comparison of Hepatic Ferritin

When hepatic ferritin preparations from the five strains of untreated mice were examined by non-denaturing PAGE, there was considerable variation between strains in levels of the holoferritin band at approximately 600 kDa (Fig. 1A). The SWR mice appeared to have the highest levels and the C57BL strains the lowest. Levels of ferritin, following non-denaturing PAGE, were quantitated by Western blotting using an antibody produced against the 600 kDa component. This confirmed that hepatic ferritin concen-

TABLE 2. Comparison of tissue iron levels in C57BL/10ScSn and SWR mice

Tissue	Non-heme iron (μg/g tissue) C57BL/10ScSn	SWR
Liver	44 ± 3	190 ± 6*
Spleen	109 ± 10	567 ± 41*
Kidney	39 ± 3	89 ± 9*
Lung	36 ± 3	61 ± 7*
Heart	80 ± 15	69 ± 10
Brain	14 ± 2	15 ± 1
Intestine	21 ± 4	27 ± 4
Muscle	35 ± 13	45 ± 13

Results are means ±SEM (N = 5). Mice were obtained from Harlan Ltd., U.K.
*Significantly different from C57BL/10ScSn mice.

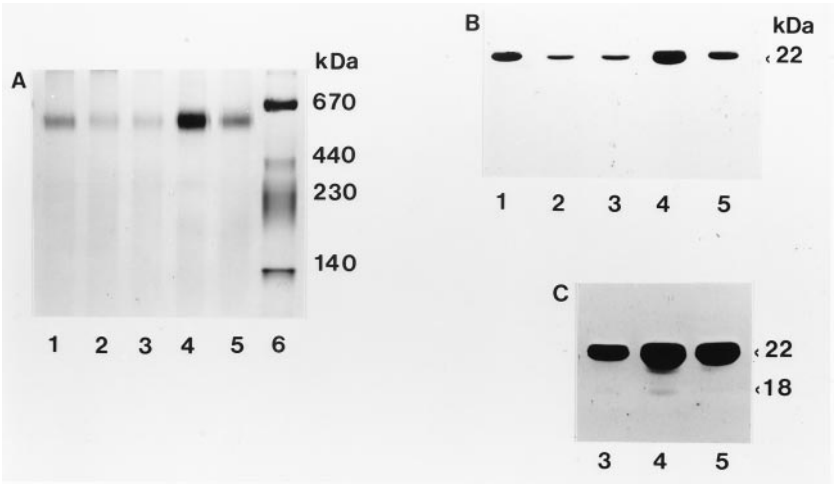

FIG. 1. PAGE of ferritin samples from the livers of untreated inbred mice. (A) Non-denaturing gel of holoferritins stained for protein with Coomassie Blue. lane 1 BALB/c, lane 2 C57BL/6J, lane 3 C57BL/10ScSn, lane 4 SWR, lane 5 DBA/2, and lane 6 molecular weight markers. (B) SDS gel of subunits immunoblotted with polyclonal antibody against 600 kDa ferritin prepared from mice with iron overload. Lanes as above. Densitometry of the 22 kDa bands gave relative values BALB/c 2.6, C57BL/6J 0.7, C57BL/10ScSn 1.0, SWR 4.7, and DBA/2 2.1. (C) High loading (4 × B) of C57BL/10ScSn, SWR, and BALB/c samples showing traces of bands at 20 kDa and 18 kDa, especially in ferritin from SWR mice. The amounts of ferritin fractions were proportional to equal amounts of fresh liver and for SWR mice was approximately 20 μg of protein.

trations were more than 3-fold higher in SWR mice compared to the C57BL strains (Fig. 2) and thus reflected the nonheme iron concentrations. Analysis of the samples by SDS-PAGE and immunoblotting using the same antibody gave similar results for the levels of 22 kDa subunits of ferritin (probably mainly the L-subunit), but this is difficult to resolve from the H-subunit [25, 26]), SWR having the highest and the C57BL strains the lowest (Fig. 1B). A trace level of the 18 kDa subunit reported in iron-loaded mouse liver and erythroleukaemic cells [25, 26, 32] was detected after heavy loading of gels, particularly in the preparation from SWR liver (Fig. 1C).

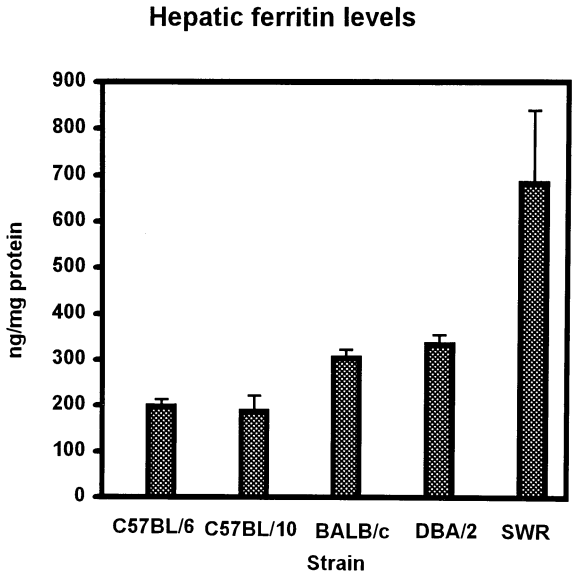

FIG. 2. Immunoquantification of total ferritin in the livers of control inbred mice. Results are means ±SEM of five animals per strain and are expressed as ng per mg of cytosolic protein. They were quantified by densitometry of non-denaturing gels Western blotted with an antibody against the 600 kDa component. Samples contained 50 μg of ferritin fraction. SWR was significantly different from other strains.

To determine whether there was any genetic variation in ferritin loading and patterns between the five strains after parenteral iron overload, mice were injected with iron–dextran and after one week the ferritin preparations obtained from the livers were examined by non-denaturing PAGE and stained with Coomassie Blue (Fig. 3A). All strains showed the major band for holoferritin with an apparent molecular weight of approximately 600 kDa. In addition, all strains of mice showed a significant, minor molecular weight lower band of approximately 500 kDa. Both bands were positive for iron by the Perls' reaction (results not shown). Bands for dimers were also observed. Examination of the ferritin preparations by SDS-PAGE gave a major band at about 22 kDa for unresolved ferritin subunits (Fig. 3B). In other gels, this band was partially resolved into a major lower molecular weight component (L-chain), with a minor higher molecular weight component possibly representing the H-chain subunit [26]. Other minor bands at approximately 20 kDa were also observed together with the component at 18 kDa. SDS electrophoresis of the purified major holoferritin gave the 22 kDa band, whereas the minor holoferritin band (500 kDa) from the native gel gave the 18 kDa subunit. In some circumstances, this latter band appeared to be resolving into two components. These findings are consistent with those of Massover [25] and Dean *et al.* [26]. Thus, no major differences in patterns between the strains of mice were observed. This demonstrated that after iron overload there appeared to be no major genetic variation in ferritin metabolism including the 18 kDa components, which are believed to be formed by cleavage of the L-chain subunits during lysosomal

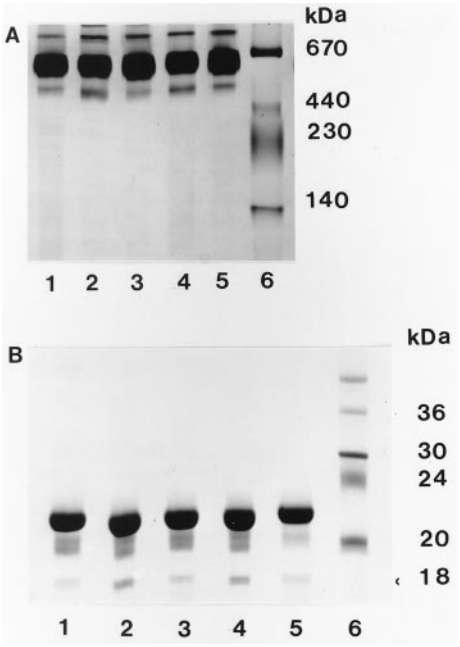

FIG. 3. PAGE of ferritin samples from the livers of inbred mice after iron overload. (A) Non-denaturing gel of holoferritin (50 μg of protein). (B) SDS gel showing subunits (20 μg of protein). Lane 1 BALB/c, lane 2 C57BL/6J, lane 3 C57BL/10ScSn, lane 4 SWR, lane 5 DBA/2, and lane 6 molecular weight markers. Samples were applied as equal amounts of total ferritin and stained for protein with Coomassie Blue.

processing of holoferritin [26]. However, slightly more 500 kDa holoferritin after non-denaturing PAGE and 18 kDa subunits after SDS-PAGE were observed with C57BL/6J and SWR strains than with the other mice.

Iron Absorption and Regulation

To determine whether the variation in basal iron loading in the liver between the strains could be explained by differential absorption from the intestine, mucosal uptake and transfer of iron were measured for C57BL/6J, DBA/2, and SWR mice (Table 3). The increased liver iron loading in the SWR strain did not appear to be attributable to increased iron absorption from the intestine. In fact, net iron absorption from the mucosa to the circulation was greatest with the DBA/2 strain. However, clearance of iron from the circulation to the liver was significantly greater in SWR than C57BL/6J mice and may have accounted in part for the strain variation in hepatic non-heme iron levels. The regulation of iron uptake and utilisation in the liver is partly regulated posttranscriptionally through IRP binding to IRE in ferritin and transferrin receptor mRNAs [4]. When IRP-1 binds four Fe atoms it has cytosolic aconitase activity, whereas in relatively iron-depleted cells it binds to IREs in mRNAs controlling expression of ferritin and transferrin receptor. Comparison between SWR and C57BL/6J mice showed no significant differences in hepatic cytosolic aconitase activity between the strains in males, but a lower level in female SWR mice. In contrast, there was a consistent significantly higher level of the total IRP binding capacity for IRE in SWR of both sexes and of actual IRE binding to IRP in male SWR mouse liver than C57BL/6J, as detected by a gel shift assay of the protein–oligonucleotide interaction (Fig. 4). The percentage binding of IRE versus total was slightly, but significantly, lower in male SWR mice.

Toxicity of Diquat

The toxicity of diquat is postulated to involve catalytically active Fe^{2+} released from ferritin. In rats, decreased ferritin-bound iron and a significant rise in chelatable iron after such treatment has been reported [33, 34]. When diquat (20 mg/kg) was administered to C57BL/10ScSn and SWR mice, there was a marked difference between the two strains in levels of hepatic ferritin-bound iron as well as non-heme iron and ferritin, although there were no changes associated with treatment in either strain. However, SWR mice showed elevated serum ALT levels as a measure of liver damage, and these animals were visibly ill whereas those of the C57BL/10ScSn strain were apparently unaffected (Table 4).

DISCUSSION

The strains of mice used in these studies are considered relatively normal and yet it seems that they have widely different levels of hepatic non-heme iron. Not only were

TABLE 3. Comparison of iron absorption between C57BL/6J, DBA/2 and SWR mice

	Iron absorption						
Strain	Mucosal retention (pmol/mg protein/10 min)	Mucosal transfer (pmol/mg protein/10 min)	Total mucosal uptake (pmol/mg protein/10 min)	Mucosal transfer (%)	Haemoglobin (g/dL)	Clearance of ^{59}Fe by liver (%)	Liver non-heme iron (μg/g)
C57BL/6J (7)	19.8 ± 1.6	8.6 ± 2.0	28.4 ± 3.3	28.8 ± 3.2	17.6 ± 0.4	8.6 ± 1.0	38 ± 4
DBA/2 (5)	27.9 ± 2.3*	19.2 ± 2.3*	47.0 ± 2.4*	40.7 ± 4.9*	17.1 ± 0.4	11.6 ± 2.3	50 ± 5
SWR (7)	27.3 ± 2.7*	10.8 ± 1.3†	38.1 ± 3.8	28.2 ± 1.4†	15.4 ± 0.4*†	13.3 ± 1.2*	130 ± 17*†

Determinations were carried out using 5 or 7 mice as shown in parentheses and are means ±SEM. Mucosal transfer (%) = mucosal transfer × 100/total mucosal uptake.
*Significantly different from C57BL/6J mice.
†Significantly different from DBA/2.

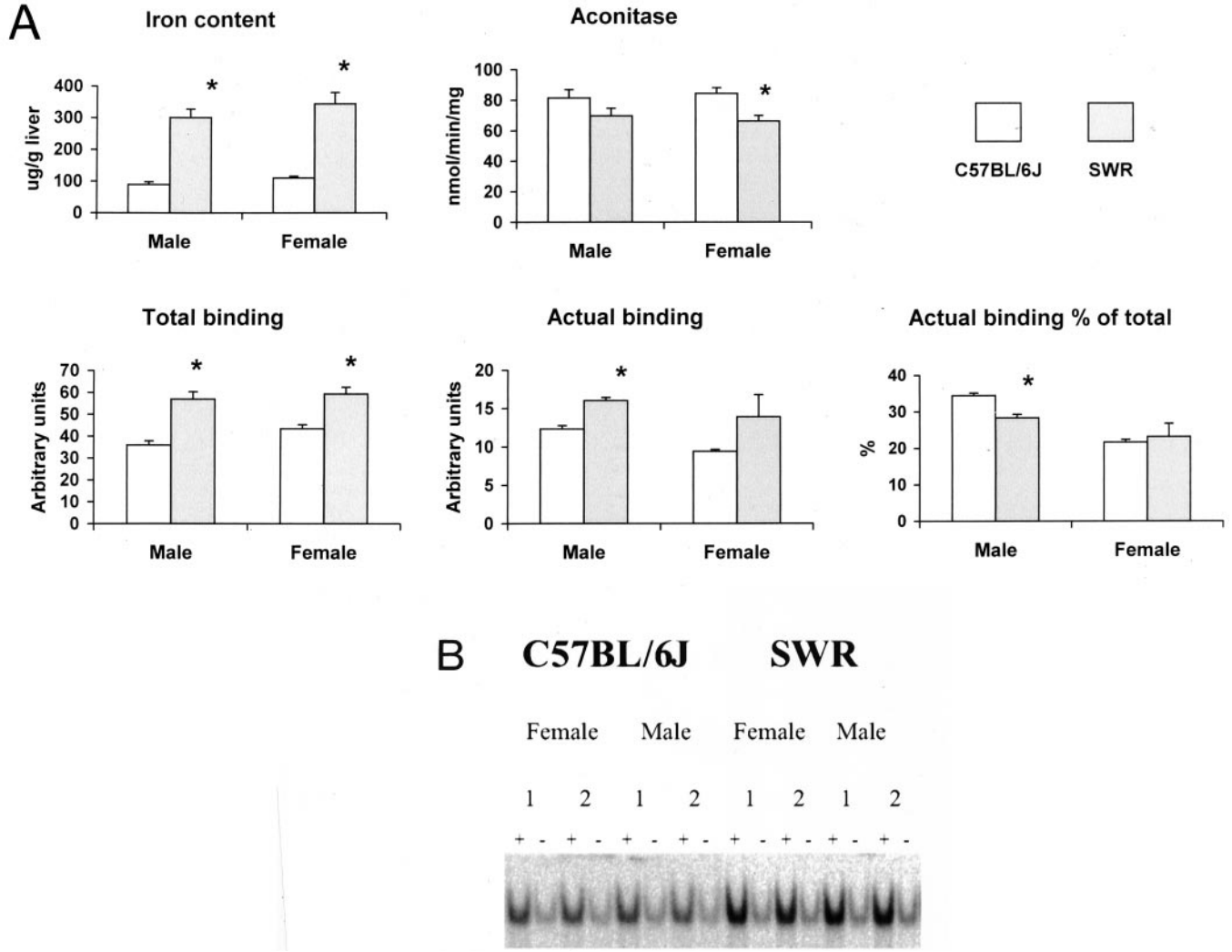

FIG. 4. (A) Comparison of iron content, cytosolic aconitase activity, total IRP binding capacity for IRE actual binding of IRE to IRP in cytosol and as a percent of total, from male and female C57BL/6J and SWR liver. Assays were as described in Materials and Methods. Values are means of 4 per group ±SEM. *Significantly different from C57BL/6J group. (B) Examples of gel shift assay for IRP/IRE interactions in cytosol of female and male C57BL/6J and SWR mouse liver. 1 and 2 represent duplicate liver samples. + and − represent with and without 2-mercaptoethanol and show total and actual IRP/IRE binding.

liver iron levels distinctly different between SWR and C57BL strains, but so too were those in the spleen and to a lesser degree in the kidney. We have no evidence that the greater levels of iron in SWR mice compared to C57BL/10ScSn or C57BL/6J mice are due to defects such as hypotransferrinemia, thalassemias, or polymorphism at the transferrin locus [14–16, 35]. Leboeuf *et al.* [19] examined females of a number of mouse strains and observed a 2-fold variation in hepatic iron levels as well as variations in serum transferrin saturation, although they noted that these were apparently not interdependent. Although these studies did not include SWR mice, it seems likely that a mechanism other than transferrin saturation must account for the strain variation. After iron overload by the oral route [19], as with our studies by a parenteral route, marked strain differences were not observed, suggesting that the variations in iron basal levels were not due to expression of polymorphisms in the *hfe* gene affecting duodenal iron uptake into the organs. In the *hfe* (−/−) null mouse compared with the wild-type (+/+), iron stores in the liver are elevated but not in the spleen [18], unlike the comparison here between C57BL/10ScSn and SWR mice. In addition, *hfe* +/− mice showed no significant elevation in iron levels. One approach to elucidate the reason for the strain difference would be by linkage analysis of an intercross between the C57BL/6J and SWR strains [36].

The differences between the strains in hepatic iron contents were reflected in the variation of ferritin levels when estimated either as the holoferritin or the ferritin subunits. Ferritin seems to act both as a source of iron to catalyse free radical reactions or as a sponge to mop up free iron [4, 37]. Mouse ferritin components have been the subject of a number of studies. Besides bands representing H- and L-subunits at about 22 kDa after SDS-PAGE, some workers have reported an 18 kDa component in siderotic liver [25, 26], differentiating erythroleukaemic cells [32,

TABLE 4. Influence of diquat on C57BL/10ScSn and SWR mice

Strain	Diquat	Serum ALT (IU/L)	Hepatic			Toxicity
			Non-heme iron (μg/g)	Ferritin (μg/g)	Ferritin iron (μg/g)	
C57BL/10ScSn	−	25.7 ± 1.1	46 ± 2	81 ± 11	12 ± 1	0/5
C57BL10/ScSn	+	20.8 ± 2.0	51 ± 2	98 ± 7	13 ± 1	0/5
SWR	−	20.1 ± 3.0	217 ± 9†	364 ± 163†	71 ± 3†	0/5
SWR	+	35.2 ± 5.1*	213 ± 12†	344 ± 13†	71 ± 4†	5/5‡

Mice from Harlan Ltd. (five per group) received diquat dibromide (20 mg/kg) i.p. in saline and were left for 48 hr.
*Alanine aminotransferase activity (ALT) was significantly different from the control group (N = 5).
†Significantly different from C57BL/10ScSn mice.
‡Mice showed gross signs of morbidity after 48 hr and were immediately culled.

38], and L cells (fibroblast-derived) [39]. We observed this band(s) in normal SWR liver as well in overload tissue. Beaumont *et al.* proposed that in erythroleukaemic cells a similar species occurred by posttranslational modification of H-ferritin subunits [38]. In contrast, Dean *et al.* showed that in siderotic liver a subunit of approximately 17 kDa was formed by posttranslational cleavage of L-chains in siderosomes [26]. Our findings are consistent with this latter proposal, but it is curious that only 600 kDa and 500 kDa holoferritins (excluding a dimer band) were observed, with no intermediate sizes. Additionally in our hands, on SDS-PAGE purified 500 kDa ferritin gave only an 18 kDa band that sometimes split into two components. If the 500 kDa ferritin is derived by cleavage of 600 kDa ferritin, it must occur in most 24 subunits, with no apparent intermediate stages [26].

Total IRP capacity and the actual IRE/IRP interaction also seemed to parallel the higher iron and ferritin status in SWR mice compared with the C57BL strains. In contrast, the degree of binding (% of total) and aconitase activity tended to be lower in the SWR strain. These findings are not necessarily what might be expected in an elevated iron scenario [4], but we are unable to provide an explanation. In studies of iron metabolism and IRP/IRE interactions, it is usually assumed that IRP levels remain constant, it being the degree of IRE binding that changes. In these analyses, we were probably not examining IRP-2, which does not have aconitase activity. However, in mouse liver the levels of IRP-2 relative to IRP-1 are very low (<10%), unlike in other tissues such as intestine [40]. It is interesting to note that IRP-1 is activated by oxidative stress *in vitro* [41], but *in vivo* oxidative stress following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity seemed to cause inactivation [31].

Variations in basal iron and ferritin levels could confer differing responses to some drugs and chemicals [2]. Hepatic injury in rats due to diquat is thought to be associated with release of toxic iron [2, 33, 34]. SWR mice were considerably more sensitive to diquat than C57BL/10ScSn mice, but we could not show that this was directly related to iron status or mobilisation. However, we did not attempt to estimate a 'free' iron pool that might be involved in toxicity and might not have been reflected in a detectable change in ferritin iron status. The higher hepatic iron status of SWR compared with C57BL/6J mice may also be a contributing factor to their susceptibility to 5-aminolaevulinate-induced uroporphyria [21]. In contrast, the higher iron of SWR compared with C57BL strains seems to protect them from the protoporphyrogenic actions of the oral iron chelator CP94 [23], and elevated ferritin content might act as a protective mechanism in resistance to oxidants or beryllium [4, 42]. Iron metabolism is believed to play an important role in the complex biological chemistry of nitric oxide (NO). Besides interaction with hemoproteins such as guanylate synthase and cytochrome P-450, NO can modify IRP operation, inactivate mitochondrial aconitase, and induce release of iron from ferritin [43]. Hence, genetic variation in basal iron pools could have significant influence on NO-mediated mechanisms.

In summary, genetic variation in the metabolism and activation of drugs and chemicals is well established in pharmacology and toxicology. Modulation of oxidative stress by genetic variations in basal iron status or mobilisation could have marked implications.

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