Copper Deficiency Increases Cytochrome P450-Dependent 7-Ethoxyresorufin-O-deethylase Activity in Rat Small Intestine (43820)

W. THOMAS JOHNSON[1] AND THERESA B. SMITH

United States Department of Agriculture, Argricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202

Abstract. **Although impaired heme synthesis during copper deficiency may limit the production and function of hemoproteins, little is known about the effects of copper deficiency on the cytochromes P450, an important family of hemoproteins, in the small intestine. A series of experiments was conducted to examine the effects of copper deficiency on cytochrome P450 content, ethoxyresorufin-O-deethylase (EROD) activity, and NADPH-cytochrome P450 reductase activity in rat small intestine. Sixteen hours after a single oral administration of 5,6-benzoflavone (BF), an inducer of cytochromes P4501A1 and P4501A2, intestinal cytochrome P450 content was elevated as indicated by the CO-difference spectrum of the reduced cytochrome and by immunoblotting using anticytochrome P4501A1/1A2. However, cytochrome P450 content, measured by either method following BF induction, was not affected by copper deficiency. Thus, copper deficiency did not impair the availability of heme for cytochrome P450 synthesis in the small intestine. Even though copper deficiency did not affect intestinal cytochrome P450 content, EROD activity, which is a cytochrome P450-associated monooxygenase activity, was 9-fold higher in copper-deficient rats compared with controls following BF treatment. The finding that copper deficiency has no effect on intestinal cytochrome P450 content suggests that the increased EROD activity results from an effect of copper deficiency on the cytochrome P450 reductase component of the monooxygenase system. Measurement of cytochrome P450 reductase activity showed a 2-fold increase in the small intestines of copper-deficient rats compared with controls. It is possible, therefore, that increased intestinal cytochrome P450 reductase activity during copper deficiency increases EROD activity by facilitating the flow of electrons to cytochrome P450 during the redox cycle that cytochrome P450 undergoes during the O-deethylation of ethoxyresorufin.**

[P.S.E.B.M. 1994, Vol 207]

Severe copper deficiency results in the development of microcytic, hypochromic anemia that is characteristic of defective hemoglobin synthesis (1, 2). This defect has been ascribed to abnormal iron utilization by the mitochondria for heme synthesis (3, 4). Because the defect in mitochondrial heme synthesis may be of a general nature, copper deficiency, in addition to reducing hemoglobin synthesis, may also affect the production of other hemoproteins.

An important group of hemoproteins belongs to the cytochrome P450 family. The cytochromes P450 are situated primarily in the endoplasmic reticulum and are components of the monooxygenase system that catalyzes the biotransformation of a variety of metabolites and xenobiotics. In the monooxygenase systems, cytochrome P450 serves as the terminal oxidase and receives electrons from NADPH through the flavoprotein, NADPH-cytochrome P450 reductase. The variety of proteins in the cytochrome P450 family

[1] To whom requests for reprints should be addressed at USDA, ARS, GFHNRC, University of North Dakota, P.O. Box 9034, Grand Forks, ND 58202-9034.

Received April 8, 1994. [P.S.E.B.M. 1994, Vol 207]
Accepted July 18, 1994.

0037-9727/94/2073-0302$10.50/0

is responsible for the diversity in the reactions catalyzed by the monooxygenase system.

Although cytochromes P450 are hemoproteins, the effect of copper deficiency on their content in various organs has not received much attention. The few reported studies describe the effect of copper deficiency on hepatic cytochrome P450 and associated monooxygenase activities. Although these reports indicate that hepatic cytochrome P450 content is not affected by copper deficiency (5, 6), some hepatic cytochrome P450-dependent monooxygenase activities are altered in copper-deficient animals. Aminopyrine demethylase (7), aniline hydroxylase, and hexobarbital oxidase activities (8) decrease with copper deficiency, while benzo[a]pyrene hydroxylase (7) and ethoxycoumarin O-deethylase (6) activities increase. The results suggest that copper deficiency may not affect the total hepatic cytochrome P450 content, but may selectively influence monooxygenase activities by differentially affecting cytochrome P450 isoforms involved in catalyzing specific monooxygenase reactions.

If copper deficiency can influence cytochrome P450 content by impairing heme synthesis, the effects may not be readily observed in liver. Hepatic cytochrome P450 content was unaffected in rats that developed anemia as a result of consuming iron-deficient diets (9–11). Although it is apparent from the development of anemia that heme synthesis was impaired in the iron-deficient rats, hepatic cytochrome P450 was apparently resilient to reduced heme production. This resiliency may at least partially explain why copper deficiency has no appreciable effect on total hepatic cytochrome P450 content. While iron deficiency has little effect on hepatic cytochrome P450, this is not the case in the intestine. Dietary iron deprivation, through a failure in maintenance of the prosthetic heme moiety, reduces intestinal cytochrome P450 content (11–14). The results obtained from examining the effects of iron deficiency suggest that intestinal cytochrome P450 is more sensitive than hepatic cyotchrome P450 to impaired heme production. Thus, impaired heme synthesis during copper deficiency may affect cytochrome P450 content and associated monooxygenase activities to a greater extent in the intestine than in liver. However, the effects of copper deficiency on intestinal cytochrome P450 and monooxygenase activities have not been examined.

Intestinal cytochrome P450-dependent monooxygenase activities are affected by a variety of nutritive and non-nutritive dietary components (15). Indole-3-carbinol and flavone derivatives found in cruciferous vegetables are potent inducers of intestinal cytochrome P450. An oral dose of either indole-3-carbinol or 5,6-benzoflavone (BF) elevated mRNA for cytochrome P4501A1 in rat colon (16), and dietary supplementation with BF increased cytochrome P450 and monooxygenase activities in rat small intestine (14). However, if copper deficiency impairs intestinal heme production, cytochrome P450 content and associated monooxygenase activities may not reach optimal levels following induction. The present study, therefore, was aimed at determining the effects of copper deficiency on intestinal cytochrome P450 and the monooxygenase activity, 7-ethoxyresorufin-O-deethylase, following induction with BF.

Materials and Methods

Diets and Animals. Copper-deficient and control diets were composed as previously described (17, 18). Both diets contained, per kg, 940.0 g of a casein-based, copper- and iron-free basal diet (#TD84469; Teklad Test Diets, Madison, WI), 50.0 g of safflower oil (Teklad Test Diets), and 10.0 g of copper-iron mineral mix. Copper-deficient and control diets were prepared by using copper-iron mineral mixes containing no added copper and 3.5 g of copper per kg of mix, respectively. As assayed by flame atomic absorption, the copper-deficient diet contained 0.4 μg Cu/g and 45 μg Fe/g. The control diet contained 5.5 μg Cu/g and 44 μg Fe/g.

Male Sprague-Dawley rats (SASCO, Inc. Omaha, NE)[2] were shipped at age 21 days. Upon arrival, they were housed in individual hanging steel cages in a temperature- and humidity-controlled room with a 12:12-hr light:dark cycle. All animals were fed the control diet for five days after which time they were divided into two weight-matched groups. Rats in the two groups were then fed either copper-deficient or control diet and deionized water for 35 days.

After a 4-hr period during which food was removed from the cage, intestinal cytochrome P450 was induced by an oral dose (80 mg/kg body wt) of BF suspended in safflower oil. The rats were then given free access to their diets until they were sacrificed 16 hr after receiving BF. Control rats in which cytochrome P450 was not induced received an oral dose of safflower oil. The volume of safflower oil used for each rat was 0.9–1.2 ml.

Contaminating hemoglobin can interfere with the spectral assay of cytochrome P450. To prevent this interference, blood was removed by perfusing the whole animal. Following anesthesia with sodium pentobarbital, the trachea was exposed and cannulated. The cannula was then attached to a rodent ventilator and ventilation performed at 60 cycles/min, 10 cm H_2O positive end inspiratory pressure, and 3 cm H_2O pos-

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

itive end expiratory pressure. Following ventilation, the chest cavity was opened to expose the heart. To avoid clotting, 1000 units of heparin were injected into the right ventricle. For the purpose of analysis, a blood sample (1–3 ml) was then taken from the right ventricle. The rat was perfused with 1L of Krebs-Henseliet buffer (Sigma Chemical Co., St. Louis, MO) by insertion of a cannula into the aorta and opening the right atrial appendage. The animal was perfused until the perfusate issuing from the atrial appendage was visibly free of blood.

Preparation of Intestinal Microsomes. Following perfusion, a 40-cm segment of the small intestine was excised starting at the pylorus. The segment was flushed with ice-cold physiological saline followed by ice-cold 0.05 *M* Tris buffer (pH 7.8) containing 20% glycerol and 1.15% KCl and then cut into two parts 30 cm from the pyloric end. The 30-cm segment was split longitudinally and the mucosa removed by scraping with the edge of a glass microscope slide. The remaining 10-cm segment was saved for trace metal analysis.

Microsomes were prepared by using a modification of the method described by *Pasoe et al.* (13). The intestinal mucosa obtained by scraping the intestine was homogenized in 5 ml of 0.05 *M* Tris buffer (pH 7.8) containing 20% glycerol, 1.15% KCl, 1.4 mg trypsin inhibitor/ml, 3 units heparin/ml, and 0.04 mg phenylmethylsulfonyl fluoride/ml. Microsomes were then isolated from the homogenate by centrifugation (13) and suspended to a final volume of 1.0 ml in 0.05 *M* Tris buffer (pH 7.8) containing 20% glycerol and 1.15% KCl.

Analytical Methods. The intestinal cytochrome P450 content was determined using an extinction coefficient of 91 cm^{-1} mM^{-1} for the carbon monoxide (CO) difference spectrum of reduced microsomes (19), which were suspended at a concentration of 2 mg protein/ml. Microsomal protein was measured by using a commercially available kit (Sigma Chemical Co., St. Louis, MO) in which bicinchoninic acid serves as the chromogen (20). The protein standard was bovine serum albumin.

Samples of microsomes containing 75 μg of protein were subjected to SDS-PAGE (5%–16% gradient gel). The proteins were then transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp., Bedford, MA) by semidry electroblotting (Trans-Blot SD semidry transfer cell, Bio-Rad Laboratories, Hercules, CA). Protein transfer was verified by staining the polyacrylamide gels with Coomassie blue R250. No proteins with molecular weights of 40,000–80,000 were detectable with Commassie blue following transfer. The blots containing transferred protein were incubated for 2 hr at room temperature in TBS (10 m*M* Tris, 150 m*M* NaCl, pH 8.0) containing 10% nonfat dry milk. They then were incubated with rabbit anticytochrome P4501A1/1A2 (Oxford Biomedical Research, Inc., Oxford, MI) for 2 hr, washed three times for 5 min in TBS containing 0.1% Tween-20 and incubated for 1 hr with anti-IgG horseradish peroxidase conjugate. After incubation with anti-IgG, the blots were washed first for 15 min and then twice for 5 min in TBS containing 0.1% Tween-20, followed by two final washes for 5 min in TBS. The blots were developed by chemiluminescence (ECL Western Blotting Detection System; Amersham Corp., Arlington Heights, IL). Scanning densitometry (GS300 Scanning Densitometer; Hoefer Scientific Instruments, San Francisco, CA) was performed on the photographic negative obtained from the blots.

Microsomal 7-ethoxyresorufin-O-deethylase activity was measured by the fluorometric assay described by Burke and Mayer (21). NADPH-cytochrome P450 reductase activity of intestinal microsomes was assayed by measuring the reduction of cytochrome c (22).

An electronic cell counter (Coulter Counter model S-plus IV; Coulter Electronics, Hialeah, FL) was used to measure hemoglobin concentrations and hematocrits in the blood sample taken from the right ventricle (see above) prior to perfusion. Plasma from this blood sample was assayed for ceruloplasmin oxidase activity by using *o*-dianisidine as substrate (23, 24).

The left lobes of the livers were excised and lyophilized. The distal 10-cm segments of small intestine not used for microsomes were also lyophilized. Following lyophilization, the copper and iron content of the liver and intestinal segments were determined by flame atomic absorption spectroscopy as previously described (25).

Statistics. The data were analyzed by ANOVA, and mean comparisons were tested by Tukey's studentized range test (SAS/STAT Version 6; SAS Institute, Inc., Cary, NC).

Results

The data representing cytochrome P450 content, 7-ethoxyresorufin-O-deethylase (EROD), and cytochrome P450 reductase were collected from three experiments. All rats fed the copper-deficient diet in these experiments developed anemia and had reduced plasma ceruloplasmin oxidase activity and liver copper concentration (Table I). Anemia, reduced ceruloplasmin and low liver copper are characteristic signs of copper deficiency and indicate that rats consuming the copper-deficient diet were indeed copper deficient.

In separate experiments, the intestinal cytochrome P450 content was determined in two ways, either by measuring the hemoprotein from the CO difference spectrum of the reduced cytochrome or by immunoblotting with antibody specific for the cytochrome P4501A proteins (1A1 and 1A2). A basal

Table I. The Effect of Copper Deficiency on Hematocrit, Hemoglobin Concentration, Ceruloplasmin Amine Oxidase Activity, and Liver Copper Concentration

	Cu-deficient	Control
Hematocrit (fractional)	0.23 ± 0.05[a] (27)	0.44 ± 0.03 (24)
Hemoglobin (g/l)	75 ± 21[a] (27)	148 ± 12 (24)
Ceruloplasmin (units/l)[b]	0.9 ± 1.2[a] (27)	93 ± 26 (24)
Liver Cu (μg/g dry wt)	1.4 ± 0.3[a] (27)	12.6 ± 1.0 (24)

Note. Values are means ± SD for the number of samples shown in parentheses.
[a] Means for Cu-deficient rats are lower than corresponding means in control rats ($P < 0.05$, t test).
[b] A unit of ceruloplasmin activity is that amount which catalyzes the oxidation of 1 μmol *o*-dianisidine/min.

amount of cytochrome P450 was present in the intestines of rats that were not treated with BF, but P4501A proteins were not detectable (Table II). The basal amount of cytochrome P450 was not influenced by copper deficiency. Following a single oral administration of BF, intestinal cytochrome P450 content was increased and cytochrome P4501A proteins were elevated to detectable levels. The P4501A proteins were detected as a single band with a molecular weight of approximately 54,000. However, the amounts of induced cytochrome P450 and P4501A proteins were not significantly different between copper-deficient and control rats.

Microsomal EROD activity (Table III), was essentially absent from the intestines of untreated rats, but was present in the intestines of the copper-deficient and control rats that were treated with BF. Although

Table II. The Effect of Copper Deficiency on Total Cytochrome P-450 Hemoprotein and on Cytochrome P-4501A Protein

Group	CYT P-450 (pmol/mg protein)[a]	CYT P-4501A protein (peak height)[b]
CuDef	58 ± 20 (10)	N.D. (5)
CuDef + BF	177 ± 17 (5)	179 ± 45 (5)
Control	88 ± 35 (8)	N.D. (3)
Control + BF	203 ± 35 (5)	185 ± 41 (3)

Note. Each sample subjected to electrophoresis and blotting contained 75 μg of microsomal protein. All values are means ± SD for the number of samples shown in parentheses. In no instance were Cu-deficient different from control means ($P > 0.05$, t test). N.D. indicates nondetectable. The lower limit of detection was 0.2 μg of antigen.
[a] Determined from the CO difference spectrum of reduced cytochrome P-450.
[b] Peak heights are integrated optical densities determined by scanning densitometry of immunoblots developed with anti-P4501A1,2.

Table III. The Effect of Copper Deficiency and 5,6-Benzoflavone (BF) Treatment on 7-Ethoxyresorufin-O-deethylase (EROD) and NADPH-Cytochrome P450 Reductase Activities

Group	EROD (pmol resorufin formed/ [min · mg protein])	Reductase (nmol cyt c reduced/ [min · mg protein])
CuDef	6 ± 14 (5)	40 ± 7 (5)
CuDef + BF	390 ± 270[a] (7)	46 ± 4 (7)
Control	N.D. (5)	20 ± 3 (5)
Control + BF	42 ± 27[b] (5)	32 ± 5 (5)
ANOVA:	*P*	*P*
Cu	0.02	0.0001
BF	0.006	0.009
Cu × BF	0.02	0.21

Note. Values for enzyme activities are means ± SD for the number of samples shown in parentheses. N.D. indicates nondetectable.
[a] EROD activities with different superscripts are significantly different ($P < 0.05$, Tukey's test).

EROD activity was highly variable following BF treatment, the induced activity was significantly higher in copper-deficient rats than in controls. Cytochrome P450 reductase activity (Table III) was significantly higher in copper-deficient compared with control rats. Treatment with BF also caused a slight but significant increase in cytochrome P450 reductase activity in copper-deficient and control rats. The effect of BF treatment on cytochrome P450 reductase activity was independent of copper status. Copper deficiency did not affect the yield of microsomal protein. Microsomes (suspended in a final volume of 1 ml) prepared from the mucosa of 30-cm intestinal segments from copper-deficient and control rats yielded 18.3 ± 5.4 ($n = 27$) and 17.4 ± 5.8 ($n = 24$) mg protein/ml, respectively ($P > 0.05$, t test). Therefore, effects of copper deficiency on the intestinal content of cytochrome P450, and EROD, and cytochrome P450 reductase activities do not represent a general effect of copper deficiency on total microsomal protein content of the mucosa.

Copper deficiency caused a significant decrease in intestinal copper concentration, but slightly increased iron concentration (Table IV). Treatment with BF had no effect on either copper or iron concentrations.

Discussion

The diet containing <1 μg Cu/g successfully produced copper deficiency as indicated by the development of anemia, decreased ceruloplasmin activities, and low liver copper concentrations in the rats consuming it. Furthermore, the presence of anemia indicates that the severity of the copper deficiency was sufficient to impair the utilization of iron for heme synthesis.

In the current study, immunological analysis using anticytochrome P4501A1/A2 indicated that cyto-

Table IV. The Effect of Copper Deficiency and 5,6-Benzoflavone Treatment on Intestinal Copper and Iron Content

Group	Cu (μg/g dry intestine)	Fe (μg/g dry intestine)
CuDef	1.7 ± 0.3 (5)	74.0 ± 12.8 (5)
CuDef + BF	1.8 ± 0.3 (7)	65.1 ± 5.8 (7)
Control	5.7 ± 1.1 (5)	64.0 ± 6.2 (5)
Control + BF	5.1 ± 0.4 (6)	60.7 ± 4.4 (6)
ANOVA:	*P*	*P*
Cu	0.0001	0.04
BF	0.27	0.07
Cu × BF	0.15	0.40

Note. Values are means ± SD for the number of samples shown in parentheses.

chrome P4501A1 and/or 1A2 proteins were elevated in the microsomes from small intestines following a single oral dose of BF. This is consistent with previous studies showing that BF selectively induces cytochrome P4501A1 in rat colon and the human intestinal cell line, CACO-2 (16, 26). However, there was no demonstrable difference in cytochrome P4501A protein content between copper-deficient and control rats following induction with BF. Because P4501A2 is not present in rat intestine (27, 28), it is most likely that the isozyme induced was P4501A1. Thus, it may be concluded that the induction of intestinal cytochrome P4501A1 protein by BF is not impaired by copper deficiency.

The induction of cytochrome P450 increases heme utilization and thus increases the rate of heme synthesis. Although copper deficiency did not affect the induction of cytochrome P4501A proteins by BF, it is possible that impaired heme production inhibited the synthesis of holocytochrome P450. Because the CO-heme complex is the chromophore detected when the CO difference spectrum is used to quantify cytochrome P450, the CO difference spectra of microsomes from copper-deficient rats should show a reduction in cytochrome P450 content, if heme incorporation were impaired. However, results from the difference spectra showed that copper deficiency did not affect either the uninduced or BF-induced intestinal cytochrome P450 content. These observations, taken with the findings from the immunoblotting data, indicate that under the conditions of these experiments, copper deficiency did not limit heme incorporation into cytochrome P4501A1 to the extent that its synthesis was impaired in the small intestine following induction with BF.

Dietary iron, by affecting the availability of heme, is an important factor in the regulation of intestinal cytochrome P450 (12–14). Although dietary iron was not a limiting factor in the present study, it was thought that dietary copper could influence intestinal iron metabolism and limit heme availability to cytochrome P450. Although copper deficiency reduced the copper concentration in the distal segment of the small intestine, it had little effect on iron concentration. This finding suggests that copper deficiency does not have a major effect on intestinal iron content and that the maintenance of intestinal iron during copper deficiency may prevent iron availability from becoming a limiting factor in the synthesis of intestinal cytochrome P450.

Because copper deficiency had no effect on intestinal cytochrome P4501A1 induction by BF, it may be expected that copper deficiency also would have little effect on induced intestinal monooxygenase reactions catalyzed by cytochrome P4501A1. The O-deethylation of 7-ethoxyresorufin is a reaction attributed primarily to cytochrome P4501A1 (26). However, even though cytochrome P4501A1 content was not affected by copper deficiency, EROD activity of intestinal microsomes was several-fold higher in copper-deficient rats than in controls following induction with BF. This is consistent with previous reports regarding the effect of copper deficiency on hepatic monooxygenase activities. These reports show that copper deficiency affects a variety of hepatic monooxygenase activities (6–8), even though it has been shown that copper deficiency does not alter hepatic cytochrome P450 content (5, 6). Because BF specifically induces cytochrome P4501A1 in the intestine, it is unlikely that the difference in EROD activity between copper-deficient and control rats following induction is caused by variation in the cytochrome P450 isoform induced. A possible explanation for the higher EROD activity may be the effect of copper deficiency on NADPH-cytochrome P450 reductase. As shown in Figure 1, cytochrome P450 reductase is the component of the monooxygenase system that transfers electrons from NADPH to cytochrome P450 which is the terminal oxidase in the catalytic process. Our results show that intestinal cytochrome P450 reductase activity in copper-deficient rats is double that of control rats. Although reasons for

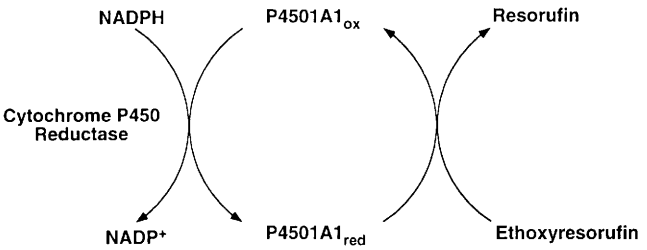

Figure 1. The relationship between cytochrome P450 reductase, cytochrome P4501A1, and the conversion of ethoxyresorufin to resorufin. Cytochrome P450 reductase transfers electrons from NADPH to the oxidized form of cytochrome P4501A1 ($P4501A1_{ox}$) forming the reduced form of the cytochrome ($P4501A1_{red}$). An electron then is transferred from $P4501A1_{red}$ to ethoxyresorufin causing O-deelkylation and the formation of resorufin.

an increase in reductase activity are not clear, the higher activity, by facilitating electron transfer during the oxidation-reduction cycle that cytochrome P450 undergoes during the O-deethylation of ethoxyresorufin, could promote higher intestinal EROD activity as observed in copper-deficient rats.

Interestingly, BF treatment resulted in somewhat higher intestinal cytochrome P450 reductase activity in both copper-deficient and control rats. The increase in reductase activity seems to be concomitant with the increase in cytochrome P450 content following BF treatment, and may reflect an increased need for reductase in order to maintain the proper stoichiometry with cytochrome P450.

The intention of the present study was to examine the hypothesis that copper deficiency, because of impaired heme synthesis, could affect intestinal cytochrome P450 content. Although the findings indicate the cytochrome P450 (most likely P4501A1) content was not influenced by copper deficiency, they did show that an increase in 7-ethoxyresorufin-O-deethylase activity occurred which may be attributed to increased cytochrome P450 reductase activity during copper deficiency. The change in reductase activity may indicate that copper deficiency can alter the structure/function relationship between the reductase and cytochrome which in turn can affect either electron transfer in the reductase-cytochrome complex or the reductase-cytochrome stoichiometry to produce an effect on monooxygenase activity. Although it is not clear why reductase activity is increased, the findings of the present study suggest that increased cytochrome P450 reductase activity could lead to increased cytochrome P450-mediated xenobiotic metabolism in the small intestine during copper deficiency. Whether or not this is harmful, depends on whether the xenobiotic involved is transformed into a hazardous or nonhazardous form by the intestinal monooxygenase system.

The authors thank Anne Thomas and David Corbine for their technical assistance, LuAnn Johnson for statistical analysis, and Marie Swenson for final preparation of the manuscript.

1. Hart EB, Steenbock H, Wadell J, Elvehjem CA. Iron in nutrition. J Biol Chem **77**:797–812, 1928.
2. Lee GR, Williams DM, Cartwright GE. Role of copper in iron metabolism and heme synthesis. In: Prasad AS, Oberleas D, Eds. Trace Elements in Human Health and Disease. New York: Academic Press, Vol **1**(Zinc and Copper):pp373–390, 1976.
3. Williams DM, Loukopoulos D, Lee GR, Cartwright GE. Role of copper in mitochondrial iron metabolism. Blood **48**:77–85, 1976.
4. Williams DM, Kennedy FS, Green BG. The effect of iron substrate on mitochondrial haem synthesis in copper deficiency. Br J Nutr **53**:131–136, 1985.
5. Williams DM, Burk RF, Jenkinson SG, Lawrence RA. Hepatic cytochrome P-450 and microsomal heme oxygenase in copper-deficient rats. J Nutr **111**:979–983, 1981.
6. Arce DS, Keen CL. Reversible and persistent consequences of copper deficiency in developing mice. Reproductive Toxicol **6**:211–221, 1992.
7. Hammermueller JD, Bray TM, Bettger WJ. Effect of zinc and copper deficiency on microsomal NADPH-dependent active oxygen generation in rat and liver. J Nutr **117**:894–901, 1987.
8. Moffitt AE Jr., Murphy SD. Effect of excess and deficient copper intake on rat liver microsomal enzyme activity. Biochem Pharmacol **22**:1463–1476, 1973.
9. Becking GC. Influence of dietary iron levels on hepatic drug metabolism *in vivo* and *in vitro* in the rat. Biochem Pharmacol **21**:1585–1593, 1972.
10. Becking GC. Hepatic drug metabolism in iron-, magnesium-, and potassium-deficient rats. Fed Proc **35**:2480–2485, 1976.
11. Dhur A, Galan P, Hercberg S. Effects of different degrees of iron deficiency on cytochrome P450 complex and pentose pathway dehydrogenases in the rat. J Nutr **119**:40–47, 1989.
12. Hoensch H, Woo CH, Raffin SB, Schmid R. Oxidative metabolism of foreign compounds in rat small intestine: Cellular localization and dependence on dietary iron. Gastroenterology **70**:1063–1070, 1976.
13. Pascoe GA, Sakai-Wong J, Soliven E, Correia MA. Regulation of intestinal cytochrome P-450 and heme by dietary nutrients. Critical role of selenium. Biochem Pharmacol **32**:3027–3035, 1983.
14. Pascoe GA, Correia MA. Structural and functional assembly of rat intestinal cytochrome P-450 isozymes. Effects of dietary iron and selenium. Biochem Pharmacol **34**:599–608, 1985.
15. Yang SC, Brady JF, Hong JY. Dietary effects on cytochromes P450, xenobiotic metabolism, and toxicity. FASEB J **6**:737–744, 1992.
16. Vang O, Jensen MB, Autrup H. Induction of cytochrome P450IAI in rat colon and liver by indole-3-carbinol and 5,6-bensoflavone. Carcinogenesis **11**:1259–1263, 1990.
17. Johnson WT, Kramer TR. Effect of copper deficiency on erythrocyte membrane proteins of rats. J Nutr **117**:1085–1090, 1987.
18. Kramer TR, Johnson WT, Briske-Anderson M. Influence of iron and the sex of rats on hematological, biochemical and immunological changes during copper deficiency. J Nutr **118**:214–221, 1988.
19. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem **239**:2370–2378, 1964.
20. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM. Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. Anal Biochem **150**:76–85, 1985.
21. Burke MD, Mayer RT. Ethoxyresorufin: Direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab Dispos **2**:583–588, 1974.
22. Williams CH, Kamin H. Microsomal triphosphopyridine nucleotide-cytochrome c reductase of liver. J Biol Chem **237**:587–595, 1962.
23. Schosinsky KH, Lehmann HP, Beeler MF. Measurement of ceruloplasmin from its oxidase activity in serum by the use of o-dianisidine hydrochloride. Clin Chem **20**:1556–1563, 1974.
24. Lehmann HP, Schosinsky KH, Beeler MF. Standardization of serum ceruloplasmin in international enzyme units with o-dianisidine hydrochloride as substrate. Clin Chem **20**:1564–1567, 1974.

25. Johnson WT, Dufault SN, Thomas AC. Platelet cytochrome c oxidase activity as an indicator of copper status in rats. Nutr Res **13**:1153–1162, 1993.
26. Boulenc X, Bourrie M, Fabre I, Roque C, Joyeux H, Berger Y, Fabre G. Regulation of cytochrome P4501A1 gene expression in a human intestinal cell line, CACO-2. J Pharmacol Exp Ther **263**:1471–1478, 1992.
27. deWaziers I, Cugnenc PH, Yang CS, Leroux JP, Beaune PH. Cytochrome P450 isozymes, epoxide hydrolase and glutathione transferase in rat and human hepatic and extrahepatic tissues. J Pharmacol Exp Ther **253**:387–394, 1990.
28. Kaminski LS, Fasco MJ. Small intestinal cytochrome P450. Crit Rev Toxicol **21**:407–422, 1992.
29. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T. Mayer RT. Ethoxy-, pentoxy-, and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. Biochem Pharmacol **34**:3337–3345, 1985.