ELSEVIER

Rapid Communication

Malnutrition sequela on the drug metabolizing enzymes in male Holtzman rats

Rashmeet K. Reen, Gustavo E.B.A. Melo, and T. Moraes-Santos

Faculdade de Farmacia, UFMG, Belo Horizonte, Brasil

The effect of food restriction on the specific activities of the drug metabolizing enzymes (DME) system was studied in Holtzman male rats by comparing DME activities in 90-day-old control rats fed ad libitum (CO), rats fed 40% restricted food (RF) from the gestation period to the day of sacrifice, and recovered rats (rRF) fed 40% restricted food from period of gestation to 45 days of age and then fed ad libitum until the day of sacrifice. In liver, total cytochrome P450 (CYP) of the RF and rRF groups was higher by approximately 50% and 28%, respectively, than in CO rats. Specific activities of individual CYP monooxygenases (MO) such as CYP2B [7-methoxycoumarin demethylase (MOCD)], CYP1A [aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin deethylase (EORD)], and CYP2E [nitrosodimethylamine demethylase (NDMAd)] were 31, 61, 43, and 56% in RF and 16, 36, 26, and 32% in rRF groups, respectively, more than the CO values. Conjugases such as UDP- glucuronosyltransferases with substrates 3-OH benzo(a)pyrene (UGT1) and 4-hydroxybiphenyl (UGT2) and glutathione S-transferase (GST) with substrate 1-chloro-2,4-dinitrobenzene were higher by 72, 69, and 33% in RF and 28, 38, and 24% in rRF groups, respectively. MO activities (MOCD and EORD) were significantly higher in lung, kidney, and intestine: MOCD by 82, 48, and 45% in RF and 40, 25, and 22% in rRF, respectively; and EORD by 84, 77, and 67% in RF and 40, 33, and 28% in rRF, respectively. However, activity of conjugases (UGT1 and GST) were significantly lower (approximately 35–45%) in RF and rRF rats (approximately 20–30%) than in the CO group in above mentioned extrahepatic tissues. These studies indicate that undernourishment during the period of gestation, weanling, and growth and development of microsomal enzymes produces a sequela of events on the DME in hepatic and extrahepatic tissues that cannot return to the control values even when fed ad libitum. (J. Nutr. Biochem. 10:615–618, 1999) © *Elsevier Science Inc. 1999. All rights reserved.*

Keywords: malnutrition sequela; liver and extrahepatic tissues; cytochrome P450; drug metabolizing enzymes

Introduction

Food is a prerequisite for the existence of humans. Although starvation may be endemic, food restriction occurs throughout the world. A major segment of the world's population is forced to practice food restriction as an economic necessity. Various metabolic alterations are known to occur due to food deprivation or food restriction.[1,2] Among these, drug metabolizing enzymes (DME) are of special significance because the balance of the functions of phase I and phase II DME, which coexist in the same cell, determines the various effects of xenobiotcs per se.[3] The cytochrome P450 (CYP) monooxygenase (MO) system ranks first in terms of catalytic versatility and the large number of xenobiotics it detoxifies or activates to reactive intermediates.[4] Although the highest concentration of CYP is found in liver endoplasmic reticulum microsomes, they are present in virtually all tissues.[5–8] Although the liver CYP enzymes play a key role in determining the intensity and duration of action of

Dr. Reen is a Visiting Researcher (FAPEMIG) on leave from Regional Research Laboratory, Jammu, India.
The above research work was financially supported by FAPEMIG, CNPq, and PRPq/UFMG.
Address correspondence to Dr. T. Moraes-Santos, Faculdade Farmacia/UFMG, Av. Olegario Maciel 2360, 30180-112 Belo Horizonte, MG, Brazil.
Received February 10, 1999; accepted June 28, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/99/$–see front matter
PII S0955-2863(99)00045-5

Table 1 Effect of early food restriction on the weights (body and liver) of adult Holtzman rats

Groups	Body wt (g)	Liver wt (g)
Control	313 ± 12[a]	12.00 ± 0.900[a]
Recovered	252 ± 15[b]	8.61 ± 0.895[b]
Restricted	188 ± 10[c]	7.35 ± 0.503[c]

Values are mean ± SEM from 15 rats/group at the age of 3 months. Control group: fed ad libitum; restricted: 60% of food consumed by the control; recovered: restricted group fed ad libitum after 45 days of age. The different superscript letters indicate statistical difference among the means ($P < 0.05$).

drugs and in the detoxification of xenobiotics, CYP enzymes in liver and extrahepatic tissues play an important role in the activation of xenobiotics to toxic or tumorogenic metabolites. Phase II enzymes are mainly involved in facilitating excretion of foreign compounds.

There is ample evidence that nutrition is a major determinant of drug action and hence drug metabolism.[9] Malnutrition and starvation affect the tissue levels of DME.[10–12] However, there are no studies on the DME that indicate the existence of a sequela in adults caused by malnutrition during gestation period until the period of growth and development of the microsomal enzyme system. The objective of the present study was to determine the activities of DME in hepatic and extrahepatic tissues of food restricted and nutritionally recovered rats.

Materials and methods

Chemicals

Chemicals included bovine serum albumin; Brij-58; dicoumarol; reduced nicotinamide adeninedinucleotide; benzo(a)pyrene; ethoxyresorufin; N-nitrosodimethylamine; 4-hydroxybiphenyl; 1-chloro-2,4-dinitrobenzene; uridine diphosphoglucuronic acid (Sigma Chemical Company, MO USA); 7-methoxycoumarin (Aldrich Chemie, Steinhein, Germany), purified according to Reen et al.[13]; and 3-hydroxy benzo(a)pyrene (Carcinogen Standard Reference Repository, National Cancer Institute, Bethesda, MD USA). All other chemicals used were of analytical grade locally available.

Animals and diet

Holtzman female rats were kept for mating for 14 days while on ad libitum lab chow diet. On the fifteenth day, these female rats were separated and divided into two groups. One group was a control that received food ad libitum and the other group received 60% of the food consumed by the control group until weaning at 21 days of life. Pups from the control (CO) group received food ad libitum until the day of sacrifice. Pups from mothers receiving restricted food were further divided into two groups, one group received 60% of the food consumed by the CO until the day of sacrifice (RF) and the other group received same restricted food as RF to 45 days of age and then fed ad libitum until the day of sacrifice (rRF). All groups were sacrificed at 90 days of age. Five rats from each group were used in three similar experiments performed at different times.

Preparation of microsomes

Animals were anesthetized with ether and opened. Saline was injected through the portal vein to thoroughly perfuse and clean blood from all the organs. The small intestines were removed and washed with chilled saline containing 1 mM dithiothreitol, cut open along their length, and mucosa was scraped. Liver, lungs, and kidneys were rapidly excised, blotted dry, weighed, and minced, and 25% whole homogenate was prepared with ice-cold 0.25 M sucrose. For lungs, kidneys, and intestinal cells, 0.1 mM EDTA was added to 0.25 M sucrose. Small portions of intestinal homogenates (IWH) were removed and stored. Microsomes were prepared by calcium precipitation according to Kamath and Rubin.[14] Briefly, the whole homogenate was centrifuged at 12,000 × g for 10 minutes to obtain supernatant, which was recentrifuged under similar conditions to obtain postmitrochondrial supernatant (PMS). A portion of PMS was removed and stored and the rest was subjected to microsomal aggregation by Ca^{++}[14]. Microsomal pellet obtained was suspended in 0.25 M sucrose. All the fractions were stored in liquid nitrogen until the time of use. Preparation of microsomes was performed at 4°C. Microsomes from lung, kidney, and intestinal mucosa were prepared by pooling respective tissues from five rats and results represent an average of three repeated experiments. Proteins were estimated according to Hartree[15] using bovine serum albumin as the standard.

Enzyme assays

CYP450 content was determined in freshly prepared microsomes according to Omura and Sato.[16] Microsomes were used to estimate the specific enzymatic activity of CYP2B by 7-methoxycoumarin demethylase (MOCD)[13]; CYP1A by aryl hydrocarbon hydroxylase (AHH)[17]; 7-ethoxyresorufin O-deethylase (EORD) according to Bock et al. as modified by Schulz-Schlage[18,19]; CYP2E1 by N-nitrosodimethylamine demethylase (NDMAd)[20]; glucuronosyltransferases with 3 hydroxy benzo(a)pyrene as substrate for UGT1; and 4 hydroxybiphenyl as substrate for UGT2.[21,22] PMS

Table 2 Malnutrition sequela in specific activities of CYP-dependent monooxygenases in liver

Groups	Total CYP	MOCD	AHH	EORD	NDMAd
Control	0.614 ± 0.12*	534.17 ± 21	0.246 ± 0.02	49.842 ± 4.70	3.091 ± 0.42
Recovered	0.786 ± 0.18	622.39 ± 70	0.335 ± 0.02	63.229 ± 5.76	4.083 ± 0.45
Restricted	0.923 ± 0.19	700.94 ± 73	0.397 ± 0.01	71.213 ± 5.51	4.813 ± 0.48

Control group: fed ad libitum; restricted: 60% of food consumed by the control; recovered: restricted group fed ad libitum after 45 days of age. Results are mean ± SEM of 15 rats/group. All the enzymes were assayed in microsomes. Units for total CYP are nmol/mg protein; for MOCD, pmol 7-hydroxy coumarin formed/min/mg protein; for AHH, nmol 4 hydroxy benzo(a)pyrene formed/min/mg protein; for EORD, pmol resorufin formed/min/mg protein; for NDMAd, nmol formaldehyde formed/min/mg protein.

*The experimental means are statistically different from those of control means ($P < 0.05$) in all enzymes studied.

CYP–cytochrome P450. MOCD–7-methoxycoumarin demethylase. AHH–aryl hydrocarbon hydroxylase. EORD–ethoxyresorufin deethylase. NDMAd–nitrosodimethylamine demethylase.

Table 3 Malnutrition sequela in specific activities of conjugases in liver

Groups	UGT1[a] (nmol/min/mg protein)	UGT2[a] (nmol/min/mg protein)	GST[b] (μmol/min/mg prot)
Control	0.727 ± 0.053*	21.051 ± 4.05	0.881 ± 0.055
Recovered	0.931 ± 0.023	29.054 ± 6.02	1.092 ± 0.050
Restricted	1.254 ± 0.180	35.576 ± 5.70	1.168 ± 0.023

Control group: fed ad libitum; restricted: 60% of food consumed by the control; recovered: restricted group fed ad libitum after 45 days of age. Results are mean ± SEM of 15 rats/group. Enzymes assayed in [a]microsomes and [b]postmitochrondrial supernatant.
*The experimental means are statistically different from those of control means ($P < 0.05$) in all the enzymes assayed.
UGT1–UDP-glucuronosyltransferase toward substrates 3-OH benzo(a)pyrene. UGT2–UDP-glucuronosyltransferase toward substrates 4-hydroxybiphenyl. GST–glutathione S-transferase toward substrate 1-chloro-2, 4-dinitrobenzene.

and IWH were used to estimate glutathione-S-transferase (GST) with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.[23]

Statistics

The results for liver are mean ± SEM of 15 rats from each experimental group. In extrahepatic tissues results are from three experiments from a pool of five rats. Statistical significance of the difference between control and experimental groups was determined by one way analysis of variance (ANOVA) and significance was also evaluated by Student's *t*-test compared with control fed rat.

Results and discussion

As shown in *Table 1*, body weights were 40% and 20% less, respectively, in the RF and rRF rats than in the ad libitum fed CO rats and liver weights were lower by 43% and 27%, respectively. In the liver, total CYP level in both the RF and rRF groups of rats was increased 50% and 28%, respectively, above CO values (*Table 2*). Estimation of individual CYP-dependent MO activities were significantly higher than CO rats in CYP2B (MOCD activity), CYP1A (AHH and EORD activity), and CYP2E1 (NDMAd activity) by 31, 61, 43, and 56%, respectively, in the RF rats and by 16, 36, 26, and 32%, respectively, in rRF rats (*Table 2*). The enzyme activities of conjugases such as uridine diphosphate (UDP)-glucuronosyltransferases toward substrates 3 hydroxybenzo(a)pyrene (UGT1) and 4 hydroxy biphenyl (UGT2), and GST toward substrate 1-chloro-2,4-dinitrobenzene (CDNB) were higher than the CO group by 72, 69, and 33%, respectively, in the RF group and by 28, 38, and 24%, respectively, in the rRF group (*Table 3*).

Specific activities of MO in lung, intestine, and kidney also were higher than in the CO group. MOCD was 82, 48, and 45% higher, respectively, in the RF group and 40, 25, and 22% higher, respectively, in the rRF rats; EORD was 84, 77, and 67% higher, respectively, in the RF group and 40, 33, and 28% higher, respectively, in the rRF group (*Table 4*). However, activities of the conjugases UGT1 and GST were significantly lower than in the CO rats in above mentioned extrahepatic tissues. In lung, intestines, and kidneys, respectively, activity of UGT1 was 60, 57, and 64% lower in RF rats and 73, 67, and 75% lower in rRF rats, and GST was 61, 74, and 50% lower in RF groups and 75, 85, and 73% in rRF groups, (*Table 4*).

The present studies demonstrate that there exists a sequela in the body and liver weight and in specific activities of DME in adult rats that suffered from early malnutrition starting from the period of gestation up to the

Table 4 Malnutrition sequela in drug metabolizing enzymes of the extrahepatic tissue

Groups	MOCD (pmol/min/mg prot)	EORD (pmol/min/mg prot)	UGT1 (nmol/min/mg prot)	GST (μmol/min/mg prot)
Lungs				
Control	53.03 ± 5.4[a]*	4.416 ± 0.48[a]	143 ± 2[a]	0.176 ± 0.01[b]
Recovered	74.74 ± 4.76[a]	6.193 ± 0.79[a]	105 ± 5[a]	0.132 ± 0.01[b]
Restricted	96.54 ± 8.93[a]	8.138 ± 0.97[a]	86 ± 2[a]	0.108 ± 0.02[b]
Intestines				
Control	2.882 ± 0.459[c]	2.882 ± 0.28[a]	845 ± 80[a]	0.152 ± 0.012[c]
Recovered	3.614 ± 0.172[c]	3.815 ± 0.13[a]	562 ± 30[a]	0.129 ± 0.007[c]
Restricted	4.257 ± 0.548[c]	5.101 ± 0.03[a]	482 ± 23[a]	0.113 ± 0.011[c]
Kidneys				
Control	6.23 ± 0.92[a]	3.380 ± 0.30[a]	220 ± 10[a]	0.150 ± 0.016[b]
Recovered	7.63 ± 0.06[a]	4.327 ± 0.32[a]	166 ± 10[a]	0.109 ± 0.002[b]
Restricted	9.07 ± 0.19[a]	5.639 ± 0.45[a]	140 ± 30[a]	0.075 ± 0.002[b]

Control group: fed ad libitum; restricted: 60% of food consumed by the control; recovered: restricted group fed ad libitum afer 45 days of age. Results are mean ± SEM of three values in duplicates/group.
[a]Enzymes assayed in microsomes; [b]enzymes assayed in postmitochondrial supernatant; and [c]enzymes assayed in intestinal whole homogenate.
*The experimental means are statistically different from those of control means ($P < 0.05$) for all the enzymes studied.
MOCD–7-methoxycoumarin demethylase. EORD–ethoxyresorufin deethylase. UGTI–UDP-glucuronosyltransferase towards substrates 3-OH benzo-(a)pyrene. UGT2–glutathione S-transferase towards substrate 1- chloro-2, 4-dinitrobenzene.

growth and development period of DME but later fed ad libidum. Our studies are in agreement with other studies reporting enhanced activities of DME due to short or prolonged food restriction.[10,24–26] Prolonged restriction of food, in this study, in RF and rRF rats show difference in body and liver weights compared with CO and higher activities of DME: monooxygenases such as CYP2B (MOCD), CYP1A (AHH & EORD), and CYP2E (NDMAd), which were in direct proportion to the higher CYP content; and conjugases such as UGT1, UGT2, and GST in liver. Although body and liver weights of both RF and rRF rats were significantly lower than the CO rats, refeeding of rRF rats ad libitum after the 45 days of age helped to improve significantly the weights of body and liver. Difference in the enzymes activities between the RF and rRF groups were observed but were not statistically significant.

Malnutrition sequela was also observed in the DME of lung, kidney, and intestines as in liver. Food restriction enhanced the specific activities of CYP-dependent MO MOCD and EORD in both RF and rRF rats. Unlike liver, the activities of conjugases UGT1 and GST were significantly reduced in both RF and rRF rats compared with the control rats in these extrahepatic tissues.

In conclusion it appears that early food restriction in rats produces a sequela on DME system of hepatic and extrahepatic tissues such as lung, intestine, and kidney. Because DME are capable of metabolizing a large number of drugs of therapeutic importance, the bioavailability of these drugs could be affected differently in adults who were malnourished during childhood. In addition, because many carcinogens, various environmental chemicals, and even endogenous steroids are candidates for these enzymes, in addition to pharmacologic response, early malnutrition could substantially modify carcinogenic susceptibility and environmental chemical insult during life.

Acknowledgments

The authors thank M.G.V. Torquato for the technical support.

References

1 Ross, M.H. (1969). Aging, nutrition and hepatic enzyme activity pattern in the rat. *J. Nutr.* **97,** 563–602
2 Ross, M.H. (1976). Nutrition and longevity in experimental animals. In *Nutrition and Ageing* (M. Winnick, ed.), pp. 43–57, Wiley & Sons, New York, NY, USA
3 Pelkonen, O. and Nebert, D.W. (1982). Metabolism of polycyclic aromatic hydrocarbons: Etiologic role in carcinogenesis. *Pharmacol. Rev.* **34,** 189–212
4 Watermann, M.R. and Johnson, E.F. (eds) (1991). *Methods in Enzymology*, Vol. 206, Academic Press, New York, NY, USA
5 Porter, T.D. and Coon, M.J. (1991). Cytrochrome P450: Multiplicity of isoforms, substrates and catalytic and regulatory mechanisms. *J. Biol. Chem.* **266,** 13469–13472
6 Dubey, R.K. and Singh, J. (1988). Localization and characterization of drug metabolising enzymes along the villus crypt surface of the rat small intestines-I. *Biochem. Pharmacol.* **37,** 169–176
7 Dubey, R.K. and Singh, J. (1988). Localization and characterization of drug metabolising enzymes along the villus crypt surface of the rat small intestines-II. *Biochem. Pharmacol.* **37,** 177–184
8 Ronis, M.J.J., Haung, J., Longo, V., Tindberg, N., Ingelman, S.M., and Badger, T.M. (1998). Expression and distribution of cytochrome P450 enzymes in male rat kidney: Effects of ethanol, acetone and dietary conditions. *Biochem. Pharmacol.* **55,** 123–129
9 Campbell, T.C. (1977). Nutrition and drug metabolising enzymes. *Clin. Pharmacol. Ther.* **22,** 699–706
10 Hashmi, R.S., Siddiqui, A.M., Kachole, M.S., and Pawar, S.S. (1986). Alterations in hepatic microsomal mixed-function oxidase system during different levels of food restriction in adult male and female rats. *J. Nutr.* **116,** 682–688
11 Ronis, M.J.J., Lumpkin, C.K., Ingelman-Sunderberg, M., and Badger, T.M. (1991). Effects of short term ethanol and nutrition on the hepatic microsomal monooxygenase system in a model utilizing total enteral nutrition in the rat. *Alcohol Clin. Exp. Res.* **15,** 693–699
12 Liu, P.T., Shavila, I.J., Symons, A.M., and Parke, D.V. (1993). Effects of ether anaesthesia on fasting on various cytochromes P450 of rat liver and kidney. *Biochem. Pharmacol.* **45,** 871–877
13 Reen, R.K., Ramakanth, S., Weibel, F.J., Jain, M.P., and Singh, J. (1991). Dealkylation of 7-methoxycoumarin as assay for measuring constitutive and phenobarbital-inducible cytochrome P450s. *Anal. Biochem.* **194,** 243–249
14 Kamath, A.S. and Rubin, E. (1972). Interaction of calcium with microsomes. A modified method for the rapid isolation of rat liver microsomes. *Biochem. Bioph. Res. Co.* **49,** 52–59
15 Hartree, E.F. (1972). Determination of protein a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* **48,** 422–427
16 Omura, T. and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its haemoprotein nature. *J. Biol. Chem.* **239,** 2370–2378
17 Weibel, F.J., Brown, S., Waters, H.L., and Selkirk, J.K. (1977). Activation of xenobiotics by monooxygenases: Culture of mammalian cells as analytical tool. *Arch. Toxicol.* **39,** 133–148
18 Burk, M.D., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T., and Meyer, R. (1985). Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologous: A series of substrates to distinguish between different induced cytochrome P450. *Biochem. Pharmacol.* **34,** 3337–3345
19 Schulz-Schlage, T., Koch, E., Schwind, K.H., Hutzinger, O., and Neubert, D. (1991). Inductive potency of TCDD, TBDD and three 2, 3, 7, 8-mixed halogenated dioxins in liver microsomes of male rats. *Chemosphere* **23,** 1925
20 Tu, Y.Y. and Yang, C.S. (1983). High affinity nitrosamine dealkylase system in rat liver microsomes and its induction by fasting. *Cancer Res.* **43,** 623–629
21 Singh, J. and Wiebel, F. J. (1979). A highly sensitive and rapid fluorometric assay for UDP-glucuronyltransferase using 3-hydroxybenzo(a)pyrene as substrate. *Anal. Biochem.* **98,** 394–401
22 Bock, K.W., Josting, D., Lilienblum, W., and Pfeil, H. (1979). Purification of rat liver microsomal UDP-glucuronyltransferase. *Eur. J. Biochem.* **98,** 19–26
23 Habig, W.H., Pabst, M.J., and Jakoby, W.B. (1974). Glutathione S-transferase. The first step in mercapturic acid formation. *J. Biol. Chem.* **249,** 7130–7139
24 Sachan, D.S. (1982). Modulation of drug metabolizm by food restriction in male rats. *Biochem. Bioph. Res. Co.* **104,** 984–989
25 Hong, J., Pan, J., Gonzalez, F.J., Gelboin, H.V., and Yang, C.S. (1987). The induction of a specific form of cytochrome P-450 (P-450j) by fasting. *Biochem. Bioph. Res. Co.* **142,** 1077–1083
26 Khatsenko, O.G., Sindhu, R.K., and Kikkawa, Y. (1991). Undernutrition during hyperoxic exposure induces CYP2E1 in rat liver. *Arch. Toxicol.* **71,** 684–689