# Effect of protein and calorie restriction on the cytochrome P-450 isozyme (P-450 IID6) activity in rats

**Hosam M. Kandil, David J. Hermann, Jinfang Lu, Mark J. Koruda, William T. Sawyer, George E. Dukes, and Lawrence J. Hak**

*Drug Development Laboratory, University of North Carolina, Schools of Pharmacy and Medicine, Chapel Hill, NC, USA*

*Dextromethorphan (DXM) is commonly used as a probe for the metabolic activity of the debrisoquine isozyme (P-450 IID6) of the cytochrome P-450 mixed-function oxidase system. Several reports have described changes in P-450 activity with dietary alteration. This study was conducted to determine the effect of protein and calorie restriction on DXM metabolism in Sprague Dawley (SD) rats. Four- to five-week-old male SD rats were fed one of four dietary regimens: a regular diet containing 22.5% protein ad libitum (REGLIB); a low protein (8%) diet fed ad libitum (PROLIB); a regular protein diet, but only 50% by weight of that consumed by the REGLIB group (REG50); a low protein (8%) diet, but only 50% by weight of that consumed by the PROLIB group (PRO50). The DXM $\log_{10}$ molar metabolic ratio (LMR) calculated as the ratio of the urinary DXM to its metabolite dextrorphan concentration was measured at the beginning of the study and after 5 weeks of feeding. Groups REG50, PROLIB, and PRO50 received 49%, 57%, and 29% of the calorie intake and 49%, 24%, and 12% of the protein intake of group REGLIB, respectively. Their weight gain was 24%, 40%, and 3% of that gained by REGLIB. LMR significantly decreased for REGLIB and PROLIB indicating an increase in DXM metabolism. The LMR of REG50 and PRO50 did not change. DXM metabolism continues to develop in these rats during the first 7 to 8 weeks of life. Energy restriction inhibited the maturation of DXM metabolism, while protein restriction did not significantly affect DXM metabolism.*

**Keywords:** dextromethorphan; protein; calorie

## Introduction

The metabolism of debrisoquine (DEB) and sparteine is genetically controlled and is bimodally distributed in two phenotypes, is transmitted as an autosomal recessive trait, and occurs primarily by a single isozyme of the cytochrome P-450 mixed function oxidase system.[1-4] Approximately 10% of Caucasians are of the homozygous poor metabolizer phenotype (PM), while 90% are extensive metabolizers.[5] Phenotypic classification of an individual's ability to metabolize compounds by this isozyme system (P-450IID6) is determined by measurement of the concentration of the parent drug: concentration of the main metabolite (metabolic ratio) in urine of DEB or other compounds similarly metabolized.

A rat model has been described for the study of DEB metabolic polymorphism.[6-8] Most rat strains are extensive metabolizers of DEB, whereas female Dark Agouti (DA) rats are poor metabolizers of the drug.[6] An isozyme specific for metabolism of DEB has been isolated from hepatic rat microsomes. Poor metabolizer DA rats are deficient in this enzyme.[8] In vivo studies in humans[3] and rats[6] have demonstrated a strong correlation between polymorphic metabolism of DEB and dextromethorphan (DXM).

In vitro studies show that both drugs are metabolized by the same cytochrome P-450 isozyme.[11] PMs of DEB are PMs of DXM in humans and rats.[1,7-10] The relative safety, specificity, and availability of DXM make it an attractive tool for pharmacogenetic studies in both humans and rats.

Address reprint requests to Dr. George E. Dukes, Associate Professor at the School of Pharmacy, CB# 7360, Beard Hall, University of North Carolina, Chapel Hill, NC 27599-7360 USA.
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Nutrition affects the metabolism of selected drugs in both humans and rats.[12] Clinical studies on normal volunteers[13] and on asthmatic children[14,15] show that high protein diets stimulate theophylline and antipyrine metabolism. Low protein intake decreases the metabolism of benzphetamine, ethylmorphine,[16] and theophylline.[17] Hepatic microsomal oxidation of antipyrine, isoniazide, and acetanilide are depressed in protein energy malnutrition.[18] Starvation of rats enhances the oxidative demethylation of different nitrosamines,[19,20] while oxidative metabolism of ethylmorphine and aniline is inhibited.[20] However, no data are available on the effect of nutrition on the disposition of drugs in which metabolism is genetically controlled. A study was therefore conducted to determine if altering nutrient intake would alter DXM metabolism in the rat model.

## Materials and methods

This study was approved by the Institutional Animal Care and Use Committee, The University of North Carolina at Chapel Hill. Forty-eight male Sprague Dawley (SD) rats, four- to five-weeks-of age and weighing 100–150 grams were studied. Rats were randomly divided into four groups of 12 rats each. Five days prior to the start of the study, each rat was placed in a separate metabolic cage, acclimated to a 12-hour dark:light cycle and fed a regular diet (Prolab, Agway Inc., Syracuse, NY, USA) ad libitum.

### *Dietary regimen*

The dietary regimens of the four groups were *(Table 1)*: a) (REGLIB) control group of rats received the same regular diet, which is 22.5% protein and 4.4 Kcal/gram, ad libitum; b) (REG50) a protein/calorie restricted group in which rats were pair-fed 50% of the average intake of REGLIB; c) (PROLIB) a protein depleted group in which rats received an 8% low protein diet (ICN Biomedical, Inc., Cleveland, OH, USA), 3.8 Kcal/gram, ad libitum; d) (PRO50) a severe protein/calorie restricted group in which rats were pair-fed 50% of the average intake of PROLIB. The macronutrient composition of the two diets is shown in *Table 2*. The rats were maintained on these diets for 35 days.

### *Drug administration*

On day 0 and day 35 of the study, 2 mg of DXM in 0.5 mL distilled water was given to each rat by gavage. Food and water were removed from all cages for 4 hours before DXM gavage. Urine was collected for a 12-hour period following each DXM dosage and was stored at −20° C until later analyzed.

### *Analytical methods*

The concentrations of DXM and its major metabolite, dextrorphan (DEX), were determined by high pressure liquid chromatography (HPLC). The HPLC system consisted of a Waters M-45 pump, a WISP 7108 autoinjector, and a Kratos spectroflow 980 programmable fluorescence detector M441. Fluorescence detection was accomplished by excitation at 200 nm without the use of an emission filter. Peaks were integrated on a Shimadzu C-R5A chromatopac integrator. A 250 mm × 4.6 mm phenyl column packed with spherisorb (5μ particle size) was used. The mobile phase consisted of a mixture of 70% buffer (10 mmol/L $KH_2PO_4$ and 2.5 mmol/L 1-Octanesulfonic acid sodium salt anhydrate, adjusted with phosphoric acid to a pH of 2.5) and 30% of organic phase (acetonitrile:methanol 2:1). Flow rate was 1.3 mL/min at 25° C. Retention time for DXM, DEX, and internal standard (Levallorphan) were 12.8, 6.8, and 8.5 minutes, respectively.

For DXM extraction, the pH of a 1.0 mL urine sample was adjusted to 9–10 with concentrated ammonium hydroxide. Twenty μL internal standard solution (concentration 100 μg/mL) and 5 mL hexane, containing 10% n-butyl alcohol, were added and the mixture was shaken on a mechanical shaker for 30 minutes. After centrifugation for 10 minutes at 3000 rpm, the aqueous layer was frozen in a dry ice-acetone bath. The organic phase was decanted into a glass centrifuge tube and evaporated to dryness using a speed vacuum concentrator, (Savant Instruments Inc., Hicksville, NY, USA). The residue was reconstituted with 500 μL of mobile phase, and 50 μL injected onto the HPLC column. The assay was linear over a concentration range of 2.5 to 2000 ng/mL of DXM ($r$ = 0.997). The day-to-day coefficient of variation was 3%.

For extraction of total free and conjugated DEX, 250 μL of urine was mixed with 20 μL of internal standard (concentration 100 μg/mL) and 20 μL bromocresol green indicator. The pH was adjusted to 3–5 with 18% acetic acid, and then 20 μL of B-glucuronidase (Glusolase, Sigma Chemical Co., St. Louis, MO, USA) was added. After an 18-hour incubation at 37° C, the pH was adjusted to 10–11 with concentrated ammonium hydroxide. Following the same procedure as for DXM, 25 μL of the reconstituted solution was injected onto the HPLC column. The assay was linear over 25–20000 ng/mL of DEX ($r$ = 0.9995). The day-to-day coefficient of variation was 9.6%.

### *Statistical methods*

The dextromethorphan metabolic ratio (MR) was calculated as the urinary concentration of DXM divided by the concentration of DEX. The log molar metabolic ratio (LMR) was calculated as $\log_{10}$ (MR/molecular weight of DXM/molecular weight of DEX). The lower detection limit for DXM

**Table 1** Dietary regimens of the four rat groups

| Group | Diet | Intake |
|---|---|---|
| REGLIB | Regular rat diet | Ad libitum |
| REG50 | Regular rat diet | 50% of mean intake of REGLIB |
| PROLIB | Low protein diet (8%) | Ad libitum |
| PRO50 | Low protein diet (8%) | 50% of mean intake of PROLIB |

**Table 2** Macronutrient composition of study diets*

| | Regular diet | Low-protein diet |
|---|---|---|
| Carbohydrates | 78 | 56.5 |
| Protein | 8 | 22 |
| Fat | 10 | 5 |

*Data are the percent by weight.

(2.5 μg/mL) in this study was used in the calculation of LMR when no DEX was detected in the urine.

The difference between groups in mean body weight and LMR were compared by analysis of variance with a value of alpha = 0.05 designated as the apriori criterion of statistical significance. Where the differences among groups were identified, post-hoc analyses were conducted using multiple paired *t* tests at an alpha level corrected for the number of group comparisons (alpha = 0.05/number of comparisons, Bonferrone' Method). Changes for each group over time were evaluated by comparing the means for the same group before and after dietary treatment utilizing paired *t* test at an alpha of 0.05. Data are presented as mean ± SD.

## Results

### *Food intake*

The mean calorie and protein intake of animals in the four groups are summarized in *Table 3*. Group REG50 received 49% of the calorie and protein intake of the control group (REGLIB). Although group PROLIB rats were fed ad libitum, both calorie and protein intake were lower than that of group REGLIB (57% and 24% of group REGLIB, respectively). Group PRO50 received 51% of the intake of group PROLIB. Calorie and protein intakes of group PRO50 were 29% and 12%, respectively, of the intakes of the control group, REGLIB.

### *Body weight*

The mean body weight for the four groups are summarized in *Table 4*. The mean body weight gain over the 35-day study period for groups REGLIB, REG50, PROLIB, and PRO50 was 215 ± 32.4, 52 ± 8.9, 85 ± 20, and 6 ± 5 grams, respectively. The body weight gain was statistically greater in group REGLIB than the three treatment groups ($P < 0.001$ for the three comparisons). At the end of the study, the mean body weight of the animals in each of the three treatment groups was significantly lower than that of animals in group REGLIB ($P < 0.001$, *Table 4*).

### *Log molar MR*

The mean LMR values for the four groups are summarized in *Table 5*. Mean LMR was statistically equivalent among the four groups at the start of the study.

**Table 3** Average calorie and protein intake

| Group | Calorie/day* | | Protein (g)/day* | |
|---|---|---|---|---|
| | Intake | % of control | Intake | % of control |
| REGLIB | 100 (8.5) | 100 | 5 (0.4) | 100 |
| REG50 | 49 (5.8) | 49 (2.4) | 2.5 (0.3) | 49 (2.4) |
| PROLIB | 58 (9) | 57 (6.2) | 1.2 (0.2) | 24 (2.6) |
| PRO50 | 30 (4.4) | 29 (2.3) | 0.6 (0.1) | 12 (1.0) |

*Mean (SD).

**Table 4** Body weight (g) of study animals*

| Parameter | Day | REGLIB | REG50 | PROLIB | PRO50 |
|---|---|---|---|---|---|
| Body | 0 | 139 (9.1) | 144 (11.1) | 135 (15.3) | 144 (11.0) |
| Weight (g) | 35 | 354 (32.8) | 196† (9.6) | 220† (19.9) | 150† (10.1) |
| Change in weight (g) | | 215 (32.4) | 52 (8.9) | 85 (20) | 6 (5) |

*Mean (SD).
†Value is significantly different from REGLIB ($P < 0.001$).

**Table 5** LMR of study animals*

| Parameter | Day | REGLIB | REG50 | PROLIB | PRO50 |
|---|---|---|---|---|---|
| LMR | 0 | −2.3 (0.6) | −2.4 (0.5) | −1.8 (0.5) | −1.9 (0.6) |
| | 35 | −2.9 (0.3) | −2.7 (0.4) | −2.8 (0.5) | −2.5† (0.5) |
| Change in LMR | | −0.6‡ (0.7) | −0.3 (0.7) | −1.0‡ (0.7) | −0.6 (0.9) |

*Mean (SD).
†Value is significantly different from REGLIB ($P < 0.001$).
‡Change is statistically significant ($P < 0.05$).

Although LMR for groups PROLIB and PRO50 tended to be lower than that of group REGLIB at time zero, the difference was not statistically significant. The mean LMR for each of the four groups decreased between the start and the end of the study *(Table 5)*, indicating increased DXM metabolism, but this decrease was statistically significant only in groups REGLIB and PROLIB ($P$ = 0.01 and 0.002, respectively). Although LMR of group PRO50 decreased to the same extent as that of group REGLIB, the change was not statistically significant due to a large variability among group REGLIB. At the end of the study, the mean LMR of group PRO50 rats was significantly higher than that of the group REGLIB rats, while the REG50 group and PROLIB group did not differ from the REGLIB group.

## Discussion

The reported correlation between the polymorphic metabolism of DXM and debrisoquin in humans[3,7–10] and rats[5] indicates that these drugs either share a common metabolic pathway or that their respective metabolic pathways share a common genetically linked rate-limiting step. In the present study, the effect of different diets, restricted to different extents in protein, calorie content, or both, on DXM metabolism in Sprague Dawley rats was evaluated. The metabolic ratio of DXM was used as a marker of cytochrome-P450IID6 enzyme activity in the study rats. Metabolic ratio has been shown to reflect changes in metabolic activity at the microsomal level.[21]

The statistically significant decrease in mean LMR of rats in the control group, who were fed a regular diet ad libitum indicates an increase in the metabolic activity of the isozyme and may reflect a normal maturation process. This continued increase in metabolic activity suggests that development of the P-450IID6 isozyme may continue during the first 7–8 weeks of age. This is contrary to the description of the development of the hydroxylation metabolism of aromatic compounds such as aniline and ethylmorphine, as well as N-dealkylation and *O*-dealkylation metabolism of other drugs.[22] Studies of metabolism of these drugs showed that cytochrome P-450 activity in rats is low at birth and increases gradually to a maximum level around day 30 after birth.

A similar pattern of decreasing LMR was observed in PROLIB rats despite their reduced protein intake (25% of REGLIB group rats). In contrast, mean LMR of rats in groups REG50 and PRO50 did not significantly change, indicating possible inhibition of the development of DXM metabolic activity of rats in these two groups. These data suggest that low caloric intake inhibited the development of the isozyme specific for DXM metabolism in these rats and that calorie intake may be more important than protein intake in the development of this isozyme.

The lack of effect of protein intake observed in this study is in contrast with the reported inhibition of the metabolism of benzphetamine, ethylmorphine, and theophylline[16,17] in rats fed a low protein diet. This suggests that the effect of dietary protein intake may be specific for different cytochrome P-450 isozymes. The decrease in mean LMR of rats in each of the three treatment groups is intriguing and suggests that factor(s) other than nutrition are also important in the development of a DXM-specific isozyme.

In summary, energy restriction inhibited the maturation of the enzyme that metabolizes DXM in young male Sprague Dawley rats, while protein restriction had less of an effect. The data also suggest that the enzyme may continue to develop in these rats during the first 7–8 weeks of life.

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