Effect of protein and calorie malnutrition on drug metabolism in rat - in vitro

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Abstract Purpose: To study the effect of protein and calorie malnutrition on *in vitro* drug metabolism of protein and calorie malnourished juvenile and adult rats. **Method**: Microsomal incubation was used as a means of monitoring drug metabolism changes, HPLC was employed to quantify metabolites and enzyme immunoassay (EIA) was used for rat growth hormone (rGH) monitoring. **Results**: Protein and calorie malnutrition significantly decreased levels of microsomal protein and total P450. Microsome of protein and calorie malnourished rats showed impaired testosterone 16α- and 2α- hydroxylation (CYP2C11), testosterone 6β-hydroxylation (CYP3A), and testosterone 7α-hydroxylation (CYP2A1). Testosterone 16β-hydroxylation (CYP2B1) did not show any significant change, neither in capacity nor affinity. The quantity and the secretion pattern of rGH were not altered in protein and calorie malnourished rats compared to those in healthy animals. **Conclusions**: Serum albumin is not a good indicator of malnutrition. The capacity and affinity of CYP2C11, CYP3A and CYP2A1 were compromised by protein and calorie malnutrition. The impairment of drug metabolism in protein and calorie malnourished rats was not caused by the alteration of rGH.

INTRODUCTION

The major nutritional disorder, which occurs globally and accounts for severe morbidity and mortality, is protein and calorie malnutrition. It was estimated that 57 out of 100 of the children in some developing countries are affected by malnutrition (1). As a consequence of numerous pathological conditions, which are encountered in malnutrition, there may be a wide range of changes in pharmacokinetic or pharmacodynamic processes of xenobiotics.

Rational use of drugs is based on sound pharmacokinetic and pharmacodynamic principles, and knowledge of these and the factors modifying the fate of the drug in the body are essential for the proper management of patients. It is now recognized that diet and nutritional status are two important variables that can alter drug metabolism and disposition (2-4).

The effect of protein and calorie malnutrition on phase I metabolism is not well characterized in literature. Most of the studies (5-9) reported in the literature used either a very low protein or calorie protocol in this type of studies. The pathway of drug metabolism altered by protein and calorie malnutrition and the effects of malnutrition on various age groups are not well understood. The objectives of this study were to determine the effect of protein and calorie malnutrition on liver metabolic protein content and total Cytochrome P450 (P450), and to investigate protein and calorie malnutrition effects on enzymes such as CYP2C11, CYP3A, CYP2A1 and CYP2B1 by using testosterone as a substrate in both juvenile and adult rats.

Effort was also made to explore the mechanism that caused the change of drug metabolism in the protein and calorie malnourished rats as this is not well investigated in literature. It was reported that growth hormone and the pituitary-thyroid axis are sensitive to protein-calorie deficit (10-12) and the constitutive male-specific CYP2C11 expression in rat liver is developmentally up-regulated at the transcriptional level by the male pattern of growth hormone (GH) secretion (12). This study was to determine whether a change in rGH secretion is a factor in diminishing drug metabolism in rats with protein and calorie malnutrition.

MATERIALS AND METHODS

Chemicals and Reagents

Testosterone, 6β-hydroxytestosterone, 16β-hydroxytestosterone, 2α-hydroxytestosterone, 7α-hydroxytestosterone, 16α-hydroxytestosterone and cortexolone were purchased from Steraloids Inc. (Newport, RI, USA). Metofane was from Janssen (North York, ON, Canada). Pituitary hormones and rGH were from UCB Bioproducts (Nanterre, France). rGH was calibrated against rGH-RP2 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, Bethesda, USA).

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Animals and Animal Care

Male Sprague-Dawley (SD) rats weighing 190-210 g (about 40 days old) were supplied by Biosciences Animal Services, University of Alberta. All animals were housed in the Dentistry-Pharmacy Building Animal Service Facility and were fed Agway Prolab RMH1000 Formula (PMI Feeds, Inc., St. Lois, MO) before starting the experiments. The composition of Agway Prolab RMH1000 Formula mainly consists of protein, fat, fiber, carbohydrates and 15 vitamins which were seen in the general rat food. From this formula, calories provided by protein was about 17.6%, by fat was about 15.8%, and by carbohydrates were 65.6%. The protocol was approved by the Animal Ethics Committee, University of Alberta. The protein and calorie malnourished diets selection were tailored to the health conditions of the rats. During the process of selecting the protein and calorie malnourished diets, juvenile rats fed with less than 10 g/day and adult rats fed with less than 12 g/day were found to be highly stressed. This condition was indicated by the change of hair color to red and the animals were hyperactive. Therefore, the Health Science Animal Welfare Committee of University of Alberta required that minimum diet for juvenile rat was 10 g/day and for adult rat was 12 g/day.

Juvenile Rats: Male SD rats (190-210 g, about 40 days old) were housed individually with a 12-hr light/dark cycle at 21-22°C. The animals had access to tap water and a nutritionally balanced Agway Prolab RMH1000 Formula *ad libitum* during an initial 4-day acclimation period. The feed was weighed daily to determine the amount of food consumed by each rat. After the acclimation period, the animals were randomly divided into two groups (n=5/group). Group 1, the control group, had free access to food (range from 20-25 g daily) and water and Group 2, the protein and calorie malnourished group, were fed 10 g of food per day and had free access to water. Rats were maintained on the assigned diet for 8 weeks.

Adult Rats: Male SD rats (190-210 g about 40 days) were housed individually with a 12-hr light/dark cycle at 21-22°C. The animals had access to tap water and Agway Prolab RMH1000 Formula *ad libitum* until the rats were 340-360 g (about three months old). By this time, the rats reached adulthood. The control animals had 20 g of Agway Prolab RMH1000 Formula daily and free access to water to maintain the body weight, while Group 2 animals were fed 12 g of Agway Prolab RMH1000 Formula and had free access to water. The assigned diets were maintained for 8 weeks.

Assessment of Nutrition Status: Rats were observed for general activity and alertness daily. Serum albumin levels were measured every two weeks and weight measurement was taken twice a week throughout the study period. For adult rats, other than serum albumin, total plasma protein was also determined during the experimental period.

Serum Albumin and Plasma Globulin Measurements: The tail clip method was used for blood sampling. After rubbing the tip of the tail with a cotton ball soaked with 75% alcohol, approximately 2 mm of the tail was clipped using a sterilized razor blade. Immediately after 50 µl of blood were taken with a capillary tube, silver nitrate sticks were used to stop bleeding. Blood was centrifuged for 5 min at 2,500 g to obtain serum and samples were frozen at –20°C until analysis. Serum albumin and globulin were determined using direct dye binding with bromocresol green. The measurement was performed by the staff at the Surgical Medical Research Institute, University of Alberta.

Serum Sampling for rGH Measurement

After the malnutrition treatment, rats were subjected to jugular vein cannulation. The animals were allowed to recover for two days. On the third day, blood samples were taken from jugular vein at 8 am, 11 am, 2 pm and 8 pm. Serum was harvested and immediately frozen at –20°C until rGH analysis.

Measurement of rGH

An enzyme immunoassay (EIA) procedure similar to the one by Ezan et al. (13) was employed for rGH measurement. Serum samples were diluted in enzyme immunoassay phosphate buffer (0.1 M at pH 7.4) containing 0.15 M NaCl, 0.001 M EDTA, 0.1% BSA, 0.01% sodium azide and 0.2% Triton X-100. The 96-well microtiter plates were coated with rabbit polyclonal antibodies specific for goat IgG at a concentration of 10 µg/ml for one night at room temperature and saturated in the EIA buffer without Triton X100. Before use, plates were extensively washed with 0.01 M phosphate buffer (pH 7.4) containing 0.1% Tween 20. The total 150 µl volume of the assay solution was added in 96-well microtiter plates. Reagents were dispensed as follows: 50 µl sample or standard rGH from UCB Bioproducts calibrated against the NIDDK standard rGH-RP2, and 50 µl rGH antiserum. After a 20 hrs incubation at room temperature, 50 µl enzymatic tracer (0.25 Ellman units/ml) were added before a further 20 hrs incubation at room temperature. Plates were again washed and Ellman's reagent (200 µl) was distributed into each well and incubated for 3 hrs. Absorbance was measured with a spectrophotometer at 414 nm.

All measurements for standards and samples were duplicated.

Preparation of Hepatic Microsomes

After 8 weeks of food restriction, SD male rats were fasted overnight and anesthetized with metofane, and then the abdominal cavity was opened for portal vein cannulation. The portal vein was cannulated and the liver was perfused *in situ* with ice-cold isotonic (1.15% w/v) potassium chloride solution for about 3 min with a speed of 30 mL/min until the liver turned yellowish (color of liver when blood was cleared). The liver was excised and weighed. The following steps were performed at 4°C. The liver was finely minced and homogenized in three volumes of ice-cold isotonic potassium chloride solution (1.15% w/v) using a glass homogenizer equipped with a glass pestle (Glas-Col, Cole-Parmer instrument Co., Terre Hautein, IN, USA). The homogenate was centrifuged at 10,000 g for 30 min using a Model IEC B-20A centrifuge equipped with a No. 870 rotor (International Equipment Company, Boston, MA, USA). The supernatant was evenly distributed into polycarbonate tubes (Ultratube, 13 × 64 mm, Nalge Company, Rochester, NY, USA) and was centrifuged at 105,000 g for 60 min in a model L-55 ultracentrifuge (rotor type 503 Ti, Beckman Instruments Inc., Palo Alto, CA, USA). The microsomal pellet was washed and resuspended in 100 mM potassium phosphate buffer (pH 7.4). After re-centrifugation at 105,000 g for 60 min, the microsomal pellet was resuspended in a volume of 100 mM potassium phosphate buffer, which contains 1 mM EDTA and 20% v/v glycerol. The final volume was equivalent to original liver weight v/w (1 g of liver to make 1 mL microsome) and stored at -80°C in 1 mL aliquots. Microsome was prepared for each individual rat.

Determination of Microsomal Protein and total P450 Content

Microsomal protein and P450 were measured for each individual rat in both malnourished and control groups. Microsomal protein was quantified using the Bradford method (14). A Standard curve was prepared using bovine plasma gamma globulin (Sigma Diagnostic, St. Louis, MO, USA). The Omura and Sato (15) method was used to measure total P450.

Microsomal Incubation and Substrate and Metabolite Extraction

A final volume of 500 μL microsomal reaction mixture containing a 10 μl aliquot of a testosterone stock solution at substrate concentrations of 5, 10, 20, 40, 80, 125, 250, 500, 1,000, and 2,500 μM, 5 mM $MgCl_2$, 5 μM $MnCl_2$, and 1 mM NADPH in 100 mM potassium phosphate buffer (pH 7.4) was pre-incubated at 37°C for 5 min. The reaction was initiated by adding a measured amount of microsomal protein to the mixture to make the microsomal reaction mixture containing 1 mg/mL microsomal protein, and carried out in air in microcentrifuge tubes (Brinkmann Instruments Inc., Westbury, NY, USA) at 37°C. The reaction was terminated by the addition of 6 mL of methylene chloride after 15 min of incubation, and then 1 nmol of cortexolone was added as an internal standard. The sample was vortexed on a vortex shaker (IKA-VIBRAX-VXR, Terrochem, setting at 1,200) for 15 min and centrifuged (1,000 *g*) for 10 min, then the methylene chloride layer was removed and dried in a speed vacuum evaporator (Speedvac SC100, Franklin Electric, USA). The residues were reconstituted in 80 μl of acetonitrile and 50 μl of water. A 100 μl of reconstituted solution was injected into HPLC.

HPLC Apparatus and Procedure for Testosterone

The HPLC system (Shimadzu, Tokyo, Japan) was equipped with a LC-600 pump, a SIL-9A auto injector, a SPD-6AV UV detector set at 247 nm and an IBM compatible PC computer system equipped with the Baseline software program (Waters, Milford, MA, USA). The column used was LiChrospher 60 RP-select B C_8 5 μm id, 125 × 4 mm (Merck, Darmstadt, Germany).

The separation system consisted of mobile phase A (23% of acetonitrile and 77% water) and mobile phase B (100% of acetonitrile). A gradient system started with 100% of A. Between 8-18 min, mobile phase B was programmed to increase linearly from 0 to 100% and the proportion of B was reduced linearly to 0 from 18-22 min. This condition was held for two minutes for equilibration. The flow rate used was 2 mL/min.

The separation of testosterone and its metabolites produced in a rat liver microsome preparation is shown in Figure 1. The results demonstrate further that there was no baseline interference associated with microsomal incubation and the chromatographic run time was shorter. All recovery rates of testosterone and its metabolites at incubate concentrations of 0.2 and 5 nmol/ml were greater than 95%. The linearity of the method was established over the concentration range 0.2-5 nmol/ml for testosterone and its metabolites. Correlation coefficients were greater than 0.99 for all the compounds measured.

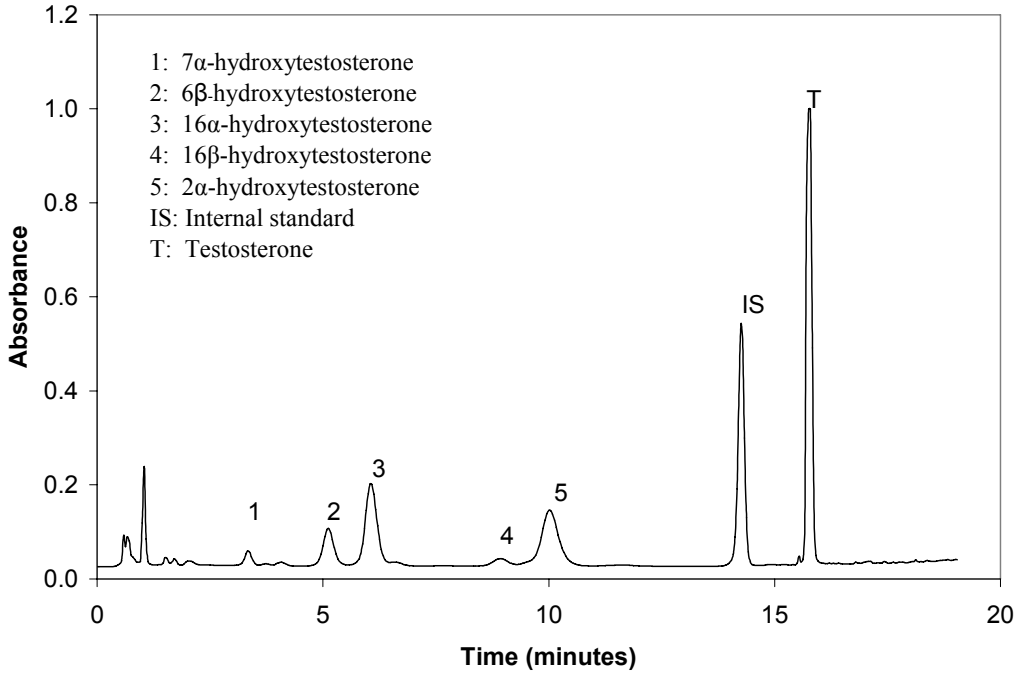

Figure 1. Representative chromatograms for testosterone metabolism in liver microsomes of male SD rats. Microsomal protein (1 mg) was incubated with testosterone (250 μM) for 15 min.

Statistic Analysis

The Lineweaver-Burke plot method (16) was used to estimate the kinetic constants (Km and Vmax) for the disappearance of testosterone for each individual rat. Mean and standard deviation (SD) were calculated from the parameters of the individual rat in the malnourished or control groups.

Student t-test was used to analyze all the parameters measured in protein and calorie malnourished and control rats. The level of statistical significance of the parameters between the experimental and control groups was identified using the ANOVA analysis. A value of $P<0.05$ was considered statistically significant. Data are expressed as mean ± SD, unless stated otherwise.

RESULTS

After 8 weeks of food restriction in juvenile and adult rats, weight loss, emaciation, loss of turgor in the skin, almost total disappearance of subcutaneous fat and atrophy of the muscles were observed in the food restricted groups. These symptoms are typical for protein and calorie malnutrition and it suggested that 8-week of food restriction induced protein and calorie malnutrition in both juvenile and adult rats. Since the diets for malnourished groups were about 40% to 60% of the control groups, the malnourished groups did not suffer from acute protein malnutrition. The malnourished conditions created in this study would rather be a mimic of the real life situation where the subjects are partially restricted in protein and calorie intake.

The physical and microsomal parameters of the malnourished and control juvenile rats are summarized on Table 1. Serum albumin levels of juvenile rats were similar in both experimental and control group after the 8 weeks of food restriction ($P>0.05$). However, the body weight, and liver weight of juvenile rats decreased to about 50% of the control values, and microsomal protein and total P450 per gram of liver decreased to about 60% of the control. The parameters derived from the testosterone metabolism in liver microsomes are summarized on Table 2. Mean and SD values were calculated from the parameters of each individual rat of the malnourished or control groups. Except for 16β-testosterone hydroxylation, the V_{max} values of the other four hydroxylation pathways decreased significantly in protein and calorie malnourished juvenile animals compared to those of control, while the K_m values of these enzymes increased significantly. The results indicate that the isozyme (CYP2C11) for catalyzing 2α-hydroxylation of testosterone has a large capacity and the catalyzing capacity was impaired by protein and calorie malnutrition.

Table 1 Physical and microsomal parameters in protein and calorie malnourished juvenile rats after 8 weeks of food restriction.

Parameters	Control (n=5)	Malnourished (n=5)
Body weight (g)	470 ± 10	230 ± 14*
Liver weight (g)	16.9 ± 0.5	7.9 ± 0.9*
Serum Albumin (g/L)	35.3 ± 3.9	34.0 ± 2.5
Microsomal Protein (mg/g liver)	43.5 ± 2.5	25.8 ± 2.6*
Total P450 (nmol/g liver)	24.1 ± 3.6	14.3 ± 3.0*

**P < 0.05 compared with controls. Values are reported as mean ± SD.*

Table 2 Mean (± SD) Michaelis-Menten parameters of testosterone metabolism in liver microsomes of juvenile rats

Pathways	V_{max} (pmol/min/mg)		K_m (μM)	
	Control (n=5)	Malnourished (n=5)	Control (n=5)	Malnourished (n=5)
16β-hydroxylation	63.4 ± 6.3	59.1 ± 5.2	38.9 ± 3.3	39.4 ± 4.3
7α-hydroxylation	348.4 ± 35.1	196.4 ± 25.5*	9.1 ± 0.76	16.1 ± 2.7*
2α-hydroxylation	5363 ± 525	2439 ± 225*	15.1 ± 1.7	22.7 ± 2.3*
16α-hydroxylation	3448 ± 345	1538 ± 165*	10.3 ± 1.8	26.6 ± 2.1*
6β-hydroxylation	833 ± 75	322 ± 35*	13.5 ± 1.1	26.3 ± 2.3*

**P < 0.05 compared with controls. Values are reported as mean ± SD.*

Table 3. Physical and microsomal parameters in protein and calorie malnourished Adult rats after 8 weeks of food restriction

Parameters	Control (n=6)	Malnourished (n=6)
Body weight (g)	445 ± 27	267 ± 10*
Liver weight (g)	13.9 ± 1.9	8.1 ± 0.4*
Serum albumin (g/L)	34.5 ± 2.1	34.0 ± 4.8
Total plasma protein (g/L)	66.1 ± 8.6	65.5 ± 10.1
Microsomal Protein (mg/g liver)	40.1 ± 4.1	27.6 ± 3.2*
P450 liver (nmol/g liver)	23.2 ± 2.8	15.8 ± 2.2*

**P < 0.05 compared with controls. Values are reported as mean ± SD.*

The physical and microsomal parameters of the malnourished and control adult rats are summarized on Table 3. After 8 weeks of food restriction, body weight of the malnourished adult rats was about 60% of the controls. Their liver weight decreased proportionally to the body weight and the liver weight of the malnourished ones was also about 60% of the control. The decrease of liver weight was not as dramatic as that in juvenile rats (40% decrease in protein and calorie malnourished adult rats while 50% decrease in protein and calorie malnourished juvenile rats). In addition to serum albumin, total plasma protein level was monitored in protein and calorie malnourished adult rats. No differences in the serum albumin and total plasma protein levels were found between the control and the malnourished rats. The lack of change in the plasma protein levels suggests that these parameters are not good indicators of malnutrition in drug metabolism studies. Changes in parameters such as total P450, microsomal protein and capacity and affinity of major enzymes better reflect a change in nutritional status.

Table 3 also shows that microsomal protein in adult malnourished rats is significantly lower than that of controls, but the decrease was not as significant as that observed in juvenile rats. Total P450 in adult malnourished rats was also significantly lower; it was about 70% of that of the control. Compared to the juvenile study in which total P450 was less than 60% of that of the controls, the impairment is not as severe in the malnourished adult rats.

Table 4 Mean (± SD) Michaelis-Menten parameters of testosterone metabolism in liver microsomes of adult rats after 8 weeks of food restriction

Pathways	V_{max} (pmol/min/mg)		K_m (μM)	
	Control (n=6)	Malnourished (n=6)	Control (n=6)	Malnourished (n=6)
16β-hydroxylation	65.4 ± 10.1	58.3 ± 7.2	35.3 ± 4.3	36.5 ± 5.0
7α-hydroxylation	396.8 ± 34.2	217.4 ± 24.3*	10.2 ± 2.4	17.4 ± 2.7*
2α-hydroxylation	5263 ± 511	2941 ± 289*	16.7 ± 2.7	23.7 ± 3.1*
16α-hydroxylation	2941 ± 299	1960 ± 210*	11.1 ± 2.7	22.5 ± 2.7*
6β-hydroxylation	753 ± 66	414 ± 46*	15.2 ± 1.7	27.3 ± 2.7*

**P < 0.05 compared with controls. Values are reported as mean ± SD.*

Table 4 summarized the values of V_{max} and K_m of 16α-, 2α-, 7α-, 6β- and 16β- hydroxylation of testosterone metabolism pathways in protein and calorie malnourished adult rats. Except for 16β-hydroxylation, the V_{max} values of 16α-, 2α-, 7α- and 6β- testosterone hydroxylation were found to be significantly lower in the malnourished group than those in the controls. Compared to the V_{max} values of those in juvenile rats, the decrease of V_{max} values of 16α-, 2α-, 7α-, 6β-hydroxylation in adult rats was not as dramatic, while the V_{max} of 16β-hydroxylation did decrease, but not significantly. The impairment of the catalyzing capacity caused by protein and calorie malnutrition is evident though the change is less than that in juvenile rats.

The K_m values of 16α-, 2α-, 7α- and 6β-hydroxylation increased significantly. The changes were similar to those observed in the juvenile rats. The K_m of 16β-hydroxylation in adult rats did not show a significant change. A similar observation was made in the juvenile study. The results demonstrate that protein and calorie malnutrition impaired the capacities and affinities of CYP2C11, 3A and 2A1 in protein and calorie malnourished rats, whereas CYP2B1 did not show any significant difference both in capacity and affinity under protein and calorie malnourished condition. The impairment of the enzyme capacities in juvenile rats was more severe than that in adult rats.

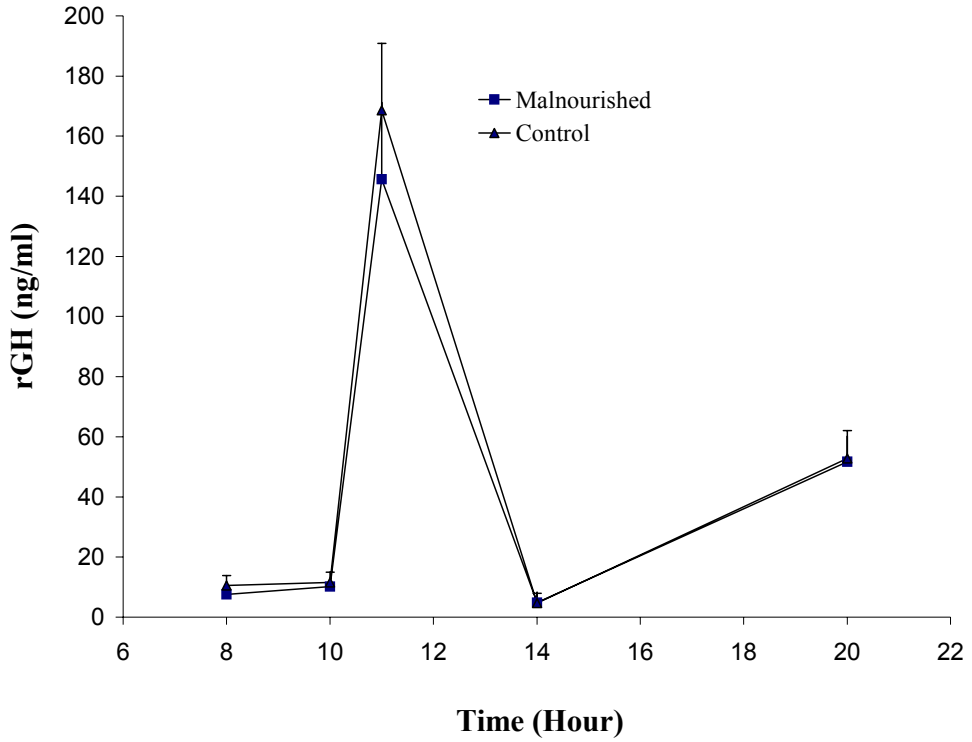

Figure 2. Circadian rhythmicity of rGH (mean ± SD) in juvenile control and protein and calorie malnourished rats after two months of food restriction (n=5/group).

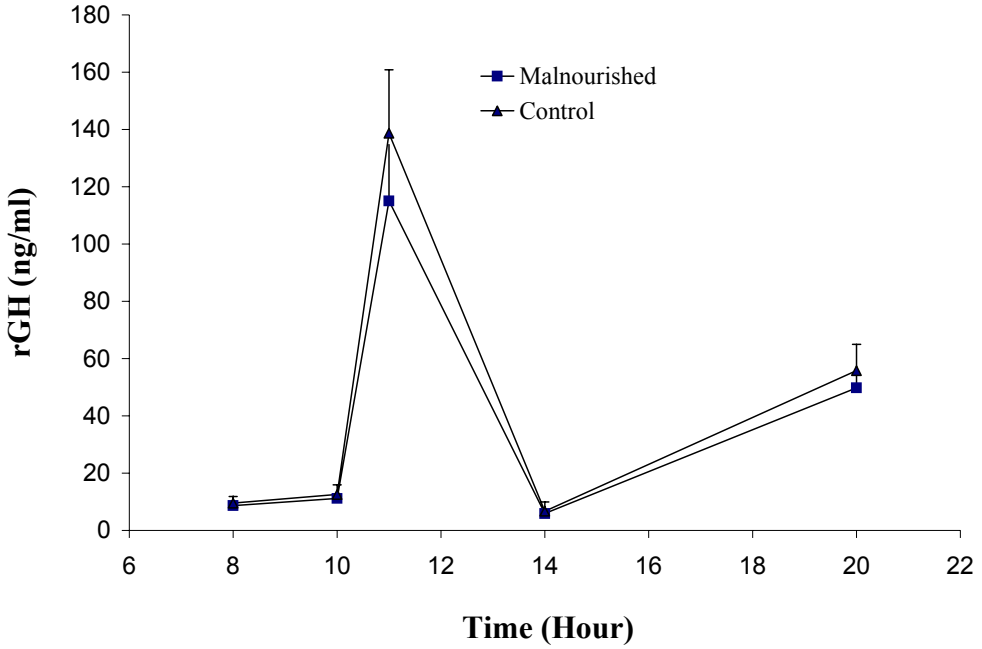

Figure 3. Circadian rhythmicity of rGH (mean ± SD) in adult control and malnourished rats after two months of food restriction (n=6/group).

Figures 2 and 3 illustrate the change of rGH levels in malnourished and controlled groups for juvenile and adult rats, respectively. Blood samples were obtained at the last day of treatment. The rGH values are expressed as Mean ± SD. Blood samples were taken at 8 am, 11 am, 2 pm and 8 pm, as according to the literature, these are the peak and trough times for rGH secretion (17). The rGH levels fluctuate during the day and have peak values at around 11 am. It reaches its trough values at around 2 pm and increases again at around 8 pm.

The results show that the secretion of rGH at peak and trough times was not significantly different between experimental and control groups in either juvenile or adult studies. The secretion pattern, as well as the concentrations of rGH in serum, was very similar in protein and calorie malnourished and control groups of juvenile and adult rats.

At the time when rGH was measured, the juvenile rats were already older than 3 months (40 days + 2 months of food control), which were in the adult rat range. At this age, it is not surprising that the rGH plateau values in the juvenile rats were not significantly different from that of the adult rats. The values of rGH reported in literature vary from study to study; data in this study are comparable with the values reported by Moberg et al (15).

DISCUSSION

The formation of 2α-hydroxytestosterone and 16α-hydroxytestosterone are catalyzed by CYP2C11 (18) and 6β-hydroxytestosterone formation is catalyzed by CYP3A, 7α-hydroxytestosterone formation is catalyzed by CYP2A1, while 16β-hydroxytestosterone formation is catalyzed by CYP2B1 (19). These results suggest that both the capacity and affinity of CYP2C11, CYP2A1, and CYP3A were impaired in protein and calorie malnourished rats while CYP2B1 did not show any significant difference in activity. The results of this study are consistent with those reported in the literature. A decrease in the levels of liver DNA together with a reduction of microsomal proteins, P450, P450 reductase, and several other microsomal enzymes in protein malnourished juvenile rats have been reported (5, 20-22). These studies also showed a decrease in CYP activity under the malnourished conditions. Bulusu and Chakravarty (23) showed that three weeks of protein deprivation (3 or 6 % protein intake) caused significant reductions in the aniline hydroxylase and aminopyrine (substrates for CYP2C and CYP3A) demethylase activities. From the results of this study, it seems that serum albumin level is not a good indicator of malnutrition for drug metabolism studies.

As after 8 weeks of food restriction in juvenile and adult rats, the symptoms of protein and calorie malnutrition (weight loss, emaciation, loss of turgor in the skin, almost total disappearance of subcutaneous fat and atrophy of the muscles) were observed, while serum albumin was virtually unchanged. This is interesting since conventionally, serum albumin has been set as a criterion for the evaluation of animal malnutrition (6, 24). However, this condition would only happen when the subject is suffered from kwashiorkor, an acute form of protein malnutrition. Kwashiorkor manifests as inadequate growth of juvenile subjects, loss of muscular tissue, edema and hepatomegaly. For kwashiorkor subjects, low serum albumin is often observed (25). The form of malnutrition that has been induced in this study is similar to a form of severe malnutrition, namely marasmus. Marasmus is a chronic inanition and is characterized by weight loss, emaciation, loss of turgor in the skin, almost total disappearance of subcutaneous fat and atrophy of the muscles. Serum albumin concentrations may or may not change under this condition. In our study, the animals did not suffer from acute protein malnutrition since the restricted diet contained 17.6% protein. Weight loss, emaciation, almost total disappearance of subcutaneous fat and atrophy of the muscles were observed while low serum albumin, edema and hepatomegaly were not present. The lack of edema in the protein and calorie malnourished animals is a distinctive characteristic of marasmus (3).

CYP2C11 is a major isoenzyme in the liver of male rats. This isoenzyme accounts for about one third of the total cytochrome P450 in SD male rat liver and it metabolizes a host of xenobiotics such as benzphetamine, aminopyrine, ethylmorphine, benzopyrene and warfarin (18). Furthermore, rat CYP2C11 shares most substrates for metabolism with human CYP3A4 (26). Human CYP3A4 is a major isoenzyme in human liver; this single isoenzyme is involved in about 60% of all oxidations of clinically used drugs, including erythromycin, nifedipine, testosterone, and lidocaine (27).

In addition to CYP2C11, the activities of CYP3A and CYP2A1 are also impaired by malnutrition. The metabolism of antipyrine, a CYP2B1 substrate in rat (28, 29), has been contradictory in malnourished studies (30-32). Interestingly, CYP2B1 did not show a significant change in this study.

In the literature, the effect of malnutrition on drug metabolism was studied under different nutritional conditions and the results were conflicting. Anthony (5) reported that protein malnutrition (malnourished groups were fed with food containing 0.5% or 1% protein while control groups were fed with food containing 18% protein) severely impaired drug metabolism. Leakey's (7) group reported that caloric restriction (with 60% of the amount of food consumed by the control group) decreased the age-related changes in hepatic testosterone metabolism (improved drug metabolism). Catz (6) found that in neonatal animals with moderate malnutrition, the difference in the rate of metabolism was not uniform. For oxidative pathways, an increase was found using aminopyrine and benzpyrene as substrates, suggesting CYP2C11 activity was elevated. However, the rate of aniline, a substrate for CYP2E1 metabolism was not altered. For reductive pathways, a decrease was observed with two substrates: p-nitrobenzoic acid and neoprontosil. The results of these studies indicate that malnutrition has different impact on drug metabolism in rats; however, the effect of the changes was not studied in detail.

The novelty of this study is the investigation of drug metabolism changes under the influence of protein and calorie malnutrition. This is different from protein-calorie malnutrition, in that the only nutritional deficiency is protein (5, 8, 9). The condition which is being mimicked here is chronic malnutrition, similar to that observed globally (1). The symptoms observed in malnourished rats in this study are consistent with that of marasmus. It is characterized by weight loss, emaciation, almost total disappearance of subcutaneous fat and atrophy of the muscles.

Malnutrition started at different stages of life has different effects on drug metabolism as shown in this study. In comparison of the results from the juvenile rats to those from the adult rats, the effect of malnutrition was more severe in the juvenile rats. The juvenile rats were in "juvenile" stage when the food restriction began. In juvenile stage, protein synthesis is more sensitive to environmental changes, the immune system is more fragile to foreign insults, and consequently the degree of protein and enzyme reduction is more severe in juvenile protein and calorie malnourished rats (7). Since similar results were observed from both protein and calorie malnourished juvenile rats and protein and calorie malnourished adult rat, age does not seem to play a significant role in producing contradictory results. However, the type of malnutrition may be a major contributing factor. Most of the metabolism studies involving malnutrition focused on protein

malnutrition and no studies have been conducted in protein and calorie malnutrition. More studies are required to evaluate the effects of various forms of nutrition deprivation on drug metabolism.

Some researchers reported a decrease of rGH in malnourished rats. According to the report of Armario et al. (33), food restriction changed the profile of rGH secretion. Rats that had food *ad libitum* showed a rGH secretion profile that fluctuated during the day and had peak values around 11 am and 8 pm and troughs around 2 pm and 2 am, while the profile for the food-restricted rats (65% of the control) was a flat line. Dickerman's group also made a similar observation with starved rats (34). Nevertheless, the study carried out by Moberg et al. (17) showed that the effects of food restriction on rGH levels were slight and transient. After 3 days of food restriction, the plasma levels of rGH were markedly depressed when compared to those of the control SD male rats. After 14 days of food restriction, the daily pattern of plasma GH levels were still depressed, but showed a trend of recovery. After 6 weeks of food restriction, the profile of daily plasma GH rhythm was essentially the same as that for the *ad libitum* fed controls. The depressed levels of GH in the 3-day and 14-day groups may be the result of adapting to the new feeding regimen since various stresses have been reported to suppress GH levels (35-39).

The discrepancy between our observation and those in the literature could be associated with the ages of the animal used and with the duration and degree of food restriction. In Armario's study (33), about 30 day old male SD rats were used. At this age, GH secretion might be very sensitive to environmental stress, like food restriction. After 30 days of food restriction, the animals had not yet adapted to the stress. In Dicherman's study (34), SD male rats were starved for 7 days. The animals had to adapt to the dramatic environmental change, and many physical parameters such as rGH changed to balance the alteration. In some dramatic or short time stress situations, animals are not able to accommodate to the aversive situation, so alteration of rGH is observed. In our study, after two months of food restriction, rats could have already adapted to the stress and the rGH level returned to normal.

Another parameter that should be considered is serum albumin. Researchers (40, 41) observed an inverse relationship of GH with serum albumin levels. They suggested that low serum albumin might act as a stimulus for the alteration of growth hormone levels. In our study, serum albumin was in the normal range, so it is not surprising that we did not see a change in rGH.

From this study, it is concluded that protein and calorie malnutrition decreases microsomal protein and total P450 levels in both juvenile and adult rats. Protein and calorie malnutrition impairs the abilities of CYP2C11, CYP3A, and CYP2A1 to hydrolyze testosterone. CYP2B1 activity did not show any significant change either in capacity or affinity in protein and calorie malnourished juvenile and in protein and calorie malnourished adult rats. Changes in protein and calorie malnourished adult rats were not as dramatic as those in juvenile rats. Neither serum albumin nor total plasma protein level is a good indicator of malnutrition. The impairment of drug metabolism in protein and calorie malnourished rats was not caused by the alteration of rGH. The quantity and the secretion pattern of rGH were not altered in protein and calorie malnourished rats compared to those in healthy animals. The mechanisms that caused the impairment of drug metabolism in malnourished subjects require further investigation.

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