THE INFLUENCE OF L-GLUTAMINE ON THE DEPRESSION OF HEPATIC CYTOCHROME P450 ACTIVITY IN MALE RATS CAUSED BY TOTAL PARENTERAL NUTRITION

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ABSTRACT:

Total parenteral nutrition (TPN) bypasses the gut leading to intestinal and hepatic dysfunction, including decreased hepatic cytochrome P450 (P450) activity. Glutamine prevents the TPN-associated changes in gut function and morphology. This study examined the effect of glutamine supplementation on hepatic P450 activities in male Sprague-Dawley rats receiving continuous TPN. Animals received continuous lipid-free TPN for 7 days with 0, 0.1%, or 4.5% glutamine. Surgical controls were allowed free access to rat chow. The Vmax/Km ratios (intrinsic clearance) for the formation of 4-hydroxymidazolam (CYP3A) were 12.8, 14.6, and 27.7 µl/min/mg for TPN treatment with 0, 0.1%, or 4.5% glutamine, respectively, compared with a chow-fed control (37.1 µl/min/mg). The corresponding values for 1’-hydroxymidazolam formation (CYP3A) were 3.7, 6.1, 11.7, and 15.2 µl/min/mg, respectively. The addition of glutamine to TPN similarly affected the formation rates for 2β- and 6β-hydroxytestosterone (CYP3A), and these metabolite formation rates were highly correlated (r = 0.865; p < 0.001). The formation rates for 2α- and 16α-hydroxytestosterone (CYP2C) were also highly correlated (r = 0.892; p < 0.001). Parenteral glutamine modified the TPN-associated suppression of CYP3A and CYP2C activities in adult male rats receiving TPN.

Total parenteral nutrition (TPN2) is a therapeutic intervention designed to provide sufficient calories and nitrogen to sustain patients who are unable to consume adequate nourishment by mouth and are therefore at risk of developing malnutrition. Unfortunately, alterations in both hepatic and intestinal function accompany therapy with TPN. Bypassing the intestine and the processes involved in nutrient absorption lead to intestinal hypoplasia and both absorptive and immunological hypofunction characterized by decreases in mucosal mass (DNA, RNA, and protein content), intestinal enzymes, villus size, and mitotic index (Hughes et al., 1980; O’Dwyer et al., 1989). These intestinal effects are similar to those observed in starvation. Gut barrier function, critical in preventing bacterial translocation, is also compromised by TPN (Kudsk et al., 1983). Hepatobiliary complications of TPN include fat accumulation, steatosis, cholestasis, cholecystitis, elevated serum transaminase activities, and serum bilirubin (Amii, 1999). TPN depresses both hepatic oxidative (Knodell et al., 1989; Rafatgianis et al., 1996a,b) and conjugative (Rafatgianis et al., 1996a,b) biotransformation.

Glutamine, the most abundant free amino acid in the body, is the preferred fuel for enterocytes and seems to be an essential amino acid for intestinal function (Grant et al., 1977; Leaseburgh et al., 1992). The etiology of TPN-associated cholestasis is poorly understood and probably due to multiple factors, including lack of enteral feeding and possible toxicity from methionine. TPN-associated cholestasis remains a critical problem for infants with intestinal failure (Moss and Amii, 1999). TPN depresses both hepatic oxidative (Knodell et al., 1989; Rafatgianis et al., 1996a,b) and conjugative (Rafatgianis et al., 1996a,b) biotransformation.

Glutamine supplementation prevents this intestinal atrophy. Moreover, glutamine protects the gut mucosa from injury due to experimental endotoxemia (Chen et al., 1994) or from 5-fluorouracil-induced toxicity (Bai et al., 1996). Glutamine-supplemented TPN diminished the intestinal bacterial translocation associated with TPN (Burke et al., 1989; Bai et al., 1996). In rats, parenteral glutamine decreased the TPN-induced lithogenic effects of hepatic bile (Li and Stahlgren, 1995). Clinically, glutamine-supplemented TPN decreased the incidence of sepsis in low-birth-weight infants (New et al., 1999) and improved intestinal function and shortened the hospital stay in adults compared with patients receiving TPN without glutamine (Morison et al., 1998).

The decrease in drug-metabolizing enzymes produced by parenteral nutrition may be related, in part, to TPN-induced changes in intestinal function (Grant et al., 1977; Leaseburgh et al., 1992). The etiology of TPN-associated cholestasis is poorly understood and probably due to multiple factors, including lack of enteral feeding and possible toxicity from methionine. TPN-associated cholestasis remains a critical problem for infants with intestinal failure (Moss and Amii, 1999). TPN depresses both hepatic oxidative (Knodell et al., 1989; Rafatgianis et al., 1996a,b) and conjugative (Rafatgianis et al., 1996a,b) biotransformation.

Results from this work were presented in poster format at the International Society for the Study of Xenobiotics 10th North American Meeting, Indianapolis, IN, October 24, 2000 and have been published in abstract form in Drug Metab Rev (2000) 32:225 (Abstract 177).

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2 Abbreviations used are: TPN, total parenteral nutrition; MDZ, midazolam; P450, cytochrome P450; HPLC, high-performance liquid chromatography.

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function. This led us to test the hypothesis that the addition of glutamine to parenteral nutrition, sufficient to reverse the TPN-induced intestinal effects, would prevent the TPN-related depression in hepatic P450 activity. The effects of administering TPN with glutamine on hepatic CYP2C and CYP3A activities were examined in a physiological relevant, chronically catheterized rat model (Uhing and Kimura, 1995; Rafiqoglanis et al., 1996a). Hepatic CYP2C and CYP3A activities were selectively probed by quantifying the formation rates of relevant monohydroxyl metabolites of testosterone and midazolam (MDZ) in microsomes from rats receiving TPN with and without glutamine and in chow-fed surgical controls.

### Materials and Methods

#### Chemicals.
Midazolam, 1'-hydroxymidazolam, and 4-hydroxymidazolam were gifts of Hoffmann-La Roche (Nutley, NJ and Basel, Switzerland). β-NADPH and L-glutamine were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Testosterone, androstenedione, and 2α, 2β, 6α, 6β-, and 16α-hydroxytestosterone were obtained from Steraloids, Inc. (Newport, RI). Xylazine was obtained from Butler Co. (Columbus, OH), and ketamine was obtained from the Parke-Davis Division of Warner Lambert Company (Morris Plains, NJ). HPLC-grade solvents and all other reagents were obtained from Fisher Scientific (Pittsburgh, PA).

#### Animal Preparation.
Male Sprague-Dawley rats weighing 225 to 250 g were obtained from Harlan Bioproducts for Science (Indianapolis, IN) and allowed free access to rat chow and water. The animals were housed on shaved bedding for 5 days under a 12-h light/dark cycle before surgery. All experimental procedures conformed to the guidelines promulgated by the National Institutes of Health and Purdue University, and the study was approved by the Institutional Animal Care and Use Committee.

Surgery was performed on all animals, as described previously (Uhing and Kimura, 1995; Rafiqoglanis et al., 1996a). Animals were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg) administered by intramuscular injection. Body temperature was maintained with Deltaphase (BrainTree Scientific, Inc., Braintree, MA) heating pads. Using an over-the-needle technique (Kimura et al., 1988), a catheter was placed in the inferior vena cava for infusion of parental nutrition. Briefly, a midline vertical incision was made from the base of the sternum to just above the suprapiaphragmatic region. The catheter was introduced into the inferior vena cava using a 22-gauge, 1-inch Insyte i.v. catheter/needle unit (BD Biosciences, San Jose, CA) and secured in place with a drop of cyanoacrylate glue. The abdominal wall and skin were each closed with a 4-0 silk suture, and the infusion set was sutured securely to the back of the rat using a 0-0 silk suture and glued together using silicone glue. The surgical procedure was completed in less than 15 min. Upon completion of the surgery, 0.1 mg of butorphanol tartrate (Fort Dodge Laboratories, Inc., Fort Dodge, IA) was administered subcutaneously for postoperative pain. Twice daily after surgery, the catheters were gently flushed with 0.5 ml of 0.9% sodium chloride containing heparin (20 IU/ml) and ampicillin (4 mg/ml).

#### TPN Administration and Glutamine Supplementation.
Surgically prepared animals were divided into four groups and received 1) TPN without glutamine, 2) TPN plus 0.1% (w/v) glutamine, 3) TPN plus 4.5% (w/v) glutamine, or 4) ad libitum rat chow. Animals receiving TPN were weaned onto the full-strength solution. On postop day 1, animals received 10% dextrose in lactated Ringer’s solution containing 1 U/ml heparin at a constant rate of infusion of 1.0 ml/h (Harvard model 22 infusion pump; Harvard Apparatus, South Natick, MA). On postop day 2, the infusion rate was increased to 2.0 ml/h. On day 3, full-strength TPN (25% dextrose, 5% Aminosyn II (Abbott Laboratories, North Chicago, IL) in a balanced electrolyte solution (sodium, 100 mEq/l; chloride, 80 mEq/l; potassium, 8 mEq/l; magnesium and phosphate, 13 mEq/l) with vitamins (2.5 ml/MVIL; Astra Pharmaceutical Products, Inc., Westborough, MA) and 1 U/ml heparin] was infused at a constant rate of 2.15 ml/h. The TPN solution contained 1.1 kcal/ml, providing the rats with approximately 220 to 250 kcal/kg/day. Aminosyn II is a glutamine-free amino acid solution. Fresh TPN solutions were prepared every 48 h and sterilized during administration using an inline 0.45-μm filter (Acrodisc; Gelman Sciences, Ann Arbor, MI). The two groups of glutamine-treated rats received TPN plus 0.1 or 4.5% (w/v) L-glutamine (L-2-aminoglutaric acid). The three groups of rats received TPN solutions that were isocaloric but were not isonitrogenous.

Rats were individually housed on shaved bedding in small Plexiglas cages that permitted the maximum allowable freedom. The animals were weighed, and the bedding changed on a daily basis. Treated animals received full-strength intravenous nutrition for 7 days and access to water ad libitum.

#### Preparation of Microsomes, Midazolam and Testosterone Hydroxylase Assays.
Before the preparation of hepatic microsomes, the infusion of TPN was discontinued, and rats were lightly anesthetized with carbon dioxide and immediately sacrificed by decapitation. The livers were rapidly perfused in situ with cold isotonic saline. Liver microsomes were prepared by differential centrifugation (Franklin and Estabrook, 1971) and stored at ~80°C in 50 mM Tris-chloride, pH 7.4, in 250 mM sucrose until assayed for activity. The protein concentration of microsomal fractions was determined colorimetrically (Lowry et al., 1951). Microsomal CYP3A activity was assessed using the formation rates of 1'- and 4-hydroxymidazolam and 2β- and 6β-hydroxytestosterone. CYP2C activity was assessed from the formation rates of 2α- and 16α-hydroxytestosterone.

The rate of midazolam hydroxylation was determined using a previously published method (Gorski et al., 1994). Briefly, 0.4-ml incubations containing 100 μg of microsomal protein, 100 mM sodium phosphate buffer, pH 7.4, 25 mM magnesium chloride, and a range of 13 midazolam concentrations (1, 2, 3, 4, 5, 10, 20, 40, 80, 100, 250, 500, and 1000 μM) were preincubated for 1 min at 37°C. The reaction was initiated by adding 100 μl of 5 mM β-NADPH (final concentration, 1 mM). The metabolism at 37°C was terminated after 1 min by the addition of 1000 μl of cold methanol containing desmethyldiazepam as the internal standard. The rate of midazolam hydroxylation was also determined with cDNA-generated CYP3A1 and CYP3A2 from a baculovirus-infected cell expression system (Supersomes; GENTEST, Woburn, MA). Supersomes contained P450 protein plus cDNA-expressed rat P450 reductase (260–310 nmol/min/mg) and human cytochrome b5 (290 pmol/mg). The concentration of CYP3A1 and CYP3A2 was 0.12 and 0.18 nmol/mg, respectively, in these preparations. Metabolite formation rates were determined at 1, 2, 4, 10, 20, 35, 50, and 100 μM midazolam concentrations.

The rate of testosterone hydroxylation were determined using modified published methods (Waxman, 1988; Chang et al., 1996). Briefly, 0.8-ml incubations containing 600 μg of microsomal protein, 100 mM sodium phosphate buffer, pH 7.4, 25 mM magnesium chloride and testosterone, and 250 μM were preincubated at 37°C for 1 min. Metabolism was initiated by the addition of 200 μl of 10 mM β-NADPH (final concentration, 1 mM). The metabolism was terminated at 10 min by the addition of 6 ml of ethyl acetate containing androstenedione as the internal standard.

#### HPLC Determination of Midazolam and Testosterone.
Microsomes incubated with MDZ were processed using a liquid-liquid extraction technique, as described previously (Gorski et al., 1994). Following evaporation of the solvent, the residue was reconstituted with 200 μl of mobile phase (acetonitrile, methanol, and 20 mM ammonium acetate, pH 7.3; 40:20:40) and a portion was injected onto an HPLC column. MDZ, 4-OH MDZ, 1’-OH MDZ, and desmethyldiazepam were separated using a Phenomenex Luna C8 column (5 μ × 4.6 mm × 150 mm) and a Brownlee RP-18 guard column. The mobile phase was delivered at a flow rate of 1 ml/min, and the eluent was delivered to a mass spectrometer (Naviator, Thermo Finnigan, San Jose, CA). The atmospheric pressure chemical ionization probe was run in the positive ion mode with source and probe temperatures of 200°C and 550°C, respectively. MDZ and desmethyldiazepam were detected in the selected ion-encoding mode at m/z 326 and 271, respectively. The m/z for the 4-OH and 1’-OH MDZ was set at 342.3. The limit of quantification was 0.25 ng/ml for MDZ and metabolites.

Microsomes incubated with testosterone were extracted with ethyl acetate, evaporated to dryness, and reconstituted with 200 μl of mobile phase (methanol:30 mM ammonium acetate, pH 6.5; 63:37); a portion was injected onto the HPLC. The mobile phase was delivered isocratically at a flow rate of 1 ml/min, and the peaks were quantified by UV spectroscopy at 254 nm. Drug and metabolites were separated using a Phenomenex Luna C18 column (5 μ × 4.6 mm × 250 mm) and SecurityGuard C18 guard column. The retention times for the 2α-, 2β-, 6β-, and 16α-hydroxytestosterone, androstenedione, and testosterone were 11.0, 11.9, 6.9, 8.0, 16.1, and 21.1 min, respectively.

#### Analysis of Kinetic Data and Statistics.
The data for each microsomal
incubation represent the mean of duplicate assays. Untransformed kinetic data from the midazolam incubations were fitted to the following equation:

$$ v = \frac{V_{\text{max}} \cdot S}{K_m + S} $$

for a single site, nonallosteric Michaelis-Menten enzyme model by nonlinear least-squares regression (WinNonLin v 1.1; Pharsight, Mountain View, CA). The appropriateness of the fit and the weighting schemes were determined by the visual inspection of residual patterns, residual sums of squares, and precision of the parameter estimates. Midazolam kinetics were performed using microsomes from a representative individual animal from each of the treatment groups. Testosterone metabolite data are reported as mean ± S.D. The effects of TPN with and without glutamine on the hydroxysterosterone metabolite formation rates were detected by single-factor analysis of variance, and the groups responsible for an effect were identified by Tukey’s multiple comparison test. Differences were judged to be due to TPN or glutamine supplementation rather than chance variation when $P < 0.05$.

### Results

**Midazolam Metabolism by Rat Liver Microsomes.** The total body weight and liver weight for the animals from each nutrient treatment group are summarized in Table 1. Animals typically lose 10 to 15% of preoperative body weight after surgery. TPN provided sufficient calories for the animals to regain and maintain their initial body weight over the course of the experiment. The liver weight was decreased in rats receiving TPN compared with chow-fed controls, and this reached statistical significant only in animals receiving the high-dose glutamine. The kinetic data were normalized to microsomal protein (i.e., nanomoles per minute per milligram). When analyzed per whole liver or per gram of liver, the results and conclusions were unchanged.

The formation rates of 4-OH MDZ and 1′-OH MDZ were characterized in hepatic microsomes from a single representative animal in each of the four nutrient treatment groups. The hydroxymidazolam metabolite formation rate versus substrate concentration plots and the transformed Lineweaver-Burk plots for the chow-fed, TPN, and TPN plus 4.5% glutamine animals are shown in Figs. 1 through 3, respectively. The TPN plus 0.1% glutamine plot is not shown. The Lineweaver-Burk plots were linear for both metabolites in all four groups. The formation rates of both 4-OH and 1′-OH MDZ in rat hepatic microsomes were linear with respect to the incubation times up to 5 min in the presence of 100 μg of protein. An incubation time of 1 min was routinely used to insure initial rate conditions. Rat liver microsomes formed mainly 4-hydroxymidazolam. The 1′,4-dihydroxymidazolam metabolite was not detected in any of the incubations. The intrinsic clearance ($V_{\text{max}}/K_m$) value for 4-OH MDZ in the chow-fed animal was 37.1 μmol/min/mg of protein. This declined to 12.8 in the animal receiving TPN without glutamine due to a decrease in $V_{\text{max}}$. The addition of 0.1% glutamine had no restorative effect on $V_{\text{max}}$ or $K_m$. The $V_{\text{max}}$ and intrinsic clearance were restored by the addition of 4.5% glutamine to 93 and 75% of the values measured in the chow-fed control.

![Fig. 1. Kinetics of formation of 4-OH midazolam (■) and 1′-OH midazolam (□) in rat liver microsomes from a chow-fed control. A, plot of formation rate versus midazolam concentration. B, plot of the transformation of the Michaelis-Menten kinetics to the Lineweaver-Burk equation.](image)

![Fig. 2. Kinetics of formation of 4-OH midazolam (■) and 1′-OH midazolam (□) in rat liver microsomes from a rat receiving TPN. A, plot of formation rate versus midazolam concentration. B, plot of the transformation of the Michaelis-Menten kinetics to the Lineweaver-Burk equation.](image)

### Table 1

<table>
<thead>
<tr>
<th>Weight</th>
<th>Chow-Fed (n = 5)</th>
<th>TPN (n = 8)</th>
<th>TPN Plus Glutamine 0.1% (n = 8)</th>
<th>TPN Plus Glutamine 4.5% (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative TBW</td>
<td>271 ± 23</td>
<td>291 ± 12</td>
<td>289 ± 16</td>
<td>272 ± 49</td>
</tr>
<tr>
<td>Final TBW</td>
<td>280 ± 27</td>
<td>291 ± 33</td>
<td>273 ± 14</td>
<td>278 ± 46</td>
</tr>
<tr>
<td>Liver weight</td>
<td>11.0 ± 2.2</td>
<td>9.3 ± 1.4</td>
<td>9.3 ± 1.9</td>
<td>8.1 ± 1.3*</td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared to chow-fed controls by analysis of variance with Tukey’s multiple comparison test.
formation rate versus substrate concentration plot and the transformed Lineweaver-Burk plots for CYP3A1 and CYP3A2 are shown in Figs. 4 and 5, respectively. cDNA-expressed CYP3A2 preferentially formed 4-OH MDZ over 1'-OH MDZ, whereas CYP3A1 enzyme formed more 1'-OH MDZ compared with 4-OH MDZ. The $K_m$ for the formation of the 4-OH MDZ was 31.0 and 30.1 $\mu$M by CYP3A1 and CYP3A2, respectively. The $K_m$ for the formation of the 1'-OH MDZ was 24.7 and 8.7 $\mu$M by CYP3A1 and CYP3A2, respectively. The $V_{\text{max}}$ values for the formation of 4-OH MDZ by CYP3A2 and CYP3A1 were 8.43 and 1.09 nmol/min/mg, respectively. The $V_{\text{max}}$ values for the formation of 1'-OH MDZ by CYP3A2 and CYP3A1 were 1.66 and 1.16 nmol/min/mg, respectively. The intrinsic clearance ($V_{\text{max}}/K_m$) for 4-OH MDZ formation by CYP3A2 and CYP3A1 was 280.1 and 35.2 $\mu$mol/min/mg of protein, respectively, and for 1'-OH MDZ was 67.2 and 133.3 $\mu$mol/min/mg.

**Testosterone Metabolism by Rat Liver Microsomes.** The formation rates for 2α-OH and 16α-OH testosterone (CYP2C) and 2β-OH and 6β-OH testosterone (CYP3A) from rats in the four nutrient treatment groups are summarized in Table 3. Compared with chow-fed controls, the CYP3A-mediated hydroxylation of testosterone was significantly decreased in all rats receiving TPN. In rats receiving TPN with 4.5% glutamine, the CYP3A-mediated hydroxylation of testosterone was significantly increased compared with rats receiving TPN alone or TPN supplemented with 0.1% glutamine. A similar pattern was observed for the formation rates of 2α- and 16α-OH testosterone. The formation rates of 2α-OH and 16α-OH testosterone were highly correlated (Fig. 6), as were the formation rates of 2β-OH and 6β-OH testosterone (Fig. 7). Conversely, the formation rates of 2α-OH testosterone with either 2β-OH ($r = 0.49$) or 6β-OH testosterone ($r = 0.61$) were weakly correlated. Similarly, the formation rates of 16α-OH testosterone with either 2β-OH ($r = 0.38$) or 6β-OH testosterone ($r = 0.49$) were only weakly correlated (graphs not shown).

Finally, we compared the amount of 1'-OH MDZ and 4-OH MDZ formed using cDNA-expressed CYP2C11, CYP3A1, and CYP3A2 proteins. Following a 1-min incubation using 50 $\mu$M midazolam, the activities of CYP2C11, CYP3A1, and CYP3A2 for the formation of 4-OH MDZ were 0.16, 6.06, and 30.7 pmol/min/pmol of P450, respectively. The respective activities for the formation of 1'-OH MDZ were 0.15, 9.33, and 6.44 pmol/min/pmol of P450.

**Discussion**

The current study was designed to evaluate whether the addition of glutamine to TPN prevented the depression in hepatic P450 activity that is a consequence of TPN. CYP2C and CYP3A are the principal hepatic drug-metabolizing enzymes in male rats, and the formation of mono- and dihydroxy metabolites of midazolam and testosterone have been used to profile these enzyme activities.

TPN produces significant intestinal and hepatic pathologies, including depression of hepatic drug metabolism. Ross et al. (1982) first reported that 7 days of lipid-free TPN decreased rat hepatic P450 content and cytochrome $p_450$ concentration. These investigators subsequently showed that TPN decreased the in vivo elimination of antipyrene by 73% (Ross et al., 1983). Knodell and colleagues (1989) found that the administration of TPN to rats for 7 days significantly reduced the constitutive amounts and activities of CYP3A and CYP2C11 but not of CYP2A1 and CYP2C6. Rafiogianis et al. (1996a,b) found that administration of TPN, with and without lipid calories, to rats for 10 to 14 days significantly reduced P450 activity and sulfate and glucuronide conjugation. The present study found that CYP2C activity decreased about 35% in rats receiving TPN, but in contrast to Knodell et al. (1989), this did not reach statistical significance.

Glutamine is a five-carbon amino acid with two amino moieties and is the most abundant amino acid in plasma. Glutamine is an important factor in nitrogen transport, in intermediary metabolism, and as a cosubstrate for the formation of glutathione (Labow and Souba, 2000). Glutamine is also an important source of fuel for enterocytes. Glutamine depletion can occur during such catabolic states as surgery, trauma, or sepsis. Standard TPN solutions are not formulated with glutamine because of the chemical instability of glutamine. Thus, TPN administration routinely produces glutamine depletion, leading to intestinal mucosal atrophy and subsequent gut and liver hypofunction. Several investigators have shown in animal studies that the TPN-induced changes in gut physiology, including increased bacterial translocation (Alverdy and Burke, 1992), can be prevented by administering glutamine-enriched parenteral solutions (Burke et al., 1989; 1996a, 1996b).

**TABLE 2**

**Effect of TPN and TPN plus glutamine on the formation of 4-hydroxymidazolam and 1'-hydroxymidazolam by rat hepatic microsomes**

Male Sprague-Dawley rats received total parenteral nutrition for 7 days containing 0, 0.1 or 4.5% (w/v) glutamine. Hepatic microsomes were prepared as described under Materials and Methods. Duplicate incubations were performed at MDZ concentrations ranging from 1 to 1000 $\mu$M incubated with $\beta$-NADPH for 1 min at 37°C in microsomes from a representative animal from each nutrient group. Kinetic parameters were determined by nonlinear least-squares regression.

<table>
<thead>
<tr>
<th>Nutrient Group</th>
<th>4-Hydroxymidazolam</th>
<th>1'-Hydroxymidazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$</td>
<td>$V_{\text{max}}$</td>
<td>$V_{\text{max}}/K_m$</td>
</tr>
<tr>
<td></td>
<td>$\mu$M</td>
<td>$\mu$mol/min/mg</td>
</tr>
<tr>
<td>ad lib chow</td>
<td>114.2</td>
<td>4.24</td>
</tr>
<tr>
<td>TPN</td>
<td>118.4</td>
<td>1.52</td>
</tr>
<tr>
<td>TPN with glutamine 0.1%</td>
<td>130.4</td>
<td>1.90</td>
</tr>
<tr>
<td>TPN with glutamine 4.5%</td>
<td>143.0</td>
<td>3.96</td>
</tr>
</tbody>
</table>

**Fig. 3.** Kinetics of formation of 4-OH midazolam (■) and 1'-OH midazolam (□) in rat liver microsomes from a rat receiving TPN plus 4.5% (w/v) glutamine.

A, plot of formation rate versus midazolam concentration. B, plot of the trans-
decreasing the elevated portal insulin to glucagon ratio produced by continuous TPN.

CYP3A2 and CYP2C11 are the major P450 proteins found in rat liver. Both CYP3A1 and CYP3A2 are expressed in the liver; however, CYP3A2 is considered the predominant hepatic form in male rats (Park et al., 1986; Cooper et al., 1993), whereas CYP3A1 is more predominately expressed in extrahepatic tissues (Debril et al., 1995). CYP3A is the only CYP3A expressed in the intestine (Zhang et al., 1996). 2β-OH testosterone is mainly formed by CYP3A, whereas 6β-OH testosterone is formed by CYP1A1, CYP2A2, CYP2C13, and CYP3A (Waxman, 1988). The metabolite formation rates of 2β-OH and 6β-OH were strongly correlated suggesting that these two metabolites are formed mainly by CYP3A. MDZ is almost exclusively metabolized by CYP3A in rats. The $V_{\text{max}}$ ratio of the 4-OH MDZ to 1'-OH MDZ ranged from 3.0 to 4.5 in rat microsomes. The $V_{\text{max}}$ ratios of 4-OH MDZ to 1'-OH MDZ for the cDNA-expressed CYP3A2 and CYP3A1 were 5.0 and 0.6, respectively, which is consistent with CYP3A2 being the predominant CYP3A expressed in rat liver. 2α-OH and 16α-OH testosterone are the major metabolites of CYP2C11, and the formation rates for these metabolites were also highly correlated.

Several groups have previously reported apparent $K_m$ values for the rat hepatic microsomal metabolism of midazolam (Ghosal et al., 1996; Takedomi et al., 1998; Higashikawa et al., 1999; Yamano et al., 1999). In the present study, we found linear Lineweaver-Burk kinetics for the formation of both 4-OH and 1'-OH midazolam using 1-min incubations. Ghosal et al. (1996) incubated microsomes for 15 min and detected linear kinetics for the formation of the monohydroxy metabolite and nonlinear Michaelis-Menten formation of the 1'-4-dihydroxy metabolite. $K_m$ values for the formation of 1'-OH and 4-OH MDZ reported by Ghosal et al. (1996) were similar to our values using the cDNA-expressed enzymes but lower compared with microsomes from rats receiving TPN or chow-fed control. Iga and colleagues (Takedomi et al., 1998; Yamano et al., 1999) and Higashikawa et al. (1999) measured the rate of midazolam disappearance and reported a midazolam $K_m$ in the range of 6 to 8 μM using incubation times ranging from 30 s to 5 min. Overall, the differences in $K_m$ values may reflect differences in nonspecific protein binding, the range of substrate concentrations studied, surgical treatment, the duration of the incubation times, and/or techniques of data fitting and analyses.

In conclusion, the present study demonstrates that supplementation of TPN with glutamine can largely prevent the TPN-induced depression in hepatic P450 activity. The inclusion of 4.5% glutamine into the TPN infusion prevented the depression in CYP3A activities as characterized by 2β-OH and 6β-OH testosterone formation rates and 4-OH and 1'-OH MDZ kinetics. The relative lack of effect of TPN or glutamine supplementation on the $V_{\text{max}}$ ratios of 4-OH MDZ to 1'-OH

### TABLE 3

<table>
<thead>
<tr>
<th>Nutrient Group</th>
<th>Formation Rate of Hydroxytestosterone (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ad lib chow (n = 5)</td>
<td>0.72 ± 0.22$^a$</td>
</tr>
<tr>
<td>TPN (n = 7)</td>
<td>0.47 ± 0.24</td>
</tr>
<tr>
<td>TPN with 0.1% glutamine (n = 8)</td>
<td>0.47 ± 0.23</td>
</tr>
<tr>
<td>TPN with 4.5% glutamine (n = 6)</td>
<td>0.73 ± 0.43</td>
</tr>
</tbody>
</table>

$^a$ Values are expressed as mean ± S.D.
$^b$ $P < 0.05$ compared to ad lib chow by analysis of variance and Tukey’s multiple comparison test.
$^c$ $P < 0.05$ compared to TPN by analysis of variance and Tukey’s multiple comparison test.
MDZ and the testosterone metabolite correlations suggests that the effects of glutamine is not enzyme-selective. The addition of glutamine to TPN is important for the maintenance of gut structure and function. This, in turn, prevents the hepatotoxic effects of parenteral nutrition, including the depression of hepatic drug metabolism.

**References**
