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 to TPN similarly affected the formation rates for 1'-hydroxymidazolam formation (CYP3A) were 3.7, 6.1, 11.7, and 15.2 l/min/mg, respectively. The addition of glutamine to TPN treated for 1'-hydroxymidazolam formation (CYP3A) were 12.8, 14.6, and 27.7 l/min/mg for TPN treatment with 0, 0.1%, or 4.5% glutamine, respectively, compared with a chow-fed control (37.1 l/min/mg). The corresponding values for 1'-hydroxymidazolam formation (CYP3A) were 3.7, 6.1, 11.7, and 15.2 l/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, cholelithiasis, elevated serum transaminase activities, and serum bilirubin (Quigley et al., 1993) and may be related to TPN-induced changes in intestinal function (Grant et al., 1977; Leaseburge 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; Rafogianis et al., 1996a,b) and conjugative (Rafogianis 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 (Platell et al., 1993; Horvath et al., 1996). However, glutamine is chemically unstable and therefore not included in standard TPN formulations. Administration of parenteral (Platell et al., 1993) or enteral (Horvath et al., 1996) nutrition without glutamine produces intestinal atrophy and ulcerations in laboratory animals. 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 pathogenic effects of hepatic bile (Li and Stahlgren, 1995). Clinically, glutamine-supplemented TPN decreased the incidence of sepsis in low-birth-weight infants (Neu et al., 1999) and improved intestinal function and shortened the hospital stay in adults compared with patients receiving TPN without glutamine (Mortion 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. 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 (Uehing and Kimura, 1995; Rafiqoglanis et al., 1996a). Hepatic CYP2C and CYP3A activities were selectively probed by quantifying the formation rates of relevant monohydroxy 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 Hoffman-La Roche (Nutley, NJ and Basel, Switzerland). β-NADPH and t-glutamine were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Testosterone, androstenedione, and 2α-, 2β-, 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 (Uehing 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 Deltagel (BrainTein 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 parenteral nutrition. Briefly, a midline vertical incision was made from the base of the sternum to just above the suprapubic region. The catheter was introduced into the inferior vena cava using a 22-gauge, 1-inch Inyte 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 secured in place with a drop of cyanoacrylate glue. The abdominal region. The catheter was introduced into the inferior vena cava using a 4.6 mm and desmethyldiazepam were separated using a Phenomenex Luna C18 column (5 μm, 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 satured 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 infused at a constant 342.3. The limit of quantification was 0.25 ng/ml for MDZ and desmethyldiazepam as the internal standard. The rate of midazolam hydroxylation was also determined with cDNA-generated CYP3A1 and CYP3A2 from a baculovirus-infected insect cell expression system (Supersomes; GENTEST, Woburn, MA). Supersomes contained 54050 protein plus cDNA-expressed rat P450 reductase (260–3100 nmol/min/mg) and human cytochrome b5 (290 nmol/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 rates 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 a range of 13 midazolam concentrations (1, 2, 3, 4, 5, 10, 20, 40, 80, 100, 250, 500, and 1000 μM) were preincubated 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 insect cell expression system (Supersomes; GENTEST, Woburn, MA). Supersomes contained 54050 protein plus cDNA-expressed rat P450 reductase (260–3100 nmol/min/mg) and human cytochrome b5 (290 nmol/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 rates 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, and desmethyldiazepam were separated using a Phenomenex Luna C8 column (5 μm, 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- recording 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 μm × 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:

\[ v = \frac{V_{\text{max}} \cdot S}{K_{\text{m}} + S} \]

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 hydroxytestosterone 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 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 hydroxytestosterone 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 \( P < 0.05 \).

**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.

\( \text{TBW} \) represents total body weight. 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.

**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.

**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.

K\(_{\text{m}}\) for the reaction expressing 4-OH MDZ in the chow-fed control animal was 1.31 nmol/min/mg and 15.2 \( \mu \)l/min/mg, respectively (Table 2). Both parameters declined by 76% in the animal receiving TPN without glutamine. TPN high-dose glutamine completely restored the V\(_{\text{max}}\) and partially restored the intrinsic clearance values to 77% of that measured in the chow-fed control animal.

cDNA-Expressed CYP3A1 and CYP3A2. The hydroxy metabolite
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 ($C_{\text{int}} = \frac{V_{\text{max}}}{K_m}$) for 4-OH MDZ formation by CYP3A2 and CYP3A1 was 280.1 and 35.2 $\mu$M/min/mg of protein, respectively, and for 1'-OH MDZ was 67.2 and 133.3 $\mu$M/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. In rats receiving TPN with 4.5% glutamine, the formation rate for 2β-OH and 6β-OH 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 ($r = 0.61$) 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 monohydroxy 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 antipyrine 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. Rafiqogianis 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; 1993).

**TABLE 2**

<table>
<thead>
<tr>
<th>Nutrient Group</th>
<th>$K_m$ (4-Hydroxymidazolam) $\mu$M</th>
<th>$V_{\text{max}}$ (4-Hydroxymidazolam) nmol/min/mg</th>
<th>$V_{\text{max}}/K_m$ (4-Hydroxymidazolam) $\mu$mol/min/mg</th>
<th>$K_m$ (1'-Hydroxymidazolam) $\mu$M</th>
<th>$V_{\text{max}}$ (1'-Hydroxymidazolam) nmol/min/mg</th>
<th>$V_{\text{max}}/K_m$ (1'-Hydroxymidazolam) $\mu$mol/min/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib chow</td>
<td>114.2</td>
<td>4.24</td>
<td>37.1</td>
<td>86.2</td>
<td>1.31</td>
<td>15.2</td>
</tr>
<tr>
<td>TPN</td>
<td>118.4</td>
<td>1.52</td>
<td>12.8</td>
<td>86.4</td>
<td>0.32</td>
<td>3.7</td>
</tr>
<tr>
<td>TPN with glutamine 0.1%</td>
<td>130.4</td>
<td>1.90</td>
<td>14.6</td>
<td>93.2</td>
<td>0.50</td>
<td>5.4</td>
</tr>
<tr>
<td>TPN with glutamine 4.5%</td>
<td>143.0</td>
<td>3.96</td>
<td>27.7</td>
<td>117.6</td>
<td>1.31</td>
<td>11.7</td>
</tr>
</tbody>
</table>
GLUTAMINE AND TPN-INDUCED DECREASE IN HEPATIC P450 ACTIVITY

O'Dwyer et al., 1989). In addition, the effects of glutamine seem to be dose-dependent. Platell et al. (1993) found that reversal of the TPN-induced small-bowel atrophy in rats required TPN concentrations of glutamine above 1.5 to 2%. At these levels, glutamine also prevented the hepatic steatosis and biliary lithogenic effects of TPN, probably by glutamine above 1.5 to 2%. At these levels, glutamine also prevented induced small-bowel atrophy in rats required TPN concentrations of dose-dependent. Platell et al. (1993) found that reversal of the TPN-O

\[ \frac{1}{V_{\text{max}}} = \frac{K_m}{V_{\text{max}}} + \frac{1}{V_{\text{max}}} \]

A. plot of formation rate versus midazolam concentration. B. plot of the transformation of the Michaelis-Menten kinetics to the Lineweaver-Burk equation.

Fig. 5. Kinetics of formation of 4-OH midazolam (■) and 1′-OH midazolam (○) in cDNA-expressed CYP3A2 microsomes containing cDNA-expressed rat P450 reductase and human cytochrome b₅. A, plot of formation rate versus midazolam concentration. B, plot of the transformation of the Michaelis-Menten kinetics to the Lineweaver-Burk equation.

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

Effect of TPN and TPN plus glutamine on the formation rate of 2α-, 2β-, 6β-, and 16α-hydroxytestosterone in rat hepatic microsomes

Male Sprague-Dawley rats received TPN for 7 days containing 0.1 or 4.5% (w/v) glutamine or allowed ad lib rat chow. Hepatic microsomes were prepared as described under Materials and Methods. Duplicate incubations were performed at testosterone concentrations of 250 μM with β-NADPH for 10 min at 37°C.

<table>
<thead>
<tr>
<th>Nutrient Group</th>
<th>Formation Rate of Hydroxysteroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2α-</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>ad lib chow (n = 5)</td>
<td>0.72 ± 0.22a</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>

* Values are expressed as mean ± S.D.

** P < 0.05 compared to ad lib chow by analysis of variance and Tukey’s multiple comparison test.

*** P < 0.05 compared to TPN by analysis of variance and Tukey’s multiple comparison test.


