Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism

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Nemours Children's Clinic, Jacksonville, Florida 32207; Centre de Recherche en Nutrition Humaine, 44093 Nantes Cedex 1, France; and United States Department of Agriculture Children's Nutrition Research Center, Houston, Texas 77030-2600

Darmaun, Dominique, Susan Welch, Annie Rini, Brenda K. Sager, Astride Altomare, and Morey W. Haymond. Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E801–E807, 1998.—The present study was designed to determine whether sodium phenylbutyrate (ΦB) acutely induces a decrease in plasma glutamine in healthy humans, and, if so, will decrease estimates of whole body protein synthesis. In a first group of three healthy subjects, graded doses (0, 0.18, and 0.36 g·kg⁻¹·day⁻¹) of ΦB were administered for 24 h before study; postabsorptive plasma glutamine concentration declined in a dose-dependent manner, achieving an ~25% decline for a dose of 0.36 g ΦB·kg⁻¹·day⁻¹. A second group of six healthy adults received 5-h infusions of L-[1-14C]leucine and L-[1-13C]glutamine in the postabsorptive state on two separate days: 1) under baseline conditions and 2) after 24 h of oral treatment with ΦB (0.36 g·kg⁻¹·day⁻¹) in a randomized order. The 24-h phenylbutyrate treatment was associated with 1) an ~26% decline in plasma glutamine concentration from 514 ± 24 to 380 ± 15 μM (means ± SE; P < 0.01 with paired t-test) with no change in glutamine appearance rate or de novo synthesis; 2) no change in leucine appearance rate (Rs), an index of protein breakdown (123 ± 7 vs. 117 ± 5 μmol·kg⁻¹·h⁻¹; not significant); 3) an ~22% rise in leucine oxidation (Ox) from 23 ± 2 to 28 ± 2 μmol·kg⁻¹·h⁻¹ (P < 0.01), resulting in an ~11% decline in nonoxidative leucine disposal (NOLD = Rs − Ox), an index of protein synthesis, from 100 ± 6 to 89 ± 5 μmol·kg⁻¹·h⁻¹ (P < 0.05); 4) the data suggest that, in healthy adults, large doses of oral phenylbutyrate can be used as a “glutamine trap” to create a model of glutamine depletion; 2) a moderate decline in plasma glutamine does not enhance rates of endogenous glutamine production; and 3) a short-term depletion of plasma glutamine decreases estimates of whole body protein synthesis.

protein metabolism; nutrition; stable isotopes; radioactive tracers

GLUTAMINE IS the most abundant amino acid in the body and comprises two-thirds of the intracellular free amino acid pool (3, 17). In human plasma, the concentration of glutamine (typically ~400–600 μM) exceeds that of all other amino acids and is four times that of leucine (3, 17). Even though glutamine can be synthesized de novo and is therefore considered a nonessential amino acid, glutamine concentration may participate in the regulation of protein homeostasis. Indeed, a twofold rise in plasma glutamine concentration, obtained through a 5-h enteral infusion of natural L-glutamine, acutely inhibited leucine oxidation and increased nonoxidative leucine disposal (NOLD), an index of whole body protein synthesis, in healthy humans in the postabsorptive state (10). Conversely, a precipitous drop in the muscle free glutamine pool is observed in life-threatening conditions that are associated with protein wasting (17, 30–32), and replenishment of muscle glutamine stores is associated with improved nitrogen balance in such conditions (15, 30, 32, 36). These data collectively suggest that the maintenance of glutamine at its high, normal concentration in most biological fluids may play a unique role to sustain protein synthesis. If so, then an acute decrease in plasma glutamine concentration may cause protein wasting in healthy humans. The purpose of this study was to test the latter hypothesis.

Sodium phenylbutyrate and sodium phenylacetate are used in the clinical management of children with urea cycle enzymatic defects as a “glutamine trap” (4, 19). As shown by Brusilow (4), phenylbutyrate does not accumulate in plasma: within minutes after ingestion of large doses of phenylbutyrate (up to 0.6 g·kg⁻¹·day⁻¹), the latter is converted to phenylacetate by β-oxidation (phenylacetylglutamine is normally produced only in small quantities from hepatic metabolism of phenylalanine). Phenylacetate in turn reacts with glutamine in liver and kidney to yield phenylacetylglutamine (Fig. 1). The latter is quantitatively excreted as such in the urine and seems to substitute for urea as a means to eliminate excess ammonia. In children with inborn errors of urea cycle metabolism and high glutamine and ammonia concentrations, administration of phenylbutyrate indeed causes an abrupt decrease in plasma glutamine. The drug caused a decline in plasma glutamine even in those among the infants whose plasma glutamine levels were within normal limits (4). Even though other investigators (20, 28) have administered small doses of phenylacetate to healthy adults with the aim of using phenylacetylglutamine as a probe of intrahepatic tracer dilution in tricarboxylic acid cycle intermediates, it is not known whether large doses of phenylbutyrate alter plasma glutamine concentration in healthy adults with an intact urea synthetic pathway.

The present studies were therefore designed to determine 1) whether phenylbutyrate treatment affects plasma glutamine in healthy adults; 2) if so, whether this effect is dose dependent; and 3) whether a phenylbutyrate-induced decline in plasma glutamine concentration will decrease estimates of whole body protein synthesis.

METHODS

Materials

Solutions of L-[1-14C]leucine (>55 mCi/mmol; New England Nuclear, Boston, MA), L-[1-13C]glutamine (99% 13C), L-[5,5,5-
GLUTAMINE DEPLETION AND PROTEIN CATABOLISM

Experimental Design

Two different protocols (A and B) were carried out. Protocol A consisted of preliminary studies designed to assess the plasma glutamine response to graded doses of sodium phenylbutyrate. Three subjects (2 male, 1 female) underwent isotope infusions in the postabsorptive state on three separate days, several days apart. Each received 0, 0.18, and 0.36 g·kg⁻¹·day⁻¹ sodium phenylbutyrate per os divided in six equal doses for 24 h before and throughout the infusion study. The dose range selected was based on doses commonly used in the treatment of patients with inborn errors of urea synthesis (2). The order of the doses was randomized. During each infusion day, subjects received unprimed, continuous infusion of L-[1-13C]glutamine as the sole tracer, and arterIALIZED venous blood samples were obtained at 30-min intervals throughout the 300 min of the isotope infusion to assess the rise of [13C]glutamine toward plateau (5, 19). Protocol B was designed to assess the effect of a chosen dose of phenylbutyrate on leucine kinetics. Eight (5 male, 3 female) subjects were studied on two separate days, at least 4 days apart, after receiving either 0.36 g·kg⁻¹·day⁻¹ sodium phenylbutyrate or no treatment at all. The order of the studies was randomized. During each of the two isotope infusion days, all eight subjects received intravenous primed, continuous infusions of L-[1-14C]leucine.

Isotope Infusion Protocol

For each protocol, the night before each infusion study day, each subject ate dinner at 1800 and then remained fasting (with the exception of water and calorie-free, caffeine-free drinks) until completion of the infusion study at 1300 the following day. On the next morning at 0700, each subject was studied as an outpatient in the Baptist Medical Center/Wolfson Children’s Hospital Clinical Investigation Unit. Two short catheters were inserted, one in a forearm vein for isotope infusion and the other one in a superficial vein of the contralateral hand; the hand was placed in a warming pad at ~30°C to obtain arterialized venous blood samples (5). Five-hour continuous infusions of tracers were administered in the postabsorptive state between 0800 and 1300: 1) unprimed infusions of L-[1-13C]glutamine (=8 µmol·kg⁻¹·h⁻¹) were administered in protocol A; and 2) primed, continuous infusions of L-[1-13C]leucine (0.08 µCi/kg; ~0.08 µCi·kg⁻¹·h⁻¹) and L-[1-13C]glutamine (=8 µmol/kg; ~8 µmol·kg⁻¹·h⁻¹) in 6 subjects were administered in protocol B. Two (male) subjects in protocol B received primed infusion of L-[5,5,5-2H₃]leucine, H₁³CO₃Na, and L-[1-14C]leucine, but no L-[1-13C]glutamine, to assess the effects of sodium phenylbutyrate on 1³CO₂ fixation and the δ-[1-2H]ketosacproprate (KIC)-to-[1-2H]leucine enrichment ratio. Arterialized venous blood samples were obtained 30 and 15 min before the start and at 30-min intervals for the entire 5 h of the infusion in protocol A and between the 2nd and the 5th h of isotope infusion in protocol B. Plasma was analyzed for the isotopic enrichment and/or specific activity of plasma leucine and glutamine. Aliquots of expired air were collected before the start and during the last 2 h of isotope infusion to determine breath 1³CO₂ enrichments. The rate of respiratory 1³CO₂ excretion (dpm/min) was determined (protocol B) by obtaining timed 2-min collections of expired air into Douglas bags using a mouthpiece equipped with a one-way valve; the CO₂ in the collected air was then quantitatively trapped by slowly bubbling the expired air into a solution of ethanolamine as described previously (24). Breath air 1³CO₂ specific activity (dpm/mmol) was determined by bubbling aliquots of expired air through 2 ml of a 0.5 mM hyamine solution as described previously (24).

Analytical Methods

Plasma amino acid concentrations were determined by ion exchange chromatography using a Beckman 6300 amino acid analyzer (Beckman Instruments, Palo Alto, CA). Breath 1³CO₂ enrichments were measured by gas chromatography-isotope ratio mass spectrometry (Isochrom III). Plasma [1³C]glutamine enrichments and [1-2H]leucine and [1-2H]KIC were determined by selected-ion monitoring GC-MS (Hewlett-Packard MSD 5970), as described previously (10, 26, 27). Plasma leucine 1³C specific activity (SA) was determined by HPLC (on a Spectraphysics SP8800 instrument equipped with a Pharmacia LKB Frac 200 fraction collector) as described previously (14). The 1³C specific activity of leucine
fractions and breath CO₂ was measured on a Beckman LS6500 scintillation counter. Because of interference of phenylacetate, the main metabolite of phenylbutyrate, with the HPLC assay of KIC, we used the “primary pool” model to calculate leucine kinetics, i.e., based on plasma leucine specific activities rather than the preferred “reciprocal pool model” in which leucine kinetics are derived from KIC specific activities (13).

Calculations

Leucine appearance rate. Leucine appearance rate into plasma (RaLeu; µmol·kg⁻¹·h⁻¹) was calculated using standard isotope dilution equations for steady-state conditions as described in earlier studies (8, 18); RaLeu = iLeu/SAleu, where iLeu is the rate of [¹⁴C]leucine infusion (dpm·kg⁻¹·h⁻¹), and SAleu is the [¹⁴C]specific activity of plasma leucine (dpm/µmol)

Leucine oxidation. Leucine oxidation (OxLeu; µmol·kg⁻¹·h⁻¹) was calculated based on the excretion of labeled CO₂ in expired air: OxLeu = F₁⁴CO₂/(SAleu × 0.7), where F₁⁴CO₂ is the rate of [¹⁴C]CO₂ excretion in expired air (µmol·kg⁻¹·h⁻¹), and 0.7 is the assumed fractional recovery of labeled carbon in expired air in the postabsorptive state (12).

NOLD. NOLD (µmol·kg⁻¹·h⁻¹) was calculated as NOLD = RaLeu − OxLeu. Because leucine is assumed to be either oxidized or incorporated into protein, NOLD is an index of whole-body protein synthesis (13, 22).

Glutamine appearance into the plasma compartment. Glutamine appearance into the plasma compartment (RaGln; µmol·kg⁻¹·h⁻¹) was calculated based on the release of glutamine from protein breakdown (BGln) and glutamine de novo synthesis (DGln) contribute to glutamine Ra. Although body protein is a nonessential amino acid, both glutamine release (47.9/61.1 mmol glutamine, since 1 mmol glutamine > 146 mg, and 7,000/146 = 47.9) or 0.78 mmol glutamine/mmol leucine (47.9/61.1 = 0.78).

Glutamine release from protein breakdown. Because glutamine is a nonessential amino acid, both glutamine release from protein breakdown (BGln, µmol·kg⁻¹·h⁻¹) and glutamine de novo synthesis (DGln) contribute to glutamine Ra. Although body protein is known to contain 13.9 g of glutamine plus glutamate per 100 g protein, the exact contribution of glutamine per se to that total amount is not precisely known (10, 21). As in recent studies, BGln was estimated as 0.78 × RaLeu. This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 8,000 mg leucine, i.e., 61.1 mmol leucine (as 1 mmol leucine = 131 mg and 8,000/131 = 61.1); and 3) glutamine contributes one-half the total glutamine plus glutamate content of body protein, i.e., ≈7,000 mg glutamine (≈47.9 mmol glutamine, since 1 mmol glutamine = 146 mg, and 7,000/146 = 47.9), or 0.78 mmol glutamine/mmol leucine (47.9/61.1 = 0.78).

DGlutamine. The fraction of glutamine Ra that cannot be accounted for by release of glutamine from protein breakdown was attributed to DGlutamine. DGlutamine was therefore estimated by DGlutamine = RaGlutamine − BGlutamine (10).

Glutamine oxidation. Glutamine oxidation (OXGlutamine) was estimated based on the respiratory excretion of [¹³C]CO₂ and was calculated as OXGlutamine = (ECO₂ × VCO₂)/(EP × 0.7), where VCO₂ is the rate of CO₂ excretion (µmol·kg⁻¹·h⁻¹), ECO₂ is steady-state [¹³C]CO₂ enrichment (mole % excess) in expired air, and 0.7 is the assumed fractional recovery of labeled carbon in expired air in the postabsorptive state (12).

Glutamine tracer-miscible pool size. For studies in protocol A, the rise in plasma [¹³C]glutamine enrichment from 0 to 300 min was fitted to a single exponential curve using nonlinear regression (7, 26) as follows: EGlutamine = ĒGlutamine (1 – e⁻ⁿ), where ĒGlutamine is a function of sampling time t (min). The fitted parameters are enrichment at plateau, ĒGlutamine (mole % excess), and the rate constant for glutamine turnover, k (min⁻¹). Tracer-miscible glutamine pool size (poolGlutamine) can be estimated as RaGlutamine/k for each individual subject in protocol A (26).

Fractional recovery of labeled bicarbonate in breath. In the two patients (patients 7 and 8) who received [¹³C]bicarbonate infusion, the fractional recovery of labeled bicarbonate in breath (FRCO₂) was calculated based on the excretion of [¹³C]CO₂ in expired air after the course of an intravenous infusion of NaH[¹³C]CO₃ as FRCO₂ = VCO₂ × (ECO₂/100)/F̄CO₂, where ECO₂ is the steady-state enrichment (mole % excess) of expired air [¹³C]CO₂ during the last 2 h of the NaH[¹³C]CO₃ infusion, VCO₂ (µmol·kg⁻¹·h⁻¹) is measured by indirect calorimetry, and F̄CO₂ (µmol·kg⁻¹·h⁻¹) is the rate of NaH[¹³C]CO₃ infusion.

Statistical Analysis

Data are presented as means ± SE. During the tracer infusions, steady state for plasma amino acid levels and enrichments were defined by the absence of a significant correlation of the measured parameter vs. time over the considered period. Data were compared using paired t-tests. Significance was established at P < 0.05.

RESULTS

Response of Plasma Glutamine to Phenylbutyrate Treatment

Plasma glutamine concentrations were near steady state during the 5 h of each isotope infusion study; the average coefficients of variation (± 100 × SD/mean) of plasma glutamine concentration were 7.1, 12.9, and 11.6% with 0, 0.18, and 0.36 g·kg⁻¹·day⁻¹ of phenylbutyrate, respectively, in the three subjects assessed in protocol A. As shown in Fig. 2, a dose-dependent reduction in plasma glutamine concentration was observed in each of the three subjects studied, with the decline in glutamine level reaching 19 ± 4 and 30 ± 3% of control values after 24 h of 0.18 and 0.36 g·kg⁻¹·day⁻¹ of phenylbutyrate, respectively. The estimated size of glutamine tracer-miscible pool was 255 ± 59 µmol/kg under baseline conditions (Table 1). Even though there was a clear decline in the estimated pool size (to an average of 69 ± 10% of baseline values) with 0.18 g·kg⁻¹·day⁻¹ of phenylbutyrate in each subject, results were less clear-cut with the higher dose: the glutamine pool returned to baseline in two subjects yet remained depressed in one subject. Overall, the average estimated pool size had returned to 93 ± 22% of baseline with the higher dose (Table 1).

Effect of Phenylbutyrate on Leucine Kinetics

Based on the preliminary experiments described above, we elected to use the dose of 0.36 g·kg⁻¹·day⁻¹ for studying the effect of phenylbutyrate-induced glutamine depletion on leucine kinetics in the second group of patients. Plasma leucine concentration was at near steady state during each of the isotope infusions, with an average coefficient of variation of 7.6 ± 1.6 and 11.9 ± 2.6% over the 5 h of the isotope infusion study on the baseline study day and the phenylbutyrate treat-
ment day, respectively. Compared with the control study day, plasma leucine concentrations (65 ± 6 vs. 132 ± 16 µM) were lower ($P$, 0.001) on the phenylbutyrate treatment day. As shown in Fig. 3, $R_{\text{aLeu}}$ did not differ between study days: 122 ± 5 vs. 117 ± 5 µmol·kg$^{-1}$·h$^{-1}$ (not significant) at baseline and after phenylbutyrate treatment, respectively. In contrast, leucine oxidation increased ($P$, 0.01) < 22% from 23 ± 2 to 28 ± 2 µmol·kg$^{-1}$·h$^{-1}$ on the phenylbutyrate day, and $N_{\text{OLD}}$ decreased ($P$, 0.05; 1-tail $t$-test) by < 11% from 100 ± 6 to 89 ± 5 µmol·kg$^{-1}$·h$^{-1}$.

Because determination of leucine oxidation is based on excretion of $^{14}$CO$_2$ during the infusion of labeled leucine, any factor affecting the recovery of labeled CO$_2$ might affect the measured rates of leucine oxidation. In additional experiments performed in two subjects (subjects 7 and 8), we verified that the recovery of labeled CO$_2$ was not significantly affected by phenylbutyrate treatment: upon primed, continuous infusion of $^{13}$HCO$_3$Na, the recovery of labeled 13C in expired air averaged 81% (76 and 87%) vs. 80% (77 and 83%) for baseline vs. phenylbutyrate treatment (subjects 7 and 8, respectively).

The steady-state plasma $[^3]$H$_{KIC}$-to-$[^3]$H$_{\text{Leu}}$ enrichment ratio was not affected by phenylbutyrate treatment in the two subjects who received an L-$[^5,5,5]$-2H$_3$-leucine infusion (1.03 ± 0.04 vs. 0.99 ± 0.04 in subject 7 and 0.72 ± 0.02 vs. 0.67 ± 0.02 in subject 8, control vs. phenylbutyrate treatment days, respectively).

**Effect of phenylbutyrate on glutamine kinetics.** The rate of appearance of glutamine into plasma, glutamine oxidation rate, and the estimated release from protein breakdown and de novo synthesis remained unaltered after phenylbutyrate treatment compared with baseline in the six subjects who received infusions of L-$[^1]$-13C-glutamine (Table 2). Because plasma glutamine was not significantly affected by phenylbutyrate treatment: upon primed, continuous infusion of H$^{13}$CO$_3$Na, the recovery of labeled 13C in expired air averaged 81% (76 and 87%) vs. 80% (77 and 83%) for baseline vs. phenylbutyrate treatment (subjects 7 and 8, respectively).

**Table 1.** Effect of phenylbutyrate on glutamine tracer-miscible pool in 3 healthy subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose of Phenylbutyrate, g·kg$^{-1}$·day$^{-1}$</th>
<th>µmol/kg</th>
<th>% of baseline</th>
<th>µmol/kg</th>
<th>% of baseline</th>
<th>µmol/kg</th>
<th>% of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>175</td>
<td>57%</td>
<td>100</td>
<td>97%</td>
<td>169</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>370</td>
<td>89%</td>
<td>330</td>
<td>199</td>
<td>281</td>
<td>100%</td>
</tr>
<tr>
<td>3</td>
<td>0.36</td>
<td>219</td>
<td>59%</td>
<td>130</td>
<td>128%</td>
<td>281</td>
<td>100%</td>
</tr>
</tbody>
</table>

Means ± SD

<table>
<thead>
<tr>
<th>µmol/kg</th>
<th>% of baseline</th>
</tr>
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<tbody>
<tr>
<td>255 ± 102</td>
<td>187 ± 125</td>
</tr>
<tr>
<td>93 ± 37%</td>
<td>69 ± 18%</td>
</tr>
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</table>

**Table 2.** Effect of phenylbutyrate treatment on glutamine kinetics in 6 healthy adults

<table>
<thead>
<tr>
<th></th>
<th>[GLN], µM</th>
<th>$R_{\text{aGLN}}$, µmol·kg$^{-1}$·h$^{-1}$</th>
<th>$B_{\text{GLN}}$, µmol·kg$^{-1}$·h$^{-1}$</th>
<th>$D_{\text{GLN}}$, µmol·kg$^{-1}$·h$^{-1}$</th>
<th>$O_{\text{GLN}}$, µmol·kg$^{-1}$·h$^{-1}$</th>
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<tbody>
<tr>
<td>Basal</td>
<td>519 ± 27</td>
<td>304 ± 25</td>
<td>96 ± 5</td>
<td>208 ± 26</td>
<td>196 ± 24</td>
</tr>
<tr>
<td>Phenylbutyrate</td>
<td>385 ± 17</td>
<td>302 ± 27</td>
<td>89 ± 5</td>
<td>213 ± 23</td>
<td>164 ± 13</td>
</tr>
<tr>
<td>$P$</td>
<td>0.0003</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. [GLN], plasma glutamine concentration; $R_{\text{aGLN}}$, glutamine appearance rate; $B_{\text{GLN}}$, glutamine release from protein breakdown; $D_{\text{GLN}}$, glutamine de novo synthesis; $O_{\text{GLN}}$, glutamine oxidation. $P$, comparison with baseline using 2-tail paired $t$-test. NS, not significant.
amine was ≈25% lower, whereas glutamine \( R_3 \) remained unaltered, glutamine metabolic clearance rate (\( = \frac{R_{Gln}}{[Gln]} \), where [Gln] is glutamine concentration) was higher on the phenylbutyrate treatment day than at baseline (776 ± 43 vs. 589 ± 46 ml·kg\(^{-1}·h^{-1} \); \( P < 0.05 \)).

**DISCUSSION**

The results of the current study demonstrate that a short course of treatment with the drug phenylbutyrate is able to induce acute glutamine depletion even in healthy adult humans with an intact urea synthetic pathway and normal baseline glutamine levels. They further show that, upon treatment with phenylbutyrate, 1) rates of glutamine de novo synthesis fail to increase, and 2) leucine oxidation increases, whereas NOLD, an index of protein synthesis, declines. This study suggests that 1) short-term treatment with phenylbutyrate can be used as an experimental model to create glutamine depletion in healthy humans and 2) an acute depletion of plasma glutamine may decrease estimates of whole body protein synthesis.

Although the “trapping effect” of phenylbutyrate only occurs in intracellular space in selected tissues (presumably liver and kidney), a highly significant, dose-dependent decline in glutamine concentration was detected in systemic plasma (Fig. 2). The main repository of glutamine in the body is, however, skeletal muscle rather than plasma (3, 17). Assuming 1) a muscle mass of ≈28 kg in a 70-kg subject, 2) an intramuscular glutamine concentration of ≈19.5 mmol/kg of intracellular water (3, 17), and 3) an intracellular water content of ≈77% in muscle, baseline intramuscular glutamine content can be estimated at ≈420 mmol. Based on the stoichiometry of phenylacetylglutamine synthesis (Fig. 1), 0.36 g·kg\(^{-1}·day^{-1} \) phenylbutyrate should “trap” an equimolar amount of glutamine, i.e., 1.935 mmol glutamine·kg\(^{-1}·day^{-1} \), or 135.5 mmol/day in a 70-kg subject. Thus the amount of glutamine presumably “trapped” by phenylbutyrate over a 24-h regimen is equivalent to ≈32% of the estimated glutamine content of skeletal muscle. As muscle is the main source of plasma glutamine, a loss of this magnitude (wherever it occurred within the body) is unlikely to have spared the intramuscular glutamine pool, especially since the loss was not compensated by any appreciable rise in rates of glutamine de novo synthesis and/or release from protein breakdown (Table 2). We therefore speculate that phenylbutyrate induced depletion of the muscle free glutamine pool.

In vitro studies suggest that glutamine itself regulates the activity of glutamine synthetase: for instance, increasing the glutamine concentration of the culture medium inhibits glutamine synthetase in cultured muscle cells (29). Accordingly, we observed in an earlier study that rates of glutamine de novo synthesis were suppressed in vivo when exogenous glutamine was infused to raise plasma glutamine concentration in healthy volunteers (10). In contrast, in the current study, the acute drop in plasma glutamine concentration was not associated with a rise in estimated rates of whole body glutamine de novo synthesis. The lack of a stimulation of glutamine de novo synthesis ultimately accounts for the fact that the subjects did not compensate for the continuous “trapping” of glutamine by phenylacetate and were therefore unable to maintain plasma glutamine concentrations at their baseline level. The current data suggest that, in this experimental model, the body's capacity to synthesize glutamine may have been limited, or, in other words, that glutamine may have become an essential amino acid. In that regard, short-term phenylbutyrate treatment may therefore be used as an experimental model for other states of glutamine depletion in which increased glutamine utilization by splanchnic tissues exceeds the endogenous capacity of glutamine synthesis, as is the case during severe illness.

Although glutamine is the most abundant amino acid in the body and can be synthesized de novo, a precipitous fall in muscle glutamine concentration indeed is a hallmark of multiple clinical situations associated with protein wasting such as burns, surgical stress, or bone marrow transplantation (17, 30–32). With stress, a large increase in glutamine synthesis occurs concomitantly with the depletion in the muscle glutamine pool (1, 2). The precise mechanisms by which both glutamine production and glutamine utilization are enhanced with stress are, however, still debated. Because glutamine is predominantly released by skeletal muscle and taken up in splanchnic tissues, the depletion of the muscle glutamine pool observed during stress could result from an initial, dramatic rise in glutamine release from muscle; increased glutamine release would, in turn, enhance glutamine uptake by the gut and liver. Alternatively, the primary event may be a dramatic surge in splanchnic glutamine uptake, which could lead to a secondary rise in glutamine outflow from muscle. Furthermore, it is not known whether glutamine depletion is simply a meaningless epiphenomenon, reflecting the severity of stress, or, alternatively, a major disturbance that has severe consequences by itself. The glutamine depletion induced by phenylbutyrate differs from stress-induced protein wasting inasmuch as it specifically depletes glutamine pools, without altering glutamine production (Table 2); it therefore creates a much simpler model of an isolated increase in glutamine “clearance.” Although the “model” of glutamine depletion created by phenylbutyrate treatment may not give any clue as to the mechanisms leading to glutamine depletion during stress, the “phenylbutyrate model” may prove useful to examine the consequences of an isolated increase in glutamine depletion per se on various functions in the body and thus help delineate some of the complex consequences of stress-induced protein wasting.

The rise in leucine oxidation observed during phenylbutyrate treatment is remarkable as it occurred at a time when plasma leucine concentration was lowered. Indeed, in most circumstances, rates of leucine oxidation are a direct function of the prevailing plasma leucine concentration (9, 23). Because neither bicarbonate retention nor the ratio of plasma [\(^{1}\text{H}_{2}\)KIC to \(^{1}\text{H}_{3}\)leucine enrichment was affected by phenylbutyr-
ate (see RESULTS), the change in measured rates of leucine oxidation must result from an actual change rather than from a change in the leucine-to-KIC isotope ratio.

It should be pointed out that, in theory, the observed increased rate of leucine oxidation could be a direct effect of sodium phenylbutyrate through stimulation of branched-chain ketoacid dehydrogenase or other mechanisms, rather than a direct effect of glutamine depletion per se. Further experiments with the simultaneous administration of phenylbutyrate and glutamine supplementation are underway to resolve this issue.

Animal studies revealed a striking correlation between the size of the intracellular glutamine pool and the rates of muscle protein synthesis in rats submitted to dietary restriction or endotoxin injection (16, 18). Improvement in nitrogen balance was observed upon replenishment of the muscle glutamine pool with intravenous glutamine supplementation in several clinical studies (30, 32, 36). Yet conflicting data have appeared more recently, as several groups failed to observe a correlation between muscle protein fractional synthetic rate and muscle glutamine concentration in various models of stress in rats (8, 25, 34, 35) and humans (15). It therefore remains to be established whether the decline in muscle free glutamine concentration observed in stress is merely a reflection of a protein catabolic state or a causative factor. Indeed, the multiple metabolic and hormonal disturbances associated with the disease process or stress per se (33) might concomitantly affect protein synthesis and glutamine concentration without implying a causal relationship between the two events. In the current study, we observed that a moderate decline in plasma glutamine concentration, in otherwise healthy adult humans, was associated with a reduction in estimates of whole body protein synthesis (Fig. 3). In an earlier study, we had associated with a reduction in estimates of whole body concentration, in otherwise healthy adult humans, was observed that a moderate decline in plasma glutamine concentration: a guide in the management of urea cycle disorders. Am. J. Clin. Nutr. 65: 256–262, 1997.


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