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Acute depletion of plasma glutamine increases leucine oxidation in prednisone-treated humans

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Abstract

Background, aims & methods—To determine whether depletion in plasma glutamine worsens the catabolic response to corticosteroids, 7 healthy volunteers received oral prednisone for 6 days on 2 separate occasions, at least 2 weeks apart, and in random order. On the 6th day of each treatment course, they received 5h intravenous infusions of L-[1-¹⁴C]-leucine and L-[1-¹³C]-glutamine in the postabsorptive state *1*) under baseline conditions (prednisone only day), and *2*) after 24h of treatment with phenylbutyrate (prednisone+phenylbutyrate day), a glutamine chelating agent.

Results—Phenylbutyrate treatment was associated with **1**) an ≈15% decline in plasma glutamine concentration (627±39 vs. 530±31 μmol.L⁻¹; *P*<0.05), **2**) no change in leucine appearance rate, an index of protein breakdown (124±9 vs. 128±9 μmol.kg⁻¹.h⁻¹; NS) nor in non oxidative leucine disposal, an index of whole body protein synthesis (94±9 vs. 91±7 μmol.kg⁻¹.h⁻¹; NS); and **3**) a ≈25% rise in leucine oxidation (30±1 vs. 38±2 μmol.kg⁻¹.h⁻¹, *P*<0.05), despite an ≈25% decline (*p*<0.05) in leucine concentration.

Conclusions—In a model of mild, stress-induced protein catabolism, depletion of plasma glutamine *per se* may worsen branched chain amino acid and protein wasting.

Keywords

nutrition; stress; protein wasting; stable isotopes; humans

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Introduction

It has long been known that glucocorticoids induce protein wasting in humans. Both cortisol (1,2) and prednisone (3) indeed are associated with increased rates of leucine release from protein breakdown, and prednisone increased leucine oxidation (3). Glucocorticoid treatment is therefore commonly used as a “model” to mimic the protein wasting that occurs with critical illness.

Critical illness is often associated with the depletion of muscle glutamine stores as well. Although glutamine is the most abundant free amino acid in the body, and can be synthesized *de novo* in most tissues, especially skeletal muscle, a profound depletion of muscle glutamine stores has been observed in patients suffering from stress or undergoing major surgery, and glutamine supplementation has been shown to attenuate protein wasting in several clinical situations (4,5-7). Taken together, data from the literature collectively suggest that glutamine depletion *per se* may have a protein catabolic, or anti-anabolic effect.

Although glucocorticoid treatment produces many of the metabolic alterations observed during severe stress, it fails to produce the plasma glutamine depletion that is commonly observed in the clinical situations associated with the most severe degree of stress, such as extensive burn injury (8). In healthy volunteers, glucocorticoid treatment, in fact, enhances plasma glutamine concentration (9), perhaps as a compensatory reaction to accommodate increased glutamine requirement. If glutamine depletion *per se* has a protein wasting effect, then both enhanced glucocorticoid excess and glutamine depletion could contribute to protein wasting in some clinical settings.

The drug sodium phenylbutyrate is known to be metabolized to phenylacetate in liver and kidney to produce phenylacetylglutamine. The latter is quantitatively excreted as such in urine, and has been used as an effective “glutamine nitrogen trap” in the management of children suffering from inborn errors of urea synthesis who have high glutamine and ammonia concentrations (10). In earlier studies, we demonstrated that large doses of phenylbutyrate, a glutamine chelating agent, can be used as a “glutamine trap” to create an experimental model of acute glutamine depletion in healthy humans with no enzymatic defect. Short-term depletion of plasma glutamine decreased estimates of whole body protein synthesis (11). The purpose of this study was therefore to determine 1) whether phenylbutyrate treatment would decrease plasma glutamine concentration in glucocorticoid-treated subjects, and 2) if so, whether this would alter the protein catabolic response to glucocorticoids.

Materials and Methods

Materials

Solutions of L-[1-¹⁴C]leucine (>55 mCi/mmol; New England Nuclear, Boston, MA), L-[1-¹³C]glutamine (99% ¹³C, Cambridge Isotope Laboratories, Woburn, MA), were mixed with 0.9% saline, and tested for chemical, isotopic, and optical purity by high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GCMS) for radioactive and stable isotope tracers, respectively, and verified to be sterile (plate culture) and pyrogen-free (Limulus lysate assay). Solutions were passed through a 0.2µm Millipore® filter and stored in sterile containers at 4°C for 24h prior to use. Sodium phenylbutyrate (0.5 gram tablets) was purchased from Triple Crown America (Perkasie, PA) after our application for its use as an investigational new drug (IND 46,010) was approved by the Food and Drug Administration.

Subjects

A total of 7 healthy, 25±1 yr.-old (1 female, 6 males) volunteers were recruited for the study. Subjects received detailed information on the purpose and potential risks of the study, and were

enrolled after signing a written consent form, according to protocols reviewed and approved by the Nemours Children's Clinic Research Committee and the Institutional Review Committee at Baptist Medical Center, Jacksonville, FL. Subjects were judged to be free of any chronic or acute illness based on detailed medical history, physical exam, and routine blood chemistry. Women had to have a negative pregnancy test within 48h of study and could not be breastfeeding. A dietary history was obtained and subjects were instructed to maintain their usual intake and their regular level of activity for 3 days prior to the initial study and between the two isotope infusions. The subjects' mean ($\pm$ SE) height, weight, and body mass index were 180 ± 4 cm, 84 ± 4 kg, and 26.1 ± 1.1 kg/m², respectively (Table 1). Even though one subject [#1] had a BMI of 30, and was therefore obese, he was not excluded as he did not present with any obvious complications of obesity, and had, for instance, normal blood pressure, as well as normal; baseline levels of blood glucose, and lipids. Moreover, removing the subject from the group did not alter the overall direction and significance of the changes observed (data not shown).

Experimental design

For 5 days prior to each study day, prednisone was administered *per os* at a dose of $40\text{mg}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ (up to a maximum of 60mg total daily dose), divided in three doses per 24h. During the 5 days preceding one study day (prednisone only day), prednisone was administered alone. In addition, for the 24h preceding the other study day (prednisone + phenylbutyrate day), each subject ingested $0.36\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ of phenylbutyrate, while continuing prednisone treatment. Each subject was thus studied under two different conditions: (a) prednisone alone; and (b) prednisone + phenylbutyrate, in a randomized order.

Isotope infusion protocol

The night before each infusion study day, each subject ate dinner at 1800h, and then remained fasting (with the exception of water and calorie-free, caffeine-free drinks) until completion of the infusion study at 1300h the following day. On the next morning at 0700h, 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 $\approx 60^{\circ}\text{C}$ to obtain arterialized-venous blood samples (12). Five-hour primed, continuous infusions of L-[1-¹⁴C]leucine ($0.0\mu\text{Ci}\cdot\text{kg}^{-1}$; $\approx 0.08\mu\text{Ci}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and L-[1-¹³C] glutamine ($\approx \mu\text{mol}\cdot\text{kg}^{-1}$; $\approx 8\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) were administered in the postabsorptive state between 0800h and 1300h. Arterialized-venous blood samples were obtained 30 and 15min before the start, and at 30min interval between the second and the fifth hour of tracer infusion, to determine the isotopic enrichment and specific activity of plasma leucine and glutamine, respectively. Aliquots of expired air were collected before the start, and during the last 2 hours of isotope infusion. The rate of respiratory ¹⁴CO₂ excretion (dpm/min) was determined 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 (13). Breath air ¹⁴CO₂ specific activity (dpm/mmol) was determined by bubbling aliquots of expired air through 2ml of a 0.5mM hyamine solution as described (13).

Analytical methods

Plasma amino acid concentrations were determined by ion exchange chromatography using a Beckman 6300® amino acid analyzer (Beckman Instruments, Palo Alto, CA). Plasma ¹³C-glutamine enrichments were determined by selected ion monitoring GCMS (Hewlett-Packard MSD 5970) as described previously (2). Plasma leucine ¹⁴C-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 ^{14}C -specific activity of leucine fractions and breath $^{14}\text{CO}_2$ was measured on a Beckman LS6500® scintillation counter. Because of interference of phenylacetate, the main metabolite of phenylbutyrate, with the HPLC assay of ketoisocaproate (KIC), we used the “primary pool” model to calculate leucine kinetics (15), *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 (16).

Calculations Leucine appearance rate into plasma (Ra_{LEU} ; $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$) was calculated using standard isotope dilution equations for steady state conditions as described in earlier studies: $\text{Ra}_{\text{LEU}} = i_{\text{LEU}} / \text{SA}_{\text{Leu}}$, where i_{LEU} is the rate of ^{14}C -leucine infusion ($\text{dpm.kg}^{-1}.\text{h}^{-1}$) and SA_{LEU} is the ^{14}C -specific activity of plasma leucine ($\text{dpm}\mu\text{mol}$) at isotopic steady state. Because leucine is an essential amino acid, the only source of leucine Ra in postabsorptive subjects is leucine release from the breakdown of body protein (15).

Leucine oxidation (Ox_{LEU} ; $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$) was calculated based on the excretion of labeled CO_2 in expired air: $\text{Ox}_{\text{LEU}} = F^{14}\text{CO}_2 / (\text{SA}_{\text{Leu}} \times 0.7)$, where $F^{14}\text{CO}_2$ is the rate of $^{14}\text{CO}_2$ excretion in expired air ($\mu\text{mol.kg}^{-1}.\text{h}^{-1}$), and 0.7 is the assumed fractional recovery of labeled carbon in expired air in the postabsorptive state (17).

Non-oxidative leucine disposal (NOLD ; $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$) was calculated as: $\text{NOLD} = \text{Ra}_{\text{LEU}} - \text{Ox}_{\text{LEU}}$. Because leucine is assumed to be either oxidized or incorporated into protein, NOLD is an index of whole body protein synthesis (15).

Glutamine appearance into the plasma compartment (Ra_{GLN} ; $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$) was calculated as: $\text{Ra}_{\text{GLN}} = i_{\text{GLN}} \times [(E_{\text{GLN}} / E_{\text{PGLN}}) - 1]$, where i_{GLN} is the rate of ^{13}C -glutamine infusion, and E_{GLN} and E_{PGLN} are the ^{13}C -glutamine enrichments (mole % excess) in the infused tracer solution and plasma at steady state, respectively. Ra_{GLN} is an index of interorgan glutamine exchange between tissues (18).

Glutamine release from protein breakdown

Because glutamine is a non essential amino acid, both glutamine release from protein breakdown (B_{GLN}) and glutamine *de novo* synthesis (D_{GLN}) contribute to glutamine Ra. B_{GLN} was estimated as $\text{B}_{\text{GLN}} = k \times \text{Ra}_{\text{LEU}}$, where k is the ratio of glutamine to leucine abundance in protein-bound amino acid residues. This approach assumes that: 1) the release of an amino acid from proteolysis is proportional to its abundance in body proteins; 2) 100g of body protein contain 8g leucine (*i.e.* 61.1 mmol leucine) and 4.5g glutamine (*i.e.*, 31mmol glutamine) (19). Thus $\text{B}_{\text{GLN}} = 0.51 \times \text{Ra}_{\text{LEU}}$.

Glutamine de novo synthesis

The fraction of glutamine Ra that cannot be accounted for by release of glutamine from protein breakdown was attributed to glutamine *de novo* synthesis (D_{GLN}). D_{GLN} was therefore estimated by: $\text{D}_{\text{GLN}} = \text{Ra}_{\text{GLN}} - \text{B}_{\text{GLN}}$ (9,19). *Glutamine oxidation* (Ox_{GLN}) was estimated based on the respiratory excretion of ^{13}C -labeled carbon dioxide, and calculated as $\text{Ox}_{\text{GLN}} = (E_{\text{CO}_2} \times V_{\text{CO}_2}) / (E_{\text{P}} \times 0.7)$, where V_{CO_2} is the rate of CO_2 excretion ($\mu\text{mol.kg}^{-1}.\text{h}^{-1}$), E_{CO_2} is steady state $^{13}\text{CO}_2$ enrichment (mole % excess) in expired air, and 0.7 is the assumed fractional recovery of labeled carbon in expired air in the postabsorptive state (17).

Statistical analysis

Data are presented as means $\pm$ SE. During the tracer infusions, steady state for plasma amino acid levels and enrichments was defined by the absence of a significant correlation of the

measured parameter versus time over the considered period. Data were compared using paired *t*-tests. Significance was established at $P < 0.05$.

RESULTS

Effect of phenylbutyrate on plasma amino acid levels in prednisone-treated subjects

Plasma glutamine and leucine concentrations were near steady state during the 5h of each isotope infusion study. The average coefficients of variation ($100 \times \text{SD}/\text{mean}$) were 5.0% and 4.2% for plasma glutamine, and 4.8% and 7.6% for leucine concentration on the prednisone and prednisone + phenylbutyrate study days, respectively. Amino acids patterns are summarized in Table 2. In our prednisone-treated subjects, the most prominent effects of adding phenylbutyrate treatment were 1) an $\approx 15\%$ drop in plasma glutamine ($P < 0.05$), 2) a reduction in the plasma concentration of all three plasma branched-chain amino acids concentration (-23% , -27% , and -33% for leucine, valine, and isoleucine, respectively), and 3) a rise in plasma alanine and cysteine concentration ($+36$ and $+35\%$, respectively), while the total plasma amino acid pool remained unaffected.

Effects of phenylbutyrate on glutamine kinetics in prednisone-treated subjects

The rates of glutamine R_a , glutamine release from protein breakdown, and *de novo* synthesis did not differ between the prednisone only and the prednisone+phenylbutyrate study days (Table 3). Glutamine oxidation, as calculated from steady state $^{13}\text{CO}_2$ in expired air, did not vary between study days. Since plasma glutamine concentration ($[\text{Gln}]$) was 15% lower on the prednisone + phenylbutyrate study day, and R_{GLN} remained unaltered, glutamine metabolic clearance rate ($R_{\text{GLN}}/[\text{Gln}]$) was higher on the prednisone + phenylbutyrate treatment than with prednisone alone (0.923 ± 0.301 vs. $0.713 \pm 0.126 \text{ L.kg}^{-1}.\text{h}^{-1}$, $P < 0.05$).

Effects of phenylbutyrate on leucine kinetics in prednisone-treated subjects

^{14}C -specific activity in expired air $^{14}\text{CO}_2$ reached isotopic plateau during the last two hours of ^{14}C -leucine infusion (Fig. 1). As shown in Table 4 and Fig. 1, R_{LEU} (124 ± 9 vs. $129 \pm 8 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$, NS) and NOLD (94 ± 9 vs. $91 \pm 7 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$, NS) did not differ between prednisone and prednisone + phenylbutyrate study days. In contrast, leucine oxidation (Ox_{LEU}) increased $\approx 26\%$ from 30 ± 1 to $38 \pm 2 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ ($P < 0.05$) on the prednisone + phenylbutyrate day. Furthermore, although CO_2 production remained unaltered (393 ± 34 vs. $413 \pm 55 \text{ mL.min}^{-1}$ in prednisone vs. prednisone + phenylbutyrate study day, NS), ^{14}C excretion into expired air, calculated as the fraction of infused ^{14}C that was excreted into breath CO_2 , was higher with the combined prednisone + phenylbutyrate treatment, than with prednisone alone (17.2 ± 1.1 vs. $20.7 \pm 0.8\%$, $P < 0.02$).

DISCUSSION

The findings of the present study show that, in healthy subjects receiving prednisone treatment, a 24h treatment with phenylbutyrate depletes plasma glutamine. They further suggest that, although the short term glutamine depletion failed to alter rates of leucine appearance rate or leucine utilization for protein synthesis, it may have detrimental effects since it was associated with increased leucine oxidation, and depletion of all three branched-chain amino acids.

In the current study, phenylbutyrate treatment was associated with a highly significant, $\approx 15\%$ drop in the concentration of glutamine in systemic circulation (Table 3). It should be noted that plasma glutamine still remained within the physiologic range (19). Moreover, as the bulk of body glutamine is intracellular, we have no definite proof that phenylbutyrate treatment induced depletion of intracellular glutamine stores, since we only measured plasma glutamine, and did not perform any tissue biopsy. Yet there is reason to believe phenylbutyrate induced

glutamine depletion not only in plasma, but, presumably, in intracellular milieu as well. The “trapping” of glutamine by phenylbutyrate is indeed supposed to occur in the intracellular space in liver and kidney (10). In addition, based on the stoichiometry of phenylacetylglutamine synthesis (10,11), the $0.36\text{g.kg}^{-1}.\text{d}^{-1}$ phenylbutyrate dose ingested represents a “trapping” of $1.935\text{ mmol glutamine.kg}^{-1}.\text{d}^{-1}$, or 155 mmol.d^{-1} in a 80kg subject. Although glutamine trapping occurs on tissues other than muscle, it is of interest to compare the amount of glutamine trapped to the size of muscle glutamine stores. Muscle indeed is both the main site of glutamine storage, and the main source of extracellular glutamine in the postabsorptive state (20). Assuming a muscle mass of $\approx 32\text{kg}$ in a 80kg patient, an intramuscular glutamine concentration of $\approx 20\text{mmol.L}^{-1}$ intracellular water (4) and an intracellular water content of $\approx 77\%$, the baseline glutamine content of skeletal muscle can be estimated at $\approx 490\text{mmol}$. Thus the approximate amount of glutamine trapped by phenylbutyrate over the 24h treatment period is equivalent to $\approx 32\%$ of the estimated muscle intracellular glutamine pool.

Although phenylbutyrate treatment depressed plasma glutamine concentration and enhanced glutamine clearance, phenylbutyrate treatment failed to alter other glutamine kinetic parameters, and especially rates of overall glutamine Ra and glutamine *de novo* synthesis (Table 3). *In vitro* studies suggest that, in some experimental settings, glutamine may regulate its own rate of synthesis since: 1) increasing glutamine concentration in the culture medium reduced glutamine synthetase activity in the L6 muscle cell line *in vitro* (22), and, conversely, growing Caco-2 cells—a model of human enterocytes—in a glutamine free medium for 20 days upregulated glutamine synthetase activity (23). *In vivo* data are more conflicting: in earlier studies, administration of large doses of exogenous glutamine suppressed *de novo* glutamine synthesis (24) in healthy volunteers, whereas in the current study, the $\approx 15\%$ decline in the size of the circulating glutamine pool was not able to enhance rates of glutamine synthesis. Although any extrapolation from *in vitro* experiments to *in vivo* conditions must be done with caution, two factors may account for the lack of stimulation of glutamine synthesis in the current study: 1) the duration of glutamine depletion lasted for $<24\text{ h}$ in our subjects, instead of 20 days in isolated enterocytes (23), and upregulation of glutamine synthesis may lag behind the onset of glutamine deprivation, and require a more sustained period of glutamine depletion; 2) alternatively, glutamine synthesis may have been maximally stimulated after 5 days of prednisone treatment in our subjects. In the latter case, the preexisting glucocorticoid treatment would have made it impossible to mount an appropriate response when facing the challenge of an acute glutamine “trapping”.

Under the experimental conditions of the current study, leucine oxidation (Ox) rose significantly after phenylbutyrate ingestion (Fig. 2, Table 4). This occurred in spite of the fact that the subjects were already receiving prednisone, a treatment known to enhance leucine oxidation (1). As net leucine balance, *i.e.*, the balance between protein synthesis and protein breakdown (NOLD-B), equals $-\text{Ox}$ in the postabsorptive state (15), the rise in Ox therefore implies a more negative leucine balance. Acute glutamine deficiency was thus associated with the waste of leucine, an essential amino acid. Accordingly, the plasma concentration of all three branched-chain amino acids declined (by -23% , -27% , and -33% for leucine, valine, and isoleucine, respectively) on the phenylbutyrate-prednisone day (Table 2). In the long run, a deficiency in any essential amino acid would obviously compromise the rate of protein synthesis, and earlier studies have documented that changes in leucine oxidation correlate with changes in protein balance (25).

Assuming 1 g of body protein contain 80 mg leucine (*i.e.* $611\mu\text{mol}$ leucine), the $8\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ change in net leucine balance associated with phenylbutyrate treatment would be equivalent to a change in nitrogen balance of $\approx (24 \times 8 \times 84) / (611/6.25) \approx 4.2\text{ g nitrogen per day}$, a substantial rise in nitrogen wasting, compared to prednisone treatment alone.

Although earlier studies have shown leucine oxidation to correlate with plasma leucine concentration in several instances (25), the rise in leucine oxidation occurred in spite of a decline in plasma leucine concentration (Table 2). In theory, a rise in leucine oxidation (Ox) could result from 1) a reduction in protein synthesis (NOLD); 2) a direct effect of phenylbutyrate; or 3) alternatively, this could be an effect of glutamine depletion *per se* on leucine oxidation. The rise in Ox observed in the current study did not result from a reduced rate of protein synthesis, since NOLD remained unaltered with phenylbutyrate. A direct, “toxic” effect of phenylbutyrate on leucine oxidation is unlikely, as we observed the effect of this drug to be reversible with glutamine supplementation in earlier studies carried out in healthy volunteers (26). A direct effect of glutamine depletion *per se* on leucine oxidation therefore appears a more likely hypothesis. In the current study, phenylbutyrate treatment induced a 18% decline in plasma glutamine concentration and a 26% increase in Ox_{LEU} in prednisone-treated volunteers; the same dose of phenylbutyrate induced a 25% decline in plasma glutamine, along with a 21% rise in Ox_{LEU} in healthy subjects receiving no other treatment in earlier studies (11). Conversely, increasing plasma glutamine concentration by means of exogenous glutamine infusion was associated with lower rates of leucine oxidation in healthy adult volunteers (27), preterm infants (28), children suffering from Duchenne muscular dystrophy (29), and healthy adult dogs (30) as well. Taken in aggregate, these studies therefore suggest that glutamine may exert a tonic, inhibitory effect on leucine oxidation, so that an acute drop in plasma glutamine level may lift this inhibition, and result in an immediate rise in leucine oxidation. *In vitro* studies have documented that glutamine deprivation induced upregulation of several genes, including that of branched-chain- α -keto-acid-dehydrogenase E1 β BCKA-dehydrogenase), a key enzyme in leucine oxidation, in a lymphocytic cell line (31). Regulation of genes by amino acids may in fact not be unique to glutamine since genes such as asparagine synthetase gene can be upregulated by amino acid deprivation through a promoter domain called amino acid response element (AARE) (32).

In summary, in an experimental model of catabolism induced by glucocorticosteroids, the induction of acute glutamine depletion was associated with an increase in leucine oxidation, and depletion of branched-chain amino acids, but no alteration in leucine release from protein breakdown or leucine utilization for protein synthesis. The current results are consistent with the view that, in patients suffering from critical illness with concomitant elevation of cortisol secretion and glutamine depletion, glutamine depletion *per se* may contribute to worsening the catabolic effect of severe illness.

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Abbreviations

Ra	rate of appearance
B	leucine release from protein breakdown
NOLD	non oxidative leucine disposal
MPE	mole percent excess

Fig 1. Effects of prednisone alone or in combination with phenylbutyrate on leucine fluxes
 Leucine appearance rate (Ra_{Leu}), oxidation (Ox), and nonoxidative leucine disposal (NOLD) under prednisone conditions (open bars) and during prednisone + phenylbutyrate treatment (filled bars). Values are mean $\pm$ SD. * $P < 0.05$ vs. prednisone value by 2-tail paired t -test.

Table 1

Characteristics of the studied population

Patient	Sex	Age (yrs)	Weight (kg)	Height (m)	BMI ^a
1	M	23	102	1.81	31.0
2	M	25	82	1.79	25.6
3	M	27	84	1.86	24.4
4	M	18	78	1.91	21.5
5	F	28	69	1.65	25.6
6	M	28	76	1.66	27.6
7	M	27	93	1.86	26.9
Mean±SD	6M/1F	25±4	84±11	1.79±0.10	26.1±2.9

^a BMI (body mass index)=weight/height²

Table 2

Plasma amino acid concentrations in healthy volunteers receiving either prednisone treatment alone, or prednisone + phenylbutyrate (ΦB)

	Prednisone only	Prednisone + ΦB	P
Aspartic acid	7±1	7±1	NS
Threonine	157±12	152±12	NS
Serine	114±6	108±6	<0.05
Asparagine	52±2	50±2	NS
Glutamic acid	52±5	70±13	NS
Glutamine	627±39	530±31	<0.05
Proline	199±40	192±31	NS
Glycine	217±20	215±18	NS
Alanine	467±42	635±49	<0.01
Valine	243±18	187±12	<0.05
Cysteine	28±4	38±3	<0.01
Methionine	48±3	50±4	NS
Isoleucine	77±7	56±5	<0.05
Leucine	150±12	100±8	<0.05
Tyrosine	70±3	76±4	NS
Phenylalanine	74±2	73±2	NS
Ornithine	59±3	62±7	NS
Lysine	226±18	200±16	NS
Histidine	75±3	74±3	NS
Arginine	113±10	102±7	NS

Values are means ± SE. Statistical comparison using 2-tail, paired *t*-test.

Table 3
Effect of phenylbutyrate (ΦB) on plasma glutamine concentration ([gln]) and glutamine kinetics in prednisone-treated volunteers

Subject	[gln] μmol.L ⁻¹	RaGln μmol.kg ⁻¹ .h ⁻¹	B _{Gln} μmol.kg ⁻¹ .h ⁻¹	D _{Gln} μmol.kg ⁻¹ .h ⁻¹	OxGln μmol.kg ⁻¹ .h ⁻¹	MCR _{Gln} L.kg ⁻¹ .h ⁻¹
Prednisone						
1	684	413	46	367	191	0.604
2	627	517	49	468	210	0.824
3	683	327	56	271	139	0.479
4	743	534	78	456	187	0.719
5	420	329	63	266	188	0.782
6	609	488	74	415	227	0.802
7	622	485	71	414	266	0.780
Mean ± SE	627±39	442±33	62±5	380±31	201±15	0.713±0.126
Pred + ΦB						
1	595,3	448,2	51	397	216	0.753
2	454,8	582,4	44	539	191	1.281
3	559,8	329,5	74	256	118	0.589
4	466,0	671,3	74	597	175	1.441
5	509,8	266,3	60	206	148	0.522
6	477,5	542,7	75	467	226	1.137
7	443,0	523,2	70	453	259	1.181
Mean±SE	517±23	442±33	62±5	380±31	201±15	0.713±0.126
P	0.012	0.146	0.565	0.180	0.197	0.015

Values are means ± SE. RaGln, glutamine appearance rate; B_{Gln}, glutamine release from protein breakdown; D_{Gln}, glutamine *de novo* synthesis; OxGln, glutamine oxidation. MCR_{Gln}, glutamine metabolic clearance rate (MCR_{Gln}=RaGln/[gln]). Statistical comparison between the two study days, using 1- or 2-tail, paired Student's *t*-test.

Table 4
Leucine kinetics in healthy volunteers receiving either prednisone treatment alone, or prednisone + phenylbutyrate (ΦB)

Treatment	Subject	[Leu] μM	¹⁴ C-excreted, % of infused	Ra _{Leu} μmol.kg ⁻¹ .h ⁻¹	Ox _{Leu} μmol.kg ⁻¹ .h ⁻¹	NOLD μmol.kg ⁻¹ .h ⁻¹
Prednisone	1	162	20.9	92	27	65
	2	140	19.8	97	27	70
	3	189	14.8	111	24	88
	4	96	13.3	156	30	126
	5	119	19.4	125	35	91
	6	145	16.3	146	34	112
	7	152	16.1	141	32	108
mean±SD		143±29	17.2±2.8	124±25	30±4	94±22
Prednisone +ΦB	1	151	21.3	102	31	71
	2	57	24.3	97	32	63
	3	96	19.3	146	41	106
	4	103	21.6	148	46	102
	5	94	20.7	120	35	84
	6	48	20.8	149	44	105
	7	87	17.1	140	34	105
mean±SD		91±33	20.7±2.2	129±22	38±6	91±18
P		<0.05	<0.05	NS	<0.05	NS