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Acute Dichloroacetate Administration Increases Skeletal Muscle Free Glutamine Concentrations After Burn Injury

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Objective
To investigate the hypothesis that the stimulation of pyruvate oxidation by dichloroacetate (DCA) administration would increase the level of intramuscular glutamine in severely burned patients.

Summary Background Data
The level of intramuscular glutamine decreases in response to severe injury, and the rate of intramuscular glycolysis and pyruvate oxidation is elevated. Intramuscular glutamine concentrations have been correlated to muscle protein synthesis.

Methods
Six studies were conducted on five patients with burns >40% total body surface area. Patients were studied in the fed state during an 8-hour stable isotope infusion. After 5 hours, DCA (30 mg/kg) was administered for 30 minutes.

Results
Analysis of muscle biopsy samples taken at 5 and 8 hours of the study revealed a 32% increase in intracellular glutamine levels after DCA administration. Increased intracellular glutamine concentrations did not affect skeletal muscle protein synthesis as determined by a three-pool arteriovenous model or by the direct incorporation of isotope into skeletal muscle protein. DCA administration resulted in a decrease in plasma lactate but no change in alanine *de novo* synthesis or intracellular concentration.

Conclusions
These results suggest that acute DCA administration can increase intramuscular glutamine concentration, but that this acute elevation does not affect muscle protein metabolism.

Although glutamine is the most abundant amino acid in the body and can be synthesized *de novo* in many tissues, it has been considered "conditionally" essential after injury.[1,2] A dramatic decline in muscle free glutamine levels has been consistently observed during injury or trauma.[2–5] Presumably, the body's intracellular free pool serves as a reservoir for the increased glutamine requirements of macrophages and lymphocytes after injury.[6,7] Trauma is also associated with a decrease in net protein synthesis and a loss of body nitrogen.[8,9] Thus, the coincident correlation of intracellular free glutamine and muscle protein synthesis[10] has prompted research into the restoration of muscle glutamine concentrations as a means of affecting increased protein accretion.

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Attempts to restore skeletal muscle glutamine concentrations in the stressed patient have not been entirely successful. Addition of glutamine to total parenteral nutrition in patients after elective cholecystectomy improved intracellular free glutamine concentrations compared with patients who were not supplemented with glutamine; however, muscle concentrations were still significantly below normal.[5] The ineffectiveness of glutamine infusion in restoring intracellular levels in the stressed patient is further complicated by the observation that infusion has no effect on inward transmembrane transport in severely burned patients,[11] as is the case with other amino acids.[12] Further, enteral supplementation of glutamine would not be expected to increase muscle intracellular concentrations because of preferential extraction by the splanchnic bed.[13,14] Almost all of the precursor, glutamate, and more than half of the glutamine are sequestered by the splanchnic bed in humans.[14] This

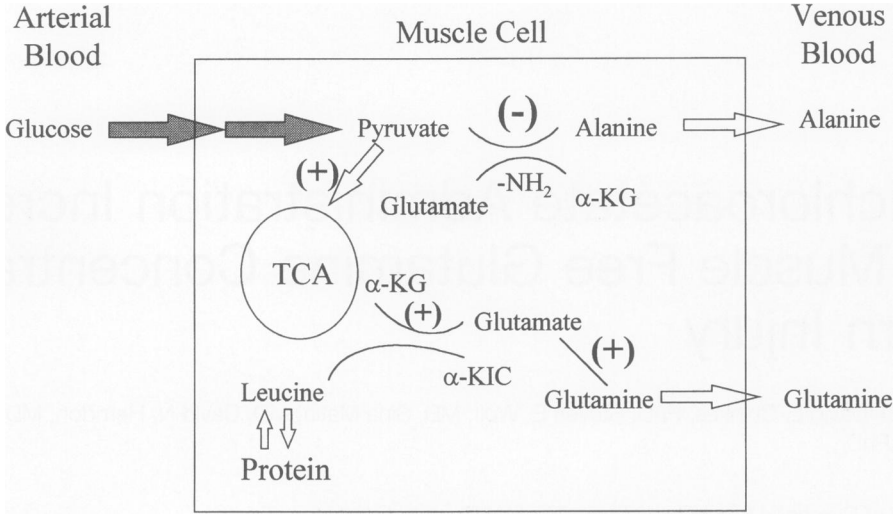

Figure 1. Proposed mechanisms involving glutamine metabolism in the burned patient. Glycolysis of arterial glucose results in pyruvate availability, which is transaminated to form alanine. DCA administration increases pyruvate oxidation and could potentially increase (+) the availability of the TCA intermediate α-ketoglutarate, and in turn the conversion of α-ketoglutarate to glutamate and glutamine. Increased pyruvate oxidation could then decrease (−) the conversion of pyruvate to alanine and decrease the requirement for glutamate nitrogen.

finding was further confirmed in the fed pig, in which it was noted that the gut metabolizes virtually all of the enterally delivered glutamate.[15] These findings indicate that restoration of intramuscular glutamine concentration in severely stressed patients must be derived at least in part from a stimulation of *de novo* glutamine synthesis.

Because the stress response entails release of glutamine from skeletal muscle at an accelerated rate,[2,5] this efflux would eventually deplete the free intracellular pool if *de novo* synthesis were not increased. However, in severe injury, *de novo* synthesis is apparently inadequate to meet the body's requirement.[2] The failure to maintain adequate glutamine concentrations, in addition to its possible effect on skeletal muscle protein synthesis, also affects the function of lymphocytes and macrophages.[7] Recent work has focused on the provision of glutamine's carbon skeleton, the tricarboxylic precursor α-ketoglutarate, in attempts to increase *de novo* synthesis of intracellular glutamine. This approach was as effective as the addition of glutamine to total parental nutrition but was not successful in restoring intracellular glutamine concentrations to presurgical levels.[16]

The suggestion that precursor availability limits the *de novo* production of glutamine is strengthened when one considers the predominant fate of pyruvate during injury. Burn injury dramatically increases endogenous glucose production and the rate of glycolysis. This leads to a 300% increase in pyruvate production.[17] As a result, pyruvate is transaminated to alanine and converted to lactate at rates twice normal.[17] This mechanism potentially limits the availability of the precursor glutamate through increased transamination to form alanine (Fig. 1). In addition, the increased pyruvate conversion to alanine may reduce the availability of TCA (tricarboxylic acid) intermediates necessary for glutamate and glutamine formation, specifically α-ketoglutarate.

The administration of dichloroacetate (DCA) in burned patients has been shown to reduce the conversion of pyruvate to alanine and lactate and to increase pyruvate oxidation.[17] Theoretically, DCA administration could then lead to an increase in glutamine precursors by reducing the requirement for transamination of glutamate, by providing for increased TCA intermediates through increased pyruvate oxidation, or by a combination of both mechanisms (see Fig. 1). Thus, we hypothesized that increased pyruvate oxidation with DCA administration would lead to an increase in intracellular glutamine.

METHODS

Subjects

Patients were admitted to the adult burn unit at the University of Texas Medical Branch within 48 hours of injury. Fluid resuscitation was provided as previously described.[18] Within 48 hours of admission, the burn wound was excised and subsequently grafted with autograft or cadaveric allograft. Patients then typically returned to the surgical suite for reharvesting of donor sites every 7 to 10 days. The present studies were conducted the day before follow-up surgery (23 ± 16 days after burn; Table 1). Five male patients were studied on a total of six occasions after severe burn injury (>40% total body surface area). Patient data and injury characteristics are summarized in Table 1. Each patient was studied during enteral feeding with Vivonex TEN (Sandoz Nutrition Corp., Minneapolis, MN).

Table 1. PATIENT CHARACTERISTICS

Study No.	Age	Weight (kg)	Type Burn	% Burn	% 3° Burn	Days Post Burn of Study
1	27	86	Flame	85	20	18
2	28	91	Chemical	70	68	6
3	74	100	Flame	45	20	5
4	53	57	Flame	55	55	32
5	31	68	Flame	73	67	34
6	31	68	Flame	73	67	45

Studies 5 and 6 conducted on same patient 11 days apart.

Studies 2, 3, and 6 were conducted with patients receiving exogenous insulin at a rate of 3 mU/kg · min^{-1}. Written consent was obtained on all patients, and all protocols were approved by the Institutional Review Board at the University of Texas Medical Branch, Galveston.

Experimental Protocol

Before each study, a 3F, 8-cm polyethylene catheter (Cook, Inc., Bloomington, IN) was inserted into the femoral vein and femoral artery under local anesthesia. Both femoral catheters were used for blood sampling, and the femoral arterial catheter was also used for indocyanine green infusion for the determination of leg blood flow. A triple-lumen central venous catheter was used for stable isotope infusion and for measurement of systemic concentration of indocyanine green. Baseline blood samples were obtained for the measurement of background amino acid enrichment and indocyanine green concentration.

Infusion studies were conducted as depicted in Figure 2. Stable isotopes were concomitantly infused at the following primed (PD) continuous infusion rates (IR) throughout the 8-hour study: L-[ring-$^2H_5$]phenylalanine, IR = 0.07 μmol/kg · min, PD = 2 μmol/kg; L-[1-^{13}C]leucine, IR = 0.08 μmol/kg · min, PD = 4.8 μmol/kg; L-[1-^{13}C]alanine, IR = 0.35 μmol/kg · min, PD = 35 μmol/kg; and L-[5-^{15}N]glutamine, IR = 0.35 μmol/kg · min, PD = 63 μmol/kg. Biopsies of the vastus lateralis were performed as previously described[19] at 2, 5, and 8 hours of tracer infusion. Fractional synthetic rate (FSR) of skeletal muscle protein was determined by the incorporation of L-[ring-$^2H_5$]phenylalanine into protein from 2 to 5 hours, and again from 5 to 8 hours.

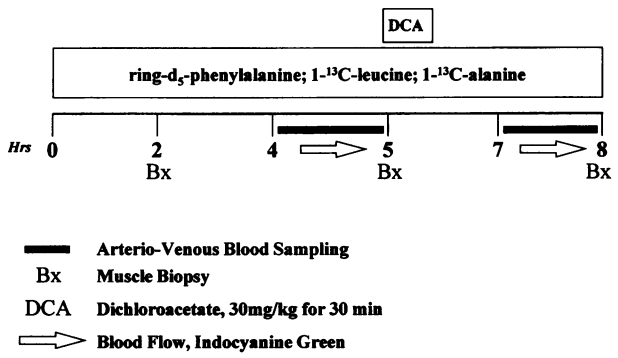

Figure 2. Stable isotope infusion protocol.

Arteriovenous blood samples were drawn at 20-minute intervals between hours 4 and 5 and again over the last hour of tracer infusion (7 to 8 hours) to determine amino acid kinetics. In addition, leg blood flow was determined by indocyanine green infusion during each sampling hour. To measure leg blood flow, a continuous infusion (IR = 0.5 mg/min) of indocyanine green was started 15 minutes before each sampling hour. Subsequent sampling was performed simultaneously from the femoral vein and the central vein over the hour. Arterial samples for amino acid kinetics were always taken after those from the femoral and central veins to avoid interference with blood flow measurement. After each sampling, indocyanine green infusion was uninterrupted for at least 10 to 15 minutes before the next blood flow measurement.

After 5 hours of tracer infusion and at the end of the sampling hour, DCA (dichloroacetic acid sodium salt, TCI America, Portland, OR) was infused at 30 mg/kg for 30 minutes. Plasma lactate and glucose concentrations were measured from the femoral vein with each arteriovenous sampling. Lactate and glucose levels were determined simultaneously on a Glucose/Lactate 2300 Stat Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH), with the average of four values reported as the concentration over each hour during which samples were taken.

Analysis of Samples

Blood

The blood concentrations of unlabeled phenylalanine, leucine, and alanine, as well as the enrichment of their isotopic counterparts, were simultaneously determined by gas chromatography–mass spectrometry (GCMS) using the internal standard approach.[19] Whole blood samples from the femoral vein and artery were immediately precipitated in preweighed tubes containing 15% sulfosalicylic acid. A

known internal standard mixture (100 μl/ml blood) was added to the tube and thoroughly mixed. The composition of the internal standard was as follows: 50.2 μmol/liter of L-[ring-$^{13}C_6$]phenylalanine, 249 μmol/liter of L-[$^2H_4$]alanine, and 120.1 μmol/liter of L-[$^2H_3$]leucine. The tubes were reweighed for determination of blood volume and were centrifuged; the supernatant was removed and frozen at −80°C until analysis. On thawing, 500 μl of the sulfosalicylic extract was passed over a cation exchange column (Dowex AG 50W-8X, 100-200 mesh H+ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum using a Speed Vac (Savant Instruments, Farmingdale, NY). To determine the enrichments of the infused tracers and internal standards, the nitrogen-acetyl-*n*-propyl esters were prepared as previously described.[19,20] The isotopic enrichment of free amino acids in blood was determined on an HP Model 5989 GCMS (Hewlett-Packard Co., Palo Alto, CA) by chemical ionization and selected ion monitoring.[21] Data were expressed as tracer/tracee ratio, with correction for overestimation of enrichment (skew) from isotopomer distribution and for the overlapping contribution of isotopomers of small weight to the apparent enrichment of isotopomers of greater mass.[21]

Muscle

Tissue samples of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then stored at −80°C until processed. On thawing, the tissue was weighed and protein precipitated with 0.5 ml of 10% perchloroacetic acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated two more times and the pooled supernatant (approximately 1.3 ml) was processed similarly to the blood samples described above. To determine intracellular enrichment of infused tracers, the TBDMS derivative was prepared as previously described[22] and was analyzed by GCMS (Model 5989B, Hewlett-Packard) using electron impact ionization. Intracellular glutamine concentration was determined with the internal standard approach by adding 2 μl/mg of wet tissue of an internal standard containing 717 μmol/liter of L-[$^{13}C_5$]-glutamine. Intracellular free glutamine concentration was calculated using the internal standard approach, as previously described.[19] The determination of intracellular water was based on the chloride method.[23]

The remaining pellet of muscle tissue was further washed twice in 0.9% saline and three times with absolute ethanol. It was then placed overnight in an oven and dried at 50°C. The dried pellet was then hydrolyzed at 110°C for 36 hours with 6N HCl. The protein hydrolysate was then passed over a cation exchange column and dried by Speed Vac as described above. To the dried samples, 500 μl of 3,5 HBr-propanol was added for esterification and heated at 110°C for 60 minutes. Samples were then sequentially dried under nitrogen, combined with 100 μl of heptafluorobutyric anhydride, and heated at 60°C for 20 minutes. To determine the enrichment of protein-bound L-[ring-$^2H_5$]phenylalanine, 200 μl of the derivatized sample was analyzed on a Finnigan MD 800 GCMS (Finnegan, San Jose, CA). Protein-bound L-[ring-$^2H_5$]phenylalanine enrichment was determined using chemical impact ionization with methane gas and by monitoring *m/z* 407 and 409. These ions are the m + 3 and m + 5 enrichments, respectively, where m + 0 is the lowest molecular weight of the ion. The ratio of m + 5 to m + 3 was used because it is more sensitive than using the traditional ratio of m + 5 to m + 0 (used for blood samples). Enrichment from the protein-bound samples was determined using a linear standard curve of known m + 5 to m + 3 ratios and corrected back to the absolute change in m + 5 enrichment over the incorporation period.

Calculations

Kinetic Model

Leg amino acid kinetics were calculated according to a three-pool compartment model that has been derived[20] and presented[19,22,24] previously. However, for simplicity and presentation of data, certain parameters will be briefly described.

Amino acids enter and leave the leg through the femoral artery (F_{in}) and femoral vein (F_{out}), respectively. Intercompartment flow of free amino acids can occur between the artery (A), vein (V), and muscle (M). For example, $F_{M,A}$ refers to the net amino acid movement *from* the artery *to* the muscle, whereas $F_{V,M}$ refers to the movement *from* the muscle *to* the vein. These terms describe inward and outward tissue transport, respectively. Thus,

$$F_{in} = C_A \cdot BF \quad (1)$$

$$F_{out} = C_V \cdot BF \quad (2)$$

where C_A and C_V are amino acid concentrations in the femoral artery and vein, respectively, and BF is leg blood flow. Tissue transport is then calculated as follows:

$$F_{M,A} = [([E_M - E_V]/[E_A - E_M]) \cdot C_V + C_A] \cdot BF \quad (3)$$

$$F_{V,M} = [([E_M - E_V]/[E_A - E_M]) \cdot C_V + C_V] \cdot BF \quad (4)$$

where E_A, E_V, and E_M are the tracer amino acid enrichments in the femoral artery, femoral vein, and muscle, respectively. $F_{V,A}$, the flow of amino acids from the artery to the vein, is calculated by

$$F_{V,A} = F_{in} - F_{M,A} \quad (5)$$

or

$$F_{V,A} = F_{out} - F_{V,M} \quad (6)$$

Intracellular amino acids can be derived from endogenous sources. However, because phenylalanine cannot be synthesized in the muscle, $F_{M,O}$ describes the phenylalanine derived from protein breakdown such that

$$F_{M,O} = F_{M,A} \cdot (E_A/E_M - 1) \quad (7)$$

$F_{O,M}$ represents the rate of disappearance of intracellular amino acids. Because the essential amino acid phenylalanine cannot be oxidized in the muscle, this term represents protein synthesis where

$$F_{O,M} = (C_A \cdot E_A - C_V \cdot E_V) \cdot BF/E_M \quad (8)$$

The total rate of appearance of the intracellular amino acid (Ra_M) is then a function of tissue transport ($F_{M,A}$) and protein breakdown ($F_{M,O}$) such that

$$Ra_M = F_{M,O} + F_{M,A} \quad (9)$$

Alanine and glutamine are synthesized from pyruvate and glutamate, respectively, in skeletal muscle. Thus, for alanine and glutamine, $F_{M,O}$ represents the sum of *de novo* synthesis and appearance from proteolysis. It is assumed that amino acids are released from proteolysis in proportion to their relative content in muscle protein. Thus, the rates of alanine and glutamine appearance from protein breakdown (PB_{Ala} and PB_{Gln}, respectively) were calculated from the value of $F_{M,O}$ of phenylalanine and the molar ratios of glutamine to phenylalanine ($MR_{Gln/Phe}$) and alanine to phenylalanine ($MR_{Ala/Phe}$) in protein as follows:

$$PB_{Ala} = F_{M,O(Phe)} \cdot MR_{Ala/Phe} \quad (10)$$

$$PB_{Gln} = F_{M,O(Phe)} \cdot MR_{Gln/Phe} \quad (11)$$

where $MR_{Gln/Phe}$ and $MR_{Ala/Phe}$ were the ratios as previously published.[19] Hence, the rates of alanine and glutamine *de novo* synthesis (DNS_{Ala} and DNS_{Gln}, respectively) have been calculated from the values of $F_{M,O}$ of alanine and glutamine ($F_{M,O(Ala)}$ and $F_{M,O(Gln)}$, respectively)

$$DNS_{Ala} = F_{M,O(Ala)} - PB_{Ala} \quad (12)$$

$$DNS_{Gln} = F_{M,O(Gln)} - PB_{Gln} \quad (13)$$

Fractional Synthetic Rate

Skeletal muscle FSR was calculated from the determination of the rate of tracer incorporation into the protein and the enrichment of the intracellular pool as the precursor:

$$FSR = [(E_{p2} - E_{p1})/(E_M \cdot t)] \cdot 60 \cdot 100 \quad (14)$$

where E_{p1} and E_{p2} are the enrichments of the protein-bound L-[ring-$^2H_5$]phenylalanine at the start (2 hours) and end (5 hours) of the sampling period (similarly, 5 and 8 hours). E_M represents the average intracellular L-[ring-$^2H_5$]phenylalanine enrichment over the time of incorporation, and t is the time in minutes. The factors 60 and 100 are required to express FSR in percent per hour.

Data Presentation and Statistical Analysis

Data are presented as mean ± SEM. DCA measures before and after administration were compared using the paired Student's t test. $P \leq 0.05$ was considered statistically significant.

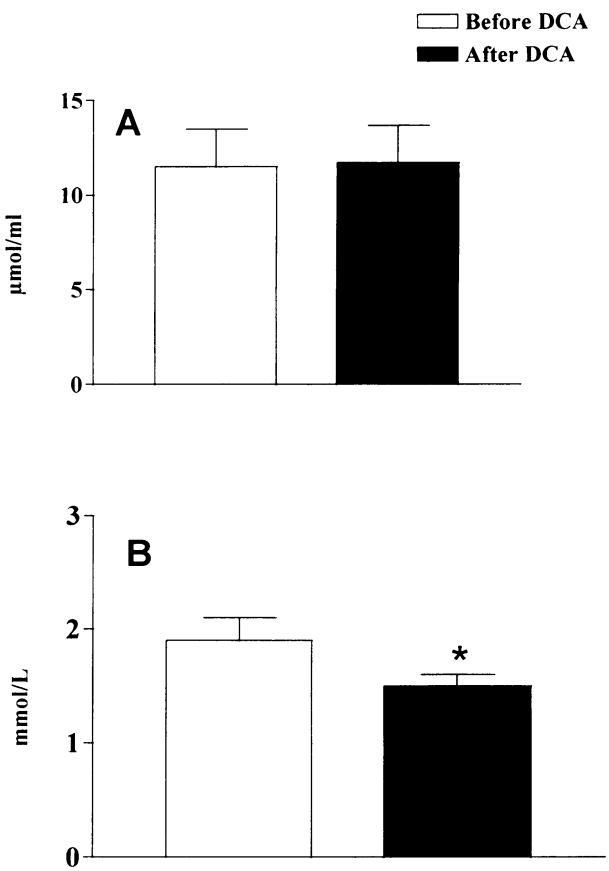

Figure 3. Femoral venous glucose (panel A) and lactate (panel B) concentrations before and after DCA administration. * $p < 0.01$ *vs.* before DCA.

RESULTS

DCA administration resulted in a decrease in femoral venous lactate concentration (1.9 ± 0.2 mmol/liter before DCA *vs.* 1.5 ± 0.1 mmol/liter after DCA, $p < 0.01$), without changing femoral venous glucose levels (11.5 ± 2 μmol/ml *vs.* 11.7 ± 2.0 μmol/ml; Fig. 3). Arteriovenous balance of glucose also did not change with DCA administration (0.33 ± 0.05 μmol/ml before DCA *vs.* 0.34 ± 0.02 μmol/ml after DCA).

Thirty minutes of DCA administration resulted in a significant increase in intracellular free glutamine concentration (4779 ± 1090 μmol/liter before DCA *vs.* 6305 ± 1143 μmol/liter after DCA, $p < 0.01$), without a concomitant change in intracellular free alanine levels (2195 ± 435 μmol/liter before DCA *vs.* 2333 ± 461 μmol/liter after DCA; Fig. 4). However, the modest increase in intracellular free glutamine concentration did not affect model-derived protein synthesis or other kinetic values (Table 2). Independent determination of muscle protein synthesis agreed with model-derived values, such that the fractional synthetic rate

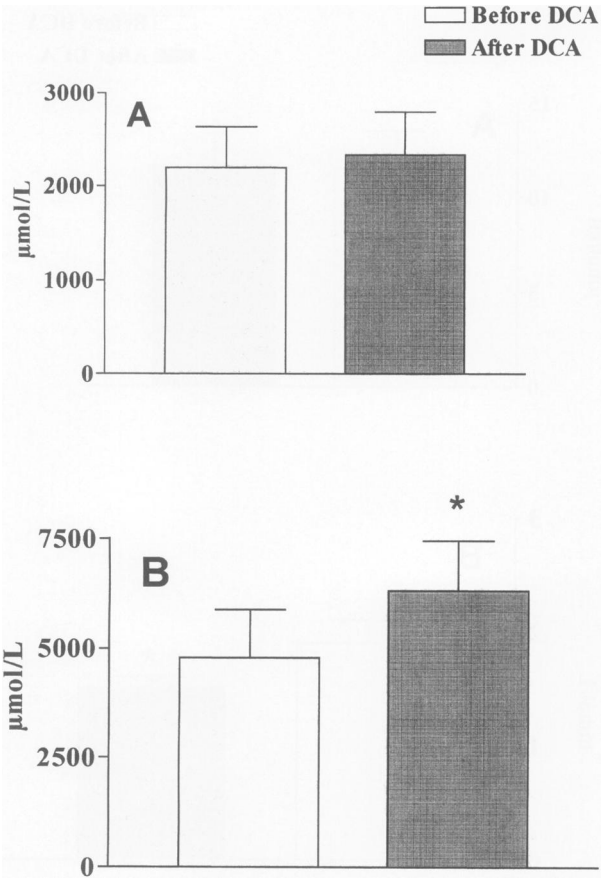

Figure 4. Intracellular alanine (panel A) and glutamine (panel B) concentrations from vastus lateralis muscle before and after DCA administration. * $p < 0.01$ *vs.* before DCA. Approximate normal intracellular concentrations[23] include alanine, 2340 μmol/liter and glutamine, 19,450 μmol/liter.

of muscle protein was unchanged (0.212 ± 0.037 %/hour before DCA *vs.* 0.141 ± 0.021 %/hour after DCA, p = 0.14).

DISCUSSION

The present study demonstrates that acute DCA administration can increase intracellular glutamine concentrations in skeletal muscle. It is apparent that this increase was not the result of a change in skeletal muscle protein breakdown ($F_{M,O}$) or increased inward amino acid transport ($F_{M,A}$). Although the glutamine precursor α-ketoglutarate was not measured directly, it is likely that DCA affected either its use or availability. The absence of change in alanine concentrations (see Fig. 4) or alanine *de novo* synthesis (see Table 2), although initially inconsistent with increased pyruvate oxidation,[17] may also point to the increased availability of α-ketoglutarate. An increase in pyruvate oxidation would be expected to provide an increase in TCA intermediates, including α-ketoglutarate. Increased availability of α-ketoglutarate, combined with nitrogen from the elevated branched chain amino acid oxidation that accompanies burn injury,[25] could then lead to an increase in glutamate formation. Thus, given a higher concentration of glutamate, a greater percentage could be used for *de novo* synthesis of glutamine, whereas alanine concentration remains the same because of the high rate of glycolysis.[17] We initially endeavored to quantify *de novo* synthesis of glutamine directly (Equation 13), but tracer methods were unsuccessful. Tracer model calculations of *de novo* synthesis assume an intracellular plateau of the glutamine tracer, which was not achieved. This inability to achieve isotopic plateau in a short period is consistent with the findings of other laboratories.[26]

Table 2. LEG MUSCLE AMINO ACID KINETICS

	Before DCA (4–5 hrs) Parameter/Amino Acid			After DCA (7–8 hrs)		
	PHE	LEU	ALA	PHE	LEU	ALA
F_{in}	1223 ± 309	1122 ± 243	2058 ± 243	1188 ± 281	1264 ± 281	1880 ± 231
F_{out}	1260 ± 305	1141 ± 236	2405 ± 301	1223 ± 282	1255 ± 276	2050 ± 232
$F_{V,A}$	1084 ± 303	651 ± 200	977 ± 100	1081 ± 275	868 ± 233	989 ± 118
$F_{M,A}$	139 ± 19	471 ± 119	1081 ± 176	106 ± 18	396 ± 112	891 ± 166
$F_{V,M}$	176 ± 23	490 ± 122	1427 ± 251	142 ± 17	387 ± 111	1060 ± 171
$F_{M,O}$	102 ± 12	354 ± 82	972 ± 173	85 ± 5	272 ± 88	937 ± 117
$F_{O,M}$	65 ± 9	335 ± 90	626 ± 135	50 ± 10	280 ± 104	767 ± 120
Ra_M	241 ± 24	825 ± 196	2053 ± 306	192 ± 22	667 ± 177	1828 ± 275
NB	−37 ± 11	−19 ± 21	−347 ± 155	−35 ± 7	9 ± 19	−169 ± 14
DNS			239 ± 27			200 ± 11

Values are mean ± SE and are expressed as nmol · min^{-1} · 100 ml leg^{-1}. F_{in} = amino acid inflow into leg from systemic circulation via femoral artery; F_{out} = amino acid outflow from leg via femoral vein; $F_{V,A}$ = direct amino acid outflow from artery to vein without entering intracellular fluid; $F_{M,A}$ = inward amino acid transport from femoral artery into free muscle amino acid pool; $F_{V,M}$ = outward amino acid transport from intracellular pool into femoral vein; $F_{M,O}$ = intracellular amino acid appearance from endogenous sources (proteolysis for phenylalanine; proteolysis plus de novo synthesis for alanine); $F_{O,M}$ = intracellular amino acid utilization (protein synthesis for phenylalanine, protein synthesis plus other fates for leucine and alanine); Ra_M = total intracellular amino acid rate of appearance (inward transport and proteolysis for phenylalanine and leucine, includes *de novo* synthesis for alanine); NB = net amino acid balance (negative numbers indicate net release from muscle). DNS = *de novo* synthesis. No significant differences in any kinetic parameters after DCA administration.

Nevertheless, our results are consistent with those of Hammarqvist et al.[16] where the addition of α-ketoglutarate to total parenteral nutrition after cholecystectomy ameliorated the decrease in intracellular glutamine without changing intracellular alanine concentrations.

The influence of DCA on pyruvate oxidation[17,27] was again demonstrated by reduced femoral venous lactate concentrations (see Fig. 3). In addition, the arteriovenous balance of glucose was unaffected by DCA administration, which is consistent with earlier work[17] demonstrating that glucose uptake in burn injury is not limited by glucose oxidation in the cell. With the increase in pyruvate oxidation, a decline in alanine appearance in the blood would be expected. Indeed, use of DCA in septic patients[27] and stressed volunteers[28] resulted in a decreased plasma alanine appearance. Previous work from our laboratory in burned children also showed that DCA reduced pyruvate-to-alanine conversion, but only with plasma glucose concentrations of approximately 7 μmol/ml or less.[17] In the same study, glucose infusion resulting in plasma glucose concentrations of approximately 11 μmol/ml did not result in decreased conversion of pyruvate to alanine with DCA. In the present study, Vivonex TEN feeding resulted in glucose concentrations of approximately 12 μmol/ml (see Fig. 3), and traditional calculations of alanine rate of appearance into blood (R_a; not shown) indicated no decrease after DCA administration. The apparent discrepancy between DCA's effects during high and low glucose concentrations in burn injury may be the result of the absolute pyruvate availability. Given an increased appearance of pyruvate with increased glycolysis,[17] stimulation of oxidation by DCA could potentially occur while alanine *de novo* synthesis is maintained. Our data support this: alanine *de novo* synthesis (see Table 2) was unchanged by DCA administration. In addition, intracellular alanine concentrations were maintained at normal values both before and after DCA (see Fig. 4),[23] demonstrating an adequate pyruvate availability under either circumstance. Thus, the results of this study are consistent with previous work in burn injury.[17]

Although this study demonstrates the ability to elevate intracellular glutamine concentrations acutely, it does not confirm a direct relation between muscle glutamine concentration and muscle protein synthesis. Acute DCA administration increased intracellular glutamine concentrations by approximately 32% (see Fig. 4) without stimulating skeletal muscle protein synthesis (see Table 2). However, despite the increased glutamine concentrations, the intracellular glutamine pool was still only 44% of normal.[23] The association of intracellular glutamine concentration and muscle protein synthesis may be strongest at levels closer to normalization. Supplementation with α-ketoglutarate for 3 days after elective cholecystectomy was capable of maintaining intracellular glutamine concentrations within 20% of presurgical values, whereas patients who did not receive supplementation had a 40% reduction.[16] Hammarqvist et al.[16] also noted that muscle protein synthesis, as determined by polyribosome concentration, was maintained in the supplemented group. However, the unsupplemented control group had a concomitant decrease in polyribosome concentration with the reduction of intracellular glutamine. Those results, combined with our results, suggest that there may be a threshold effect at which point intracellular glutamine concentrations may affect, or correlate with, muscle protein synthesis. Indeed, Wernerman et al.[29] have reported a correlation between changes in intracellular glutamine and polyribosome concentrations, and also between intracellular glutamine concentration and nitrogen balance. Taken together, the present study and others investigating glutamine precursors[16,29] suggest that a certain restoration or maintenance of intracellular glutamine concentration is required to affect muscle protein synthesis.

The hypercatabolism that accompanies large burn injury is greater than that of elective cholecystectomy. Further, burn injury entails a greater requirement for glutamine because of its role in wound healing[6] and immune function.[7] Thus, burn injury may require more chronic DCA treatment to restore intracellular glutamine concentrations to those found in lesser stress models.[16,29] However, the present study confirms the findings of others in that the provision of TCA intermediates provides a mechanism for increased intracellular glutamine synthesis. This finding is clinically significant because glutamine delivery to skeletal muscle is difficult. Glutamine has limited stability in parenteral formulas and when delivered enterally is taken up in large part by the splanchnic bed.[13,14] Further, the immediate precursor, glutamate, is not used because of its reported neurotoxicity at higher concentrations.[30] Thus, the ability of DCA to stimulate *de novo* synthesis of glutamine in skeletal muscle introduces a possible therapy to counteract injury-induced muscle catabolism.

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