# *Muscle and Plasma Amino Acids After Injury:*

## *The Role of Inactivity*

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**This study is part of an investigation of whether injury is associated with a unique amino acid pattern in muscle and plasma using the total hip replacement as a model for injury. The role of inactivity is examined as a factor in producing the changes observed with injury and associated bed rest. Nineteen preoperative patients and 16 normal subjects received a muscle biopsy following an overnight fast. Blood for plasma amino acids was drawn at the time of the biopsy. Seven patients (Group I) were treated with 90 g/day of dextrose for the first four postoperative days following which a second biopsy was performed. Eight normals were placed on four days of strict bed rest. Four (Group II) received a regular diet and four (Group III) received 90 g of dextrose/day I.V. as the sole nutrient. Both Groups I and III showed increases in valine, leucine, and isoleucine in both muscle and plasma on the postoperative biopsy. The postoperative pattern differed from that observed in either group of normal subjects in that significant decreases occurred in muscle glutamine and histidine, plasma alanine, lysine and glycine. Phenylalanine, tyrosine, methionine and threonine were increased in muscle postoperatively while only phenylalanine was increased in either Group II or III of the normal subjects. Plasma phenylalanine increased in the patients while remaining unchanged in normal subjects. The pattern reported here for the patient group differs from that reported for other catabolic states (uremia, starvation, etc.), as well as inactivity with or without partial starvation. This study suggests that injury in the form of a total hip replacement is associated with a unique amino acid pattern of muscle and plasma which differs from that observed in other catabolic states. Bed rest plus partial starvation causes a pattern with certain similarities but cannot account for the postoperative changes observed.**

A PROMINENT FEATURE of the postinjury state is increased nitrogen excretion which is felt to reflect mainly the breakdown of muscle protein. This breakdown is in excess of what could be explained on the basis of inactivity and starvation, however both may play a partial role. Practically every condition associated with weight loss and nitrogen wasting is thought to be associated with a translocation of amino acids from muscle to liver. The mechanism and significance of these changes are incompletely understood. The percutaneous muscle biopsy technique of Bergstrom[2] has been used extensively to study changes in muscle composition with various disease states and exercise. Studies[1,3,4,14,18] have indicated that the intracellular amino acid pattern of muscle differs from plasma and that various disease states are associated with their own specific patterns (*i.e.* uremia, starvation, lactic acidosis) in muscle.

Vinnars et al.[1] have recently suggested that a unique pattern may be associated with injury. The classical work of Deitrick suggests inactivity to be associated with muscle wasting even if nutrition is adequate,[10] while semistarved but active muscle is preserved.[9] Although muscle wasting during severe injury or infection is greater than could be accounted for on the basis of inactivity and starvation both may play a role in the amino acid patterns obtained.

This study compares plasma and muscle amino acid changes of patients undergoing total hip replacement whose sole nutrient was 90 grams of dextrose/day to normal subjects on 1) bed rest plus a regular diet and 2) bed rest plus 90 g of dextrose/day only.

### Methods

Nineteen patients (Group I) undergoing total hip replacement were hospitalized on a metabolic research unit for two to three days before and five days after operation. The nature, purpose and possible risks involved in the muscle biopsy technique were explained to the patients and their voluntary written consent obtained. All patients were active and healthy except for

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pain and disability associated with bony degeneration of the hip. All patients appeared well nourished, on a regular diet and had normal SMA-6, SMA-12, urinalysis and chest film. Patients with evidence of diabetes, renal or hepatic disease were excluded. On the morning of the operation, each patient had a percutaneous muscle biopsy taken immediately after the induction of anesthesia. Seven patients received 90 g of dextrose/day for the first four postoperative days as the sole nutrient and were biopsied a second time. Eight hours prior to the second biopsy the dextrose infusion was changed to normal saline at a rate of 40 ml/hr. Local anesthesia (xylocaine 1%) was used for the second biopsy and confined to the skin only. Appropriate amounts of water, electrolytes and vitamins were given daily. All patients were on strict bed rest. All biopsies were from the non-operated thigh.

Sixteen normal subjects received a muscle biopsy following an overnight fast. All subjects were aware of the risks of the study and gave signed written consent. Medical history, physical exam and blood chemistries were within normal limits. Eight subjects were maintained on a protocol of strict bed rest for four days following which the muscle biopsy was repeated. Members of Group II (four subjects) were on a regular diet. Members of Group III (four subjects) received 90 g of dextrose I.V. per day as the sole nutrient plus an appropriate quantity of water and electrolytes. The dextrose infusion was changed to normal saline at a rate of 40 ml/hr eight hours prior to the second biopsy.

The muscle biopsies were taken from the lateral portion of the quadriceps femoris muscle, about 15–20 cm above the knee, after local anesthesia of the skin. The wet biopsy material was dissected carefully to remove visible fat and connective tissue. The material was then divided into several portions. Two smaller portions (10–15 mg) were used for determination of water, fat and electrolytes. A larger amount (20–30 mg) was used for measuring free amino acids. The methodology for weighing the samples and for the determination of neutral fat, water, chloride and free amino acids in muscle tissue and plasma has been described in detail[3] and is briefly summarized here:

The muscle samples were weighed on an electrobalance (Cahn RG) for five minutes after biopsy and the wet weight is extrapolated to zero time. Water is determined by weighing before and after drying at 90°. Neutral fat is extracted with petroleum ether. The dried fat free pieces are extracted in 0.25 ml 1 N $HNO_3$. The wet weight muscle tissue specimen is homogenized. The protein is directly precipitated in 0.5 ml ice cold sulphosalicylic acid. The precipitated sample is stored for 60 minutes at 4° and thereafter centrifuged, decanted and the pH of the supernatant solution is adjusted to 2.2 by addition of 2.5 N LiOH. The neutralized supernatant is freeze dried and reconstituted with Li-citrate buffer and analyzed with an amino acid analyzer modified for use with small samples from one previously described.[11]

The determinations of extra- and intracellular muscle water was based on the chloride method. Chloride is freely diffusible across the skeletal muscle fiber membrane and is distributed according to Nernst's equation. Taking the resting membrane potential of muscle in normal man to be −87.2 mV,[6] the $Cl_e/Cl_i$ ratio is calculated from Nernst's equation to be 26:1. Thus if the total water and chloride content of the muscle tissue and the extracellular concentration of chloride (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water[4]) are known, extra- and intracellular water volumes can be calculated.[4]

The intracellular concentration of each amino acid was calculated by subtracting the free extracellular part from the total amount assuming the plasma concentration to be equal to the concentration in the interstitial fluid. The calculation of these relationships has also been described in detail.[3]

TABLE 1. *Muscle Amino Acids Following Total Hip Replacement Versus Inactivity (mMoles/Kg Intracellular $H_2O$)*

| | VAL | LEU | ISOL | PHE | TYR | METH | THR | LYS |
|---|---|---|---|---|---|---|---|---|
| Preop | .356 | .214 | .097 | .092 | .111 | .041 | .82 | 1.15 |
| ± SEM | .023 | .018 | .009 | .007 | .013 | .005 | .07 | .06 |
| Postop | .682‡ | .487‡ | .300‡ | .201‡ | .203‡ | .148‡ | 1.21† | .92 |
| ± SEM | .088 | .041 | .028 | .028 | .017 | .020 | .19 | .12 |
| Active NL | .350 | .283 | .134 | .077 | .106 | .050 | .85 | .773 |
| ± SEM | .021 | .023 | .010 | .004 | .006 | .007 | .08 | .051 |
| Bedrest | .487* | .358 | .153 | .114* | .143 | .057 | .85 | .889 |
| ± SEM | .074 | .050 | .033 | .016 | .037 | .012 | .08 | .156 |
| Bedrest ($D_5W$) | .540* | .407† | .248‡ | .131* | .136 | .082 | .72 | .738 |
| ± SEM | .054 | .053 | .033 | .041 | .035 | .025 | .08 | .092 |

* $p < .01$. †$p < .05$, Students t-test. ‡$p < .001$.

TABLE 2. *Muscle Amino Acids Following Total Hip Replacement Versus Inactivity (mMoles/Kg Intracellular $H_2O$)*

| | GLN | ALA | GLY | ARG | HIST | ORN | TAU | ASP | SER | GLU | PRO | AAB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Preop | 18.8 | 2.41 | 1.68 | .72 | .324 | .345 | 20.2 | 2.25 | .95 | 4.06 | .76 | .145 |
| ± SEM | 1.17 | .14 | .11 | .06 | .019 | .032 | 1.5 | .20 | .09 | .25 | .11 | .012 |
| Postop | 9.50‡ | 2.34 | 1.94 | .53 | .247† | .170† | 22.3 | 1.64 | 1.00 | 4.53 | .68 | .163 |
| ± SEM | .71 | .29 | .15 | .08 | .024 | .026 | 1.5 | .38 | .07 | .74 | .15 | .021 |
| Active NL | 20.5 | 2.72 | 1.56 | .603 | .390 | .417 | 15.9 | 2.07 | .91 | 3.13 | 1.02 | .129 |
| ± SEM | 1.2 | .15 | .14 | .041 | .038 | .056 | 2.2 | .20 | .12 | .25 | .10 | .021 |
| Bedrest | 18.6 | 3.10 | 1.83 | .427 | .478 | .600 | 14.7 | 2.43 | 1.20 | 3.17 | .237 | .129 |
| ± SEM | 1.4 | .33 | .37 | .108 | .059 | .070 | 2.5 | .58 | .12 | .05 | .050 | .021 |
| Bedrest ($D_5W$) | 14.2† | 2.31 | 1.51 | .441 | .293 | .302 | 12.5 | 2.00 | 1.00 | 2.72 | .630 | .243† |
| ± SEM | 2.7 | .31 | .04 | .092 | .041 | .091 | 1.3 | .38 | .39 | .31 | .130 | .034 |

* $p < .01$. †$p < .05$, Students t-test. ‡$p < .0001$.

## Results

The postoperative group was compared to the preoperative group using an unpaired t-test. The group of normals on bed rest is compared to the data for active normals using an unpaired t-test. Postoperatively muscle amino acids show significant increases in valine, leucine, isoleucine, phenylalanine, tyrosine, threonine and methionine (Table 1) and significant decreases in glutamine and ornithine (Table 2). Plasma levels of valine, leucine, isoleucine and phenylalanine (Table 3) increase while alanine and lysine decrease (Table 4).

Figure 1 shows the changes in branched chain amino acids in the patient and normal group. The normal subjects on bed rest plus dextrose (Group III) show similar effects to the operative group; however, muscle changes are less marked, while plasma changes are more marked. Figure 2 shows a difference between the postoperative group and the bed rest group. Postoperatively there are increases in muscle phenylalanine, tyrosine and methionine while the normal subjects show either minimal or no changes. Plasma phenylalanine is increased following surgery but not secondary to bed rest alone.

Figure 3 shows the two key gluconeogenic amino acids alanine and glutamine as well as glycine. In contrast to bed rest, the patient group shows a significant decrease in muscle glutamine and plasma alanine and glycine. Figure 4 shows the patients to have a marked decrease in plasma lysine with a small but significant decrease in muscle histidine.

Bed rest alone had minimal effects on muscle or plasma amino acids, the only clear changes being an increase in muscle valine and phenylalanine (Tables 1 and 2) with no significant changes in plasma amino acids. The values for muscle in active normal subjects did not differ significantly from the preoperative group of patients with the exception of an unexplained increased preoperative level of muscle lysine ($p < .001$) and an increased glutamic acid ($p < .02$). Plasma levels of active normal subjects differed from the preoperative patient group in that the patients showed a decreased level of arginine ($p < .02$).

## Discussion

Clearly bed rest plus partial starvation for four days can reproduce some changes seen in the postopera-

TABLE 3. *Plasma Amino Acids Following Total Hip Replacement Versus Inactivity (mMoles/Liter)*

| | VAL | LEU | ISOL | PHE | TYR | METH | THR | LYS |
|---|---|---|---|---|---|---|---|---|
| Preop | .246 | .146 | .062 | .065 | .074 | .025 | .139 | .212 |
| ± SEM | .014 | .008 | .003 | .003 | .004 | .001 | .008 | .009 |
| Postop | .328* | .233‡ | .102‡ | .089‡ | .077 | .030 | .149 | .067* |
| ± SEM | .019 | .014 | .008 | .006 | .003 | .002 | .013 | .004 |
| Active NL | .294 | .166 | .078 | .070 | .078 | .029 | .164 | .220 |
| ± SEM | .020 | .012 | .006 | .005 | .005 | .002 | .010 | .014 |
| Bedrest | .297 | .198 | .089 | .089 | .091 | .037 | .168 | .258 |
| ± SEM | .043 | .002 | .009 | .010 | .005 | .003 | .010 | .021 |
| Bedrest ($D_5W$) | .411* | .300‡ | .146‡ | .079 | .083 | .025 | .164 | .227 |
| ± SEM | .022 | .014 | .014 | .004 | .002 | .003 | .020 | .025 |

* $p < .01$. †$p < .05$, Students t-test. ‡$p < .001$.

TABLE 4. *Plasma Amino Acids Following Total Hip Replacement Versus Inactivity (mMoles/Liter)*

| | GLN | ALA | GLY | ARG | HIST | ORN | TAU | ASP | SER | GLU | PRO | AAB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Preop | .694 | .346 | .269 | .104 | .086 | .059 | .079 | .008 | .130 | .042 | .192 | .036 |
| ± SEM | .029 | .018 | .019 | .006 | .004 | .005 | .004 | .001 | .006 | .004 | .016 | .002 |
| Postop | .592 | .227* | .192† | .097 | .067 | .074 | .070 | .006 | .115 | .035 | .172 | .036 |
| ± SEM | .035 | .019 | .014 | .009 | .004 | .008 | .005 | .001 | .008 | .004 | .018 | .004 |
| Active NL | .780 | .438 | .273 | .129 | .097 | .063 | .072 | .012 | .126 | .039 | .248 | .037 |
| ± SEM | .045 | .034 | .014 | .008 | .006 | .004 | .005 | .002 | .009 | .008 | .029 | .005 |
| Bedrest | .774 | .475 | .272 | .122 | .109 | .067 | .069 | .007 | .136 | .037 | .236 | .035 |
| ± SEM | .020 | .032 | .025 | .012 | .004 | .009 | .005 | .002 | .007 | .008 | .043 | .006 |
| Bedrest ($D_5W$) | .690 | .308 | .235 | .114 | .091 | .053 | .080 | .005 | .130 | .017 | .174 | .069† |
| ± SEM | .040 | .027 | .031 | .012 | .008 | .008 | .008 | .001 | .012 | .003 | .036 | .006 |

* $p < .01$. † $p < .05$, Students t-test.

tive group, particularly in the branched chain amino acids. However the aromatic amino acids (phenylalanine, tyrosine) lysine and methionine among the essentials and the nonessential amino acids, glutamine, alanine, glycine, and histidine show changes in plasma and/or muscle following hip replacement which are not seen secondary to bed rest either with adequate nutrition or dextrose alone.

This suggests the changes occurring following injury cannot be explained on the basis of inactivity or nutrition alone. Corticosteroids cause a general increase in plasma levels of many amino acids[5,16,19] and cannot account for the plasma patterns observed in the patient group. Untreated diabetes is associated with an increased plasma leucine, isoleucine and valine with a concomitant reduction in alanine, glycine, threonine and serine.[14] This is similar in some respects to the changes seen postoperatively with the exception of phenylalanine which increases postoperatively, threonine which remains unchanged and glycine which decreases. Insulin may act to decrease mobilization of all, including ketogenic, amino acids and inhibit hepatic uptake of gluconeogenic amino acids,[14] hence a relative insulin deficiency could account for some of the changes observed in the patient group. Starvation alone can cause increases in plasma valine, leucine and isoleucine but does not change plasma levels of tyrosine or phenylalanine and causes de-

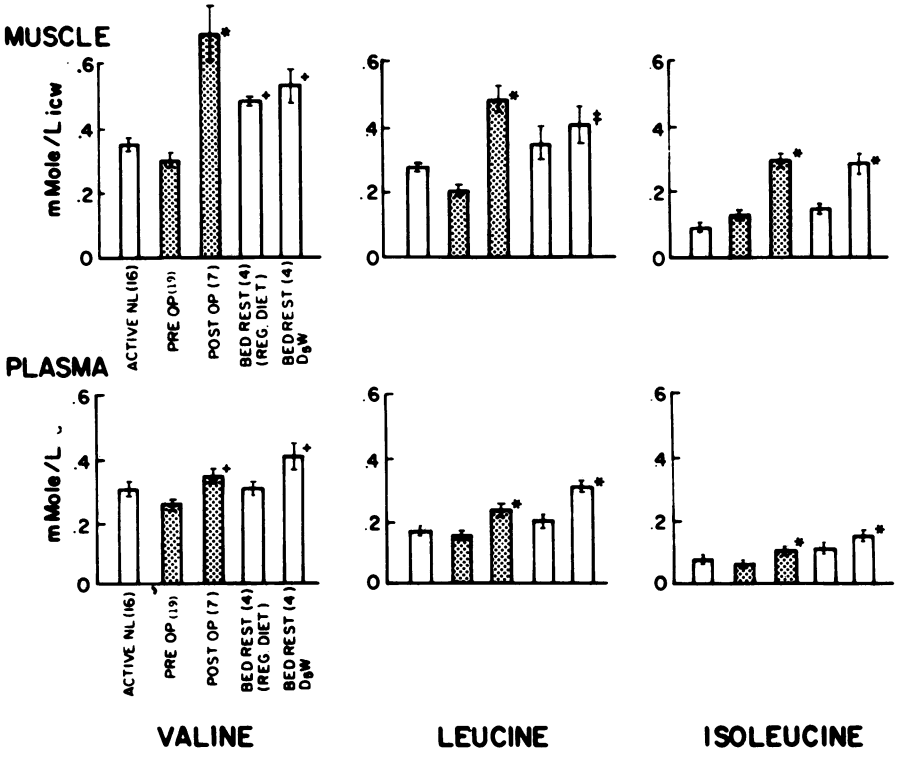


FIG. 1. *p < .001. †p < .01. ‡p < .05.

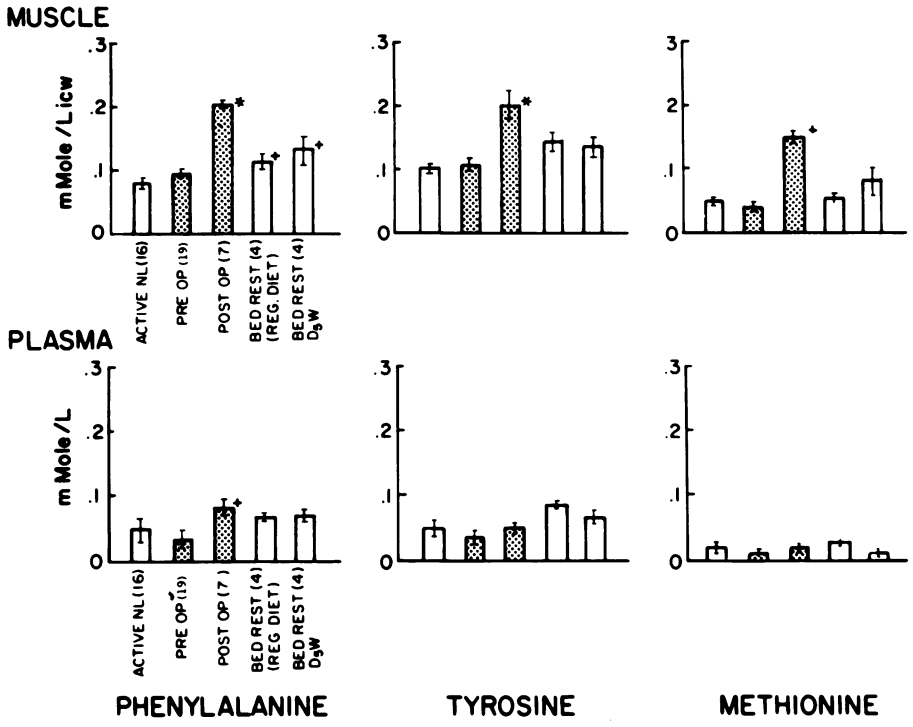


FIG. 2. *p < 0.001. †p < 0.01.

creased levels of alanine.[12] The postoperative group showed a decreased level for plasma alanine while the normal subjects receiving dextrose did not. This suggests that nutrition was not the cause of decreased plasma alanine.

Untreated uremia is associated with low plasma levels of valine, leucine and isoleucine. Muscle valine is decreased in addition.[4] In contrast to uremia, injury and inactivity is associated with increases in the branched chain amino acids which have been sug-

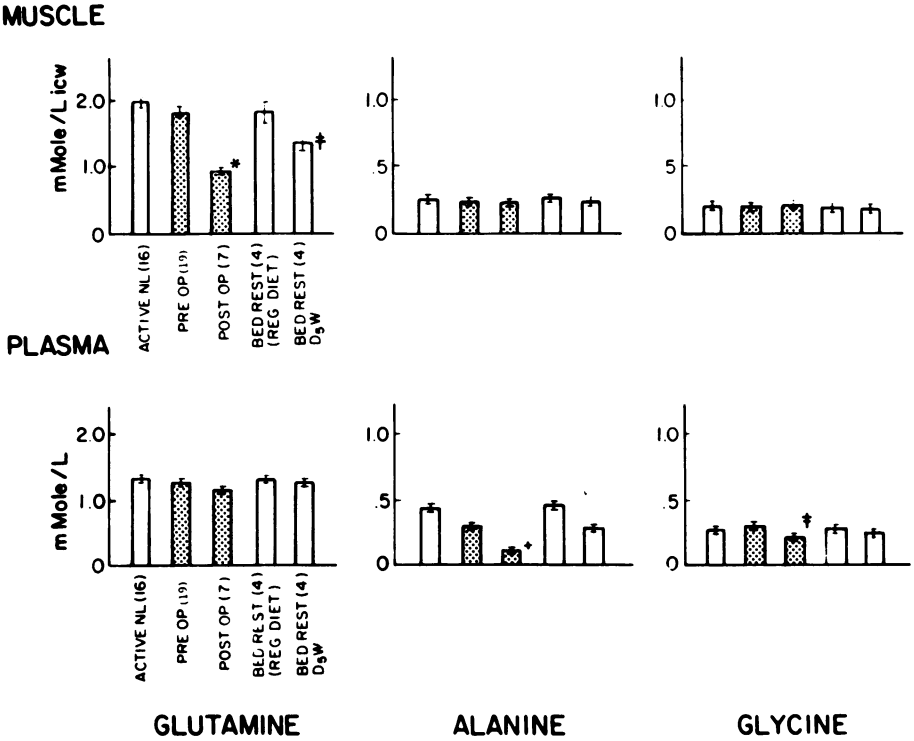


FIG. 3. *p < 0.001. †p < 0.01. ‡p < 0.05.

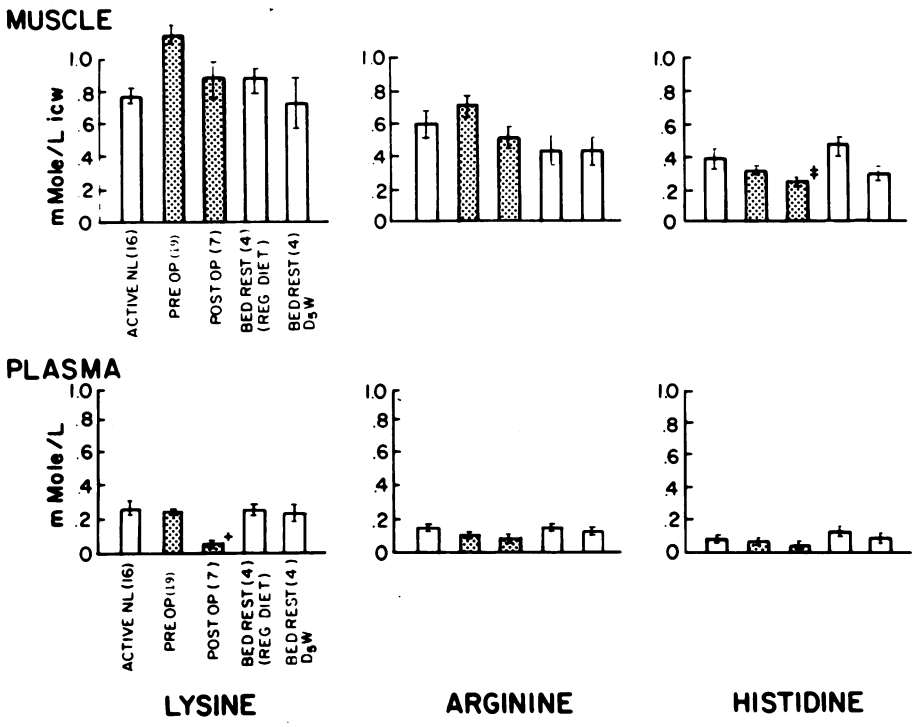


FIG. 4. †$p < 0.05$. ‡$p < 0.01$.

gested to be a characteristic of posttraumatic catabolism.[20,21] Inactivity plus starvation in this study was associated with a large decrease in muscle glutamine although less so than the patient group. The depression of essential–nonessential amino acid ratio seen in protein deficiency was not seen in either the patient or normal subjects. This may be due to the fact that this study period was limited to four days and all the subjects were previously well nourished.

Electrolyte deficiency, particularly potassium, can increase levels of lysine, arginine and histidine.[15,16,17] All subjects and patients received adequate amounts of electrolytes and had normal values for serum electrolytes checked on a daily basis. Although there are few reports on amino acid changes in response to inactivity, exercise has been relatively well studied. Brief periods of exercise are associated with increases in alanine levels only.[7,13] With heavier loads isoleucine, leucine, methionine, tyrosine and phenylalanine are increased in plasma.[13]

The pattern reported here in the postoperative group appears to be unique for injury. Clearly some changes can be reproduced by inactivity plus starvation, particularly in the branched chain amino acids. Although certain catabolic states have similarities there are distinct differences following injury from that secondary to starvation, uremia and untreated diabetes.


## Summary

The amino acid pattern following total hip replacement is characterized by increases in muscle of the branched chain amino acids (leucine, isoleucine and valine), the aromatics (phenylalanine and tyrosine) as well as methionine. The nonessential amino acids in muscle tend to decline, glutamine having the most marked change. Plasma levels of the essential amino acids increase while the nonessentials tend to decrease. This pattern differs from that observed in other catabolic states (uremia, starvation, untreated diabetes) and is significantly different from the effects of inactivity and starvation combined. This suggests that injury can be characterized by a unique pattern of muscle and plasma amino acids.


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