Muscle and Plasma Amino Acids after Injury

Hypocaloric Glucose vs. Amino Acid Infusion

J. ASKANAZI, M.D., P. FURST, M.D.,* C. B. MICHELSEN, M.D., D. H. ELWYN, PH.D., E. VINNARS, M.D.,† F. E. GUMP, M.D., F. E. STINCHFIELD, M.D., J. M. KINNEY, M.D.

From the Departments of Surgery, Anesthesiology and Orthopedics, College of Physicians and Surgeons, Columbia University, New York, New York

This study examines the effect of three different hypocaloric diets on the patterns of muscle and plasma amino acids in patients undergoing total hip replacement. Group I (seven patients) received 90 g/day of glucose, Group II (seven patients) received 70 g/day of amino acids, Group III (eight patients) received both 90 g of glucose and 70 grams of amino acids per day. Utilizing the percutaneous biopsy technique of Bergström, free amino acid patterns in muscle and plasma were analyzed pre- and postoperatively (day 4). The postoperative pattern of amino acids was characterized by elevated levels in muscle and plasma of the branched chain amino acids, phenylalanine, tyrosine and methionine. There was a marked decrease in muscle glutamine and smaller decreases in the basic amino acids in both muscle and plasma. Muscle:plasma concentration ratios increased for the neutral amino acids, decreased for glutamine and the basic amino acids and were unchanged for the acidic amino acids. The patterns seen after hip replacement are almost identical to those seen after colectomy or accidental injury. There was little effect of diet on amino acid concentrations in muscle. In plasma, concentrations of leucine, isoleucine, valine and proline were higher in Group II in the absence of glucose intake, than in the other groups. Lysine was lower in Group I with no amino acid intake than in the other groups. Thus, there is a unique amino acid pattern associated with operative trauma which is relatively unaffected by hypocaloric, intravenous nutrition.

THE METABOLIC RESPONSE to trauma is usually associated with a negative nitrogen balance. The severity of the condition is usually reflected by the extent of negativity which is largely the result of protein breakdown in muscle.[30] This catabolic condition is thought to be associated with translocation of amino acids from muscle to viscera resulting in abnormalities in amino acid metabolism. The mechanism and significance of these changes are incompletely understood. There are numerous reports of derangement of plasma amino acid metabolism in trauma.[13,31,42] However, when discussing plasma amino acid concentrations, it should be remembered that the largest pool of free amino acids are not in the extracellular space but within the cells. A fundamental question is whether a unique pattern of free amino acids exists in muscle which is distinctive for the disease or condition studied. The development of the percutaneous needle biopsy technique provides a safe method for quantitative measurement of the composition of human muscle under a variety of physiologic and pathologic conditions.[5] Using this technique, characteristic changes of muscle amino acids in different catabolic states (uremia, diabetes, injury and immobilization) have been demonstrated[2,8,22,23,38,40] suggesting that a unique pattern of muscle free amino acids appears to be characteristic for each condition.

Current developments have made it possible to support traumatized patients with energy sources and crystalline amino acids by intravenous means. Although many studies have been conducted on the amounts and proportion of nutrients[10,24,26,32,43] to be used in surgical patients, the optimal composition of the nutrition for an injured patient remains unclear.

In the present study our aims were to investigate the effect of severe operative trauma on the intracellular free amino acid pattern of muscle and plasma and to examine the effect of hypocaloric postoperative nutrition with glucose and amino acids alone or in combination.

* Metabolic Research Labs., St. Erik Hospital, Stockholm, Sweden.

† Department of Anesthesia, St. Erik Hospital, Stockholm, Sweden.

Reprint requests: J. Askanazi, Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

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Materials and Methods

Patients and Experimental Protocol

Twenty-two patients undergoing total hip replacement were hospitalized on a metabolic research unit for two days prior to and five days after operation. The study was approved by the Institutional Review Board. The nature, purpose and possible risks involved in the study and in particular the muscle biopsy technique were explained to the patients and their voluntary written consent obtained. All patients were active and healthy except for pain and disability associated with bony degeneration of the hip. All patients were on a regular diet prior to the study and appeared to be well nourished. Routine chemical analyses of plasma (SMA-6, SMA-12), analyses of urine, blood chemistry, EKG, chest x-ray and CBC revealed normal findings. There was no evidence of diabetes, cardiac insufficiency, or kidney, liver or thyroid diseases. Information concerning the age, sex distribution and body size is given in Table 1.

On the morning of the operation, 19 of the 21 patients had a percutaneous muscle biopsy (vida infra) after induction of anesthesia (pentothal). Following the operation, each patient was kept in the recovery room for two to three hours and then returned to the surgical metabolism unit where the assigned nutritional regimen was started. The three nutritional regimens used are specified in Table 1.

A repeat biopsy was performed on the morning of the fourth postoperative day in the postabsorptive state. The infusion was changed to normal saline at 40 ml/hr eight hours prior to the second biopsy. Local anesthesia (xylocaine 1%) was used, confined to the skin only.

Muscle Biopsy and Analyses

The muscle biopsies were taken from the lateral portion of the quadriceps femoris muscle, about 15–20 cm above the knee. The wet biopsy material was dissected carefully to remove visible fat and connective tissue.[4] The material was then divided into four portions. Two smaller samples (10–15 mg) were used for determination of water, fat and electrolytes[7] and the other two (15–20 mg) were used for measurements of free amino acids.[6,8,9]

The calculation of extra- and intracellular water was based on the chloride method[25] according to Nernst's equation,[12] assuming a normal resting transmembrane potential of −87.2 mv.[11] Knowing the total water and chloride content in plasma, one can calculate the extracellular water volumes.[15] The intracellular concentration of each individual 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. In this calculation, it was also assumed that none of the amino acids (except tryptophan) are bound to proteins.[27] These calculations are described *in extenso*.[9] Although the lack of measured values of membrane potential may lead to error, it does not invalidate the qualitative conclusions. The problems in using this calculation in surgical patients have been discussed in detail.[23,24,40]

The free amino acids of plasma and muscle were determined after precipitation with sulphosalicylic acid[29] by using an automated amino analyzer, modified from one previously described[18] and using resin from Dianex Co. (Sunnyvale, Calif.) and Li Citrate buffer. Serum chloride and albumin were measured by routine methods.

Statistics

Statistical calculations were carried out by using a Prime 300 computer, applying routines derived from the Scientific Subroutine Package Library (SSPL). Results are expressed as mean values ± SEM. Statistical significance was assessed by both unpaired and paired t-tests. The preoperative values in the hip patients were compared with values observed in male subjects in the age range of 21–45.[2] Only muscle lysine was found to be significantly elevated in the preoperative patient group ($p < 0.001$). A comparison between the three preoperative groups revealed no significant differences. Hence all the preoperative data were combined into one group, and unpaired t-tests were used to compare the values in the three postoperative groups to the preoperative control values. Paired t-tests within respective groups showed essentially the same direction of change as the unpaired t-tests although with less significance due to the relatively limited number of observations.

Results

The concentration of each of the branched chain amino acids (valine, leucine, isoleucine) in muscle was increased in all three groups compared to preoperative values (Table 2, Fig. 1), as were the aromatic amino acids (phenylalanine, tyrosine). The rise in the valine level in Group II (amino acids alone) was greater than in Groups I and III ($p < 0.01$). A significant increase is seen in methionine concentrations (Fig. 2).

Plasma concentrations of the corresponding amino acids were also increased. The elevations observed for valine and leucine in Group II were greater than those found in Groups I and III.

TABLE 1. *Patient Description, Postoperative Nutrition and Corresponding Postoperative Nitrogen Balance in 22 Patients Undergoing Total Hip Replacement (Mean ± SD)*

Group	n	Sex Distribution F	Sex Distribution M	Age (yrs)	Body Surface Area (m^2)	Postop Nutrition	Postop[3] Daily N-balance ($g/day/m^2$)
I	7	3	4	54 ± 14	1.74 ± .14	Glucose (90 g/day)*	−5.0 ± 1.7
II	7	2	5	56 ± 9	1.71 ± .17	Amino Acids (70 g/day)†	−5.15 ± 1.4
III	8	4	4	57 ± 9	1.78 ± .18	Glucose (90 g/day) & Amino acids (70 g/day)	−4.1 ± .95

* Given as 5% Dextrose.
† Given as 3.5% Freamine.

There was a profound decrease in glutamine in muscle, postoperatively, with no corresponding alteration in plasma (Fig. 3, Table 3). Alanine levels in muscle decreased only in Group II, while the plasma concentrations were reduced in all 3 Groups. Glycine levels increased in muscle in Groups II and III (Fig. 3, Table 3). In contrast, plasma concentrations were decreased in Group I and were unaltered in the other groups. There was also a decrease in muscle lysine and arginine in both groups receiving amino acids. The plasma concentration (Fig. 4) of lysine was found to be decreased in Group I ($p < 0.05$), but not in the other groups.

As a consequence of the postoperative changes in plasma and muscle concentrations, many of the resultant muscle/plasma ratios were found to be significantly changed as indicated in Tables 2 and 3. It is important to note that the basic amino acids exhibited decreased muscle/plasma gradients, while the neutral amino acids showed a tendency toward increased gradients.

Discussion

Hypocaloric glucose administration by peripheral vein is the primary way of providing postoperative nutrition in the United States. Since a key component of the metabolic response to injury is a translocation of amino acids from muscle to plasma, nutrition with hypocaloric carbohydrate as the sole nutrient has been criticized.[17] Nitrogen sparing through the use of peripheral amino acid infusions has been advocated by Blackburn et al.[10] In this concept glucose has been postulated to be detrimental, owing to an increased

TABLE 2. *Essential Amino Acids (Mean ± SEM)*

	Preoperative			Postoperative Group I (N = 7)			Postoperative Group II (N = 7)			Postoperative Group III (N = 8)		
	M	P	M/P	M	P	M/P	M	P	M/P	M	P	M/P
VAL	.356	.246	1.65	.682*	.328†	2.07	.728*	.525*	1.38	.688*	.381*	1.82
	.023	.014	.29	.088	.019	.19	.047	.025	.10	.059	.027	.13
LEU	.214	.146	1.52	.487*	.233*	2.04‡	.512*	.343*	1.45	.493*	.250*	2.04
	.018	.008	.14	.041	.014	.13	.051	.018	.15	.036	.019	.21
ISOL	.097	.062	1.65	.300*	.102*	2.90‡	.301*	.165*	1.91	.307*	.135*	2.32
	.009	.003	.18	.028	.008	.31	.032	.013	.20	.027	.009	.22
PHE	.092	.065	1.44	.201*	.089*	2.26*	.167*	.087*	1.72	.167*	.086†	2.56*
	.007	.003	.10	.028	.006	.28	.020	.005	.23	.020	.006	.30
TRY	.111	.074	1.50	.203*	.077	2.65*	.173	.094*	1.99	.220*	.082	2.73*
	.013	.004	.14	.017	.003	.24	.022	.006	.28	.029	.006	.24
METH	.041	.025	1.73	.148*	.030	4.88*	.151*	.046	3.38*	.131*	.037*	3.64*
	.005	.001	.23	.020	.002	.38	.024	.009	.55	.021	.003	.63
THR	.82	.139	5.8	1.21	.149	8.5	.90	.120	7.5	1.06	.148	7.5
	.07	.008	.3	.19	.013	1.8	.10	.013	.9	.12	.018	.9
LYS	1.15	.212	5.5	.92	.067	14.5†	.57*	.180	3.3	.71*	.194	3.9
	.06	.009	.3	.12	.004	1.1	.02	.013	.5	.07	.020	.5

* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

M = Muscle Amino Acids (m Moles/liter intracellular H_2O).
P = Plasma Amino Acids (m Moles/liter plasma H_2O).
M/P = Muscle/Plasma ratio.

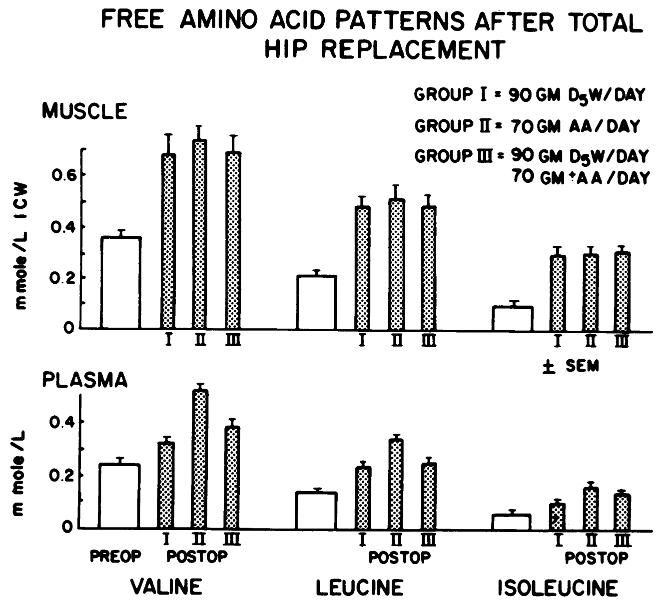

FIG. 1. Valine, leucine and isoleucine patterns after total hip replacement.

insulin secretion with concomittant decrease in fat mobilization. This contention has been supported[28] and disputed.[16,19] In Europe a balanced normocaloric form of total parenteral nutrition (TPN) is preferred with the simultaneous use of intravenous carbohydrate, fat and amino acids. Thus, the optimal amount and composition of postoperative nutrition remains to be established.

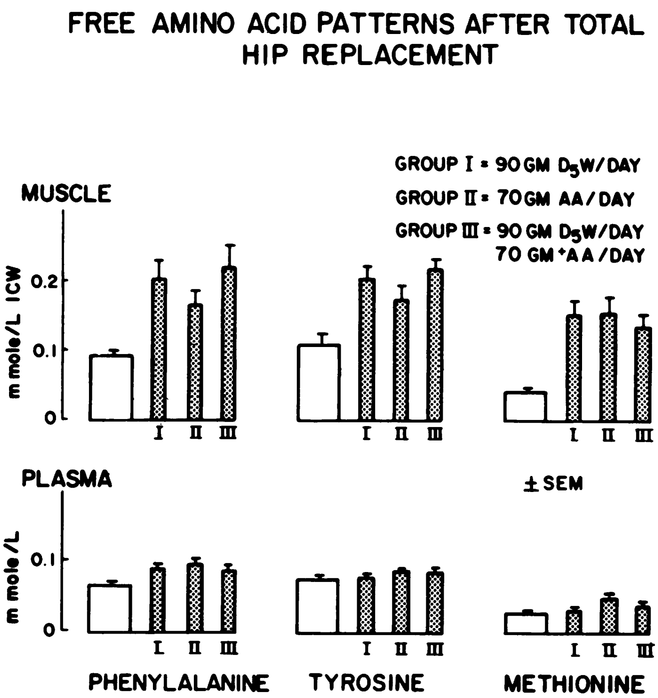

FIG. 2. Phenylalanine, tyrosine and methionine patterns after total hip replacement.

The present study is an attempt to determine whether postoperative trauma produces characteristic changes in plasma and muscle free amino acids and whether specific alterations can be attributed to different hypocaloric nutritional regimens using glucose and amino acids alone or in combination.

The pattern of amino acids is known to be influenced by numerous factors including age related changes,[1,44] sex distribution,[34] physical activity[2] and the dietary intake prior to the investigation. As given in Table 1, the three different groups were similar in these respects, thus allowing an objective comparison of postoperative results with those seen preoperatively. In addition, net negative nitrogen balances were about the same, irrespective of the nutritional regimen used (Table 1).

The alterations observed in intracellular amino acid patterns were in good agreement with earlier findings,[2,24,40] emphasizing that typical changes occur in the posttraumatic state. It appears that injury is characterized by increased muscle concentrations of the branched chain amino acids, phenylalanine, tyrosine, methionine and glycine, and decreased concentrations of lysine, arginine and glutamine. As shown in Tables 2 and 3 and Figures 1–4, the corresponding changes in plasma amino acids may parallel those in muscle but frequently differ either quantitatively or in the direction of change. Thus, it appears that the intracellular free amino acid pattern of muscle is characterized by a unique "pattern of trauma."

The most striking finding in the present work was, however, that the "pattern of trauma" was maintained irrespective of nutritional regimens, thus there were no major differences between the three postoperative groups. This would suggest that the hormonal milieu seen in the injury state causes changes in muscle which nutrition can only affect in minor ways. Exceptions were muscle lysine and arginine which seemed to be influenced by the glucose supply but not by amino acid administration. Muscle alanine concentrations were significantly decreased in Group II, who received amino acids alone, compared both with Groups I and III as well as with the preoperative mean value. In contrast, with muscle, plasma free amino acid were affected by the difference in nutritional treatment. Carbohydrate administration either with or without amino acids was associated with lower plasma levels of the branched chain amino acids than following treatment with amino acids alone, though this was not reflected in muscle. Plasma lysine concentration was significantly lower in Group I, given glucose only than in the other two groups. These specific effects of carbohydrate were probably mediated via insulin in agreement with several investigations showing that administration of glucose or insulin was associated with depression of certain amino acids, especially the branched chain amino acids, and glycine.[14,33,45] It has also been postulated that muscle is the primary site of amino acid uptake during carbohydrate administration.[35] Thus, a closer correlation between muscle and plasma amino acid patterns might have been expected then was actually found. It is noteworthy that the intracellular pools of lysine and arginine, both basic amino acids, were found to be depleted, in good agreement with earlier published data.[24,40] These depletions were greater in Group II and III, than in Group I where the slight decreases were not statistically significant, suggesting that the presence or absence of amino acids in the diet may influence the transport of these basic amino acids. Lysine depletion in Group II and III might be expected, since it was shown earlier that it was necessary to supply 77 mg lysine/kg body weight for a normalization of this pool.[24] In contrast, in the present study, less than 10 mg lysine/kg body weight was given which was probably insufficient to meet the postoperative lysine requirement.

Elevated levels of the branched chain amino acids in both muscle and plasma are a consistent finding, observed in this investigation, in earlier studies in postoperative patients[24,40] and in patients suffering from severe injuries or burns.[23] The concentrations of these amino acids in plasma were more elevated when only amino acids were given. It was suggested by Odessey et al.[37] that branched chain amino acids inhibit the flux of amino acids from muscle, effectively decreasing muscle breakdown. It was also reported that these amino acids induce improvement in N balance through a branched chain amino acid–alanine cycle.[37] Recently, Freund et al. reported better nitrogen balance when infusing branched chain amino acids in septic and injured patients.[20,21] Thus, it seems possible that an improved muscle protein synthesis is mediated by an increased concentration of branched chain amino acids in muscle and plasma. However, as shown in Figure 1, the opposite occurs in trauma, in which increased concentrations are seen in both muscle and plasma. This suggests that changes in branched chain amino acids do not mediate but rather result from changes in protein synthesis. Thus in trauma, increased net catabolism results in increased release of amino acids including the branched chain amino acids. Furthermore, the concentration of the branched chain amino acids were elevated both in muscle and plasma while the large negative nitrogen balances revealed net protein catabolism in all three groups. This would argue against the proposed action of branched chain amino acids in this study. On the other hand, data analysing 3 Me Histidine excretion in trauma showed that postoperative muscle protein breakdown

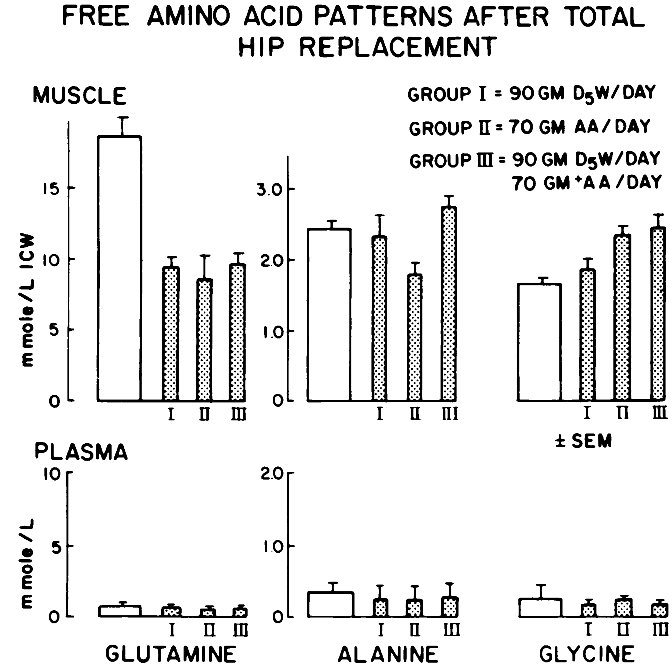

FIG. 3. Glutamine, alanine and glycine patterns after total hip replacement.

was correlated to the body protein loss as measured by nitrogen balance.[36] Amino acid administration improved nitrogen balance in spite of increased turnover of muscle protein. This strongly suggests that in trauma, amino acids improve nitrogen balance in stimulating protein synthesis.[37,39] Consequently, it may be expected that the observed increase in branched chain amino acids provides a stimulus to protein synthesis and thus limits rather than mediates the extent of muscle protein catabolism brought about by trauma.

High plasma phenylalanine levels are frequently found in catabolic situations[41,42] and it is suggested that this increment might be due to an increased release of phenylalanine from skeletal muscle.[41,42] We have observed an increased level of phenylalanine in plasma and an intracellular accumulation of muscle free phenylalanine. There is also the possibility that the high concentration of aromatic amino acids and methionine is at least partly dependent on impaired liver function.[20,42]

A profound decrease (about 50%) in muscle glutamine concentration was a consistent finding in all three groups. This finding seems to be one of the most typical features after injury. This is important because glutamine comprises more than 50% of the total free amino acid pool. Intracellular glutamine was reported to be reduced by 40% when measured four days after colectomy while receiving normocaloric TPN.[24] A 50% decrease in intracellular glutamine was also seen in severe multiple trauma and burns four to eight days after in-

TABLE 3. *Nonessential Amino Acids*

	Preoperative			Postoperative								
				Group I			Group II			Group III		
	M	P	M/P	M	P	M/P	M	P	M/P	M	P	M/P
GLN	18.8	.694	27.3	9.5*	.592	16.4*	8.8*	.513	16.1*	9.8*	.553	17.8†
	1.17	.029	1.5	.71	.035	1.4	1.95	.033	2.3	.86	.035	1.7
ALA	2.41	.346	7.1	2.34	.227*	10.3‡	1.90	.226*	8.3	2.77	.262*	11.1*
	.14	.018	.4	.29	.019	.8	.17	.018	.8	.15	.022	1.1
GLY	1.68	.269	6.5	1.94	.192†	10.5*	2.32†	.225	11.2	2.36†	.205	12.1*
	.11	.019	.4	.15	.014	1.2	.16	.02	1.7	.15	.024	1.0
ARG	.72	.104	7.2	.52	.097	6.0	.30*	.085	3.7†	.43*	.091	5.1‡
	.06	.006	.6	.08	.009	1.5	.05	.008	.7	.05	.008	.8
HIST	.324	.086	3.8	.247†	.067†	3.7	.262	.070	3.6	.284	.073	3.9
	.019	.004	.2	.024	.004	.3	.043	.004	.6	.025	.003	.3
ORN	.345	.059	6.4	.170†	.074‡	2.4	.248	.068	4.0	.229‡	.055	4.3
	.032	.005	.9	.026	.008	.4	.052	.008	1.0	.038	.004	.8
TAU	20.2	.079	254	22.3	.070	335	15.9	.069	230	17.3	.072	293
	1.5	.004	15	1.5	.005	43	1.8	.006	22	1.3	.004	40
ASP	2.25	.008	389	1.64	.006	280	2.35	.006	472	2.15	.006	557
	.20	.001	66	.38	.001	70	.19	.001	145	.28	.001	157
SER	.95	.130	7.5	1.00	.115	9.0	1.36	.118	12.0	1.54	.134	12.0
	.09	.006	8	.07	.008	.9	.20	.01	2.2	.14	.015	1.1
GLU	4.05	.042	128	4.53	.035	156	3.28	.034	116	3.29	.032	111
	.25	.002	21	.74	.004	42	.50	.005	24	.34	.003	15
PRO	.76	.192	4.4	.68	.172	4.2	.72	.275	3.2	.51	.142	4.4
	.11	.016	.7	.15	.018	1.0	.20	.118	.7	.06	.017	8
AAB	.145	.036	4.1	.163	.036	4.8	.158	.049	3.2	.196	.052*	3.9
	.012	.002	.3	.021	.004	1.2	.021	.005	.4	.021	.003	.6

* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

M = Muscle Amino Acids (m Moles/liter intracellular H_2O).
P = Plasma Amino Acids (m Moles/liter plasma H_2O).
M/P = Muscle/Plasma ratio.

jury.[22] This occurred regardless of whether amino acids were given or not. This consistent decrease in the intracellular glutamine pool occurs irrespective of the degree of trauma or the mode of nutrition, suggesting that the observed depletion is virtually obligatory. Whatever the dietary regimen, any injury above a moderate degree seems to stimulate a maximal response. The lack of this most important nonessential amino acid might be a serious limiting factor for optimum protein synthesis. Increased glutamine transport, due to accelerated gluconeogenesis in the liver may serve as a possible explanation. An additional factor might be a relative inability to reutilize available amino groups for glutamine synthesis. Increased tissue catabolism alone cannot explain the posttraumatic amino acid pattern, since as shown in Tables 2 and 3 and Figures 1–4, certain amino acids show no change or reveal a decreased concentration following trauma. On the other hand, such selective changes could be the result of altered amino acid transport. When evaluating the postoperative amino acid alterations on a qualitative basis, there were significant increases in the muscle concentrations of nine amino acids over preoperative values. These include most of the essential and four nonessential amino acids. For most of these amino acids, there is a corresponding increase in plasma concentration, although for three amino acids the change is not statistically significant. Glycine shows a significant decrease in plasma along with an increase in muscle. One result of these changes is an increased gradient or concentration ratio after trauma which is significant for each of the amino acids except leucine and valine. Since the relationship of muscle to plasma amino acids is a function of the amino acid pumps in the membrane wall, these results suggest a change in the setting of the amino acid transport system or "pumps" which are responsible for transport of amino acids across cell membranes and which serve

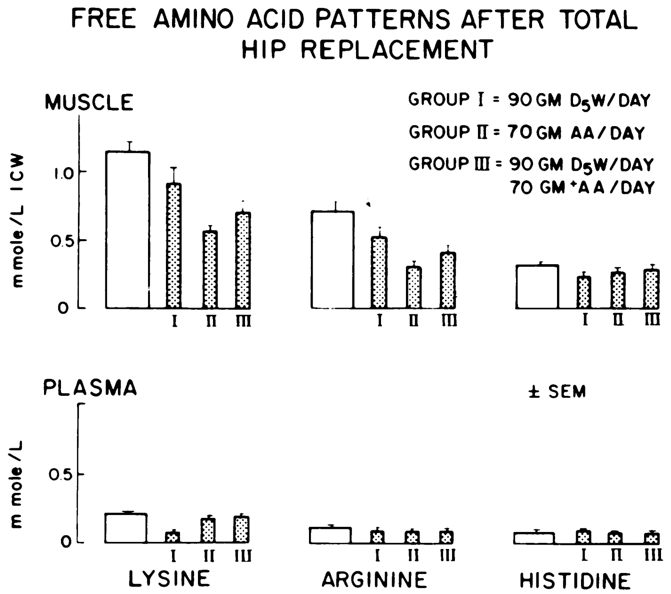

FIG. 4. Lysine, arginine and histidine patterns after total hip replacement.

to maintain concentration gradients of amino acids between intra and extracellular fluids. There are two such pumps responsible for transport of the neutral amino acids and it would appear that the setting of both is increased by trauma. These increased pump settings may account for the change in gradient but do not by themselves explain the greatly increased amino acid concentrations. These changes may also arise from the increase in net catabolism of muscle protein after trauma.

There are five amino acids which show substantial and significant decreases in muscle postoperatively. These include glutamine, and the four basic amino acids —arginine, lysine, histidine and ornithine. Decreases in plasma are either small or nonexistent, and the muscle/plasma ratio is decreased in all cases except histidine. This means that the setting must be lowered for those amino acid pumps which transport the basic amino acids and glutamine. Since there is nevertheless an increased net release of these amino acids from muscle proteins, this change in pump setting, although it may facilitate removal of these amino acids from muscle, cannot account alone for the lower or unchanged plasma concentrations. Furthermore, it is reasonable to believe that some other tissue, probably the liver, kidney and gut are actively removing more of these amino acids as well as alanine which was markedly reduced in plasma (30%), indicating that it also was being actively removed by the liver or some other tissue.

While the amino acid pattern in trauma differs from other states, it is very similar to that seen in fasting (unpublished data). Since our total hip replacement patients were without oral intake for four days postoperatively, one may question whether the changes in amino acid concentration were due solely trauma or at least partly due to dietary causes. However, our previous studies have demonstrated that 5% dextrose as the sole nutrient for four days in healthy subjects is associated with similar but smaller changes which cannot account for the entire pattern of changes seen in trauma.[2]

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