

Plasma and Cerebrospinal Fluid Amino Acid Levels in Diabetic Ketoacidosis Before and After Corrective Therapy

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SUMMARY

To evaluate the effect of insulin-saline-bicarbonate therapy on amino acid metabolism in diabetic ketoacidosis, arterial and venous blood samples as well as cerebrospinal fluid (CSF) were obtained from six patients before and after initiation of corrective therapy. Levels of CSF glutamine were decreased while alanine, α -amino-n-butyrate, valine, isoleucine and leucine were increased significantly compared to a control group composed of six normal, postabsorptive adults free of any neurologic disease. Following therapy, CSF levels of alanine, α -amino-n-butyrate, valine, isoleucine, and leucine declined while glutamine levels did not change.

Admission arterial plasma levels of the glycogenic amino acids were lower than normal while the branched-chain amino acids were elevated. Plasma alanine and glutamine arterio-venous (A-V) differences across forearm tissue were larger. After four hours of corrective therapy, arterial plasma levels of most of the amino acids had declined sharply and A-V differences for glutamine and alanine were markedly reduced ($p < .025$ and $p < .01$, paired *t*, respectively). Coincident with the decrease in A-V amino acid differences, plasma glucagon and free fatty acid levels declined significantly. These data suggest that the effect exerted by insulin-saline-bicarbonate therapy on amino acid metabolism is manifested by diminished A-V plasma alanine and glutamine differences across forearm tissue. Thus, the role played by the splanchnic bed both before and following corrective measures may be secondary to substrate availability. *DIABETES* 24:463-67, May, 1975.

Inappropriately low insulin¹ and elevated plasma glucagon levels² are characteristic of diabetic ketoacidosis. These hormone levels appear to favor the mobilization of man's limited protein stores (primarily muscle) and subsequent conversion of the stores into glucose by the liver. The former process is apparently facilitated by the total or near total absence of insulin's metabolic effects^{3,4} while the latter appears

to be enhanced by low insulin and high levels of glucagon.⁵ The resulting hyperglycemia and ketosis subsequently induce an osmotic diuresis and a metabolic acidosis that result in both fluid and electrolyte depletion.

The work of Felig et al.⁶ indirectly suggested that the low levels of insulin characteristic of patients in ketoacidosis permitted increased mobilization of amino acids from peripheral tissues that is manifested by elevated levels of branched-chain amino acids (valine, isoleucine, leucine) together with relatively decreased concentrations of the glycogenic amino acids (alanine, glycine, threonine, serine), the latter probably being due to enhanced hepatic uptake. Support for this concept is based in part upon the work in vitro^{7,8} and in man^{9,10} demonstrating that insulin is capable of decreasing the release of or actually increasing the uptake by muscle of various amino acids.

Based upon the above observations and concepts, it would be expected that initiation of the usual therapeutic maneuvers (insulin-saline, insulin-saline-bicarbonate) would greatly diminish both the release and uptake of amino acids by muscle and liver, respectively. Therefore, in order to further characterize the role of peripheral tissues, primarily muscle, in the pathogenesis of diabetic ketoacidosis, arterial and venous blood samples were obtained across forearm tissue in six patients in diabetic ketoacidosis both before and after four hours of insulin-saline-bicarbonate therapy. (These patients were a part of a larger group of nine patients that formed the basis of a recent report¹¹ in this journal.) In addition, plasma levels of glucagon and free fatty acids, and amino acid levels in cerebrospinal fluid were also monitored.

MATERIALS AND METHODS

Patients

Patients A.C., F.F., B.G., B.S., A.R., and R.C. of the previously cited study (ref. table 1) were in-

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Accepted for publication January 8, 1975.

cluded in the present investigation, and informed consent was obtained from each individual after description of possible risks and benefits. Six nondiabetic, postabsorptive adults who had diagnostic lumbar punctures and who were found to be free of any neurologic disorders served as controls.

Collection, Preparation, and Analysis of Blood and CSF Samples

Collection of blood (arterial) and cerebrospinal fluid (CSF) samples have been previously described.^{11,12} Blood from an antecubital vein was drawn simultaneously with arterial sampling. Flow was not measured; hence, flux cannot be calculated; however, none of these patients had signs or symptoms of hypovolemia or decreased perfusion. Both blood and CSF were deproteinized, volume for volume, with 10 per cent sulfosalicylic acid, and the filtrates were then stored at -20° C. until analyzed on a Beckman 120C amino acid analyzer. Glutamine and glutamate determinations were performed enzymatically.¹³ Plasma glucagon and free fatty acid levels were determined as previously described.¹⁴

RESULTS

Cerebrospinal Fluid

Comparison of amino acid levels in cerebrospinal fluid of normal subjects with that of untreated diabet-

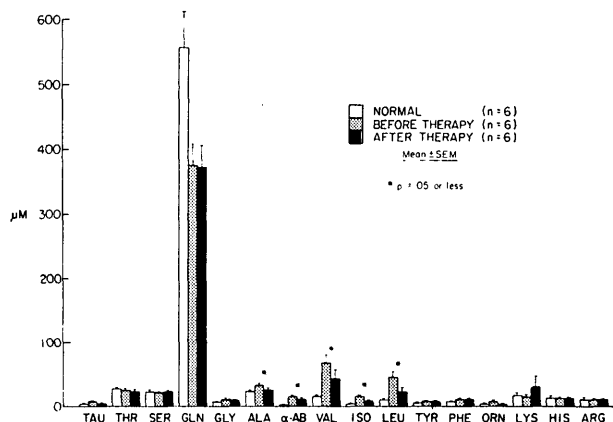


FIG. 1. Comparison of normal cerebrospinal fluid amino acid levels with that of diabetics in ketoacidosis both before and after four hours of insulin-saline-bicarbonate therapy.

ics in ketoacidosis (figure 1) reveals that while glutamine levels are significantly reduced, levels of the branched-chain amino acids, alanine, and α-amino-n-butyrate are significantly elevated (p < .05 or less, unpaired t, two tailed). A number of amino acids including aspartic acid, proline, half-cystine, and methionine could not be detected consistently.

Following treatment, a significant decline in CSF alanine, α-amino-n-butyrate, valine, isoleucine, leucine, and a slight but significant increase in

TABLE 1

Arterial and venous plasma amino acid levels across forearm tissue of diabetics in ketoacidosis (N=6)

Amino Acid	Before Treatment			After Treatment			A ₀ -V ₀ vs. A ₁ V ₁ (p value)
	Artery ₀	Vein ₀	A ₀ -V ₀	Artery ₁	Vein ₁	A ₁ -V ₁	
Taurine	50±8	52±6	- 2±9	45±5	51±4	- 6±7	
Threonine	64±11	77±12	- 14±7	49±7	56±8	- 7±4	
Serine	45±6	59±7	- 14±6*	40±4	46±7	- 5±3	<.05
Glutamine	288±83	411±69	-123±52*	205±56	204±43	+ 1±13	<.025
Proline	104±17	120±21	- 16±13	65±10	76±17	+10±9	
Glutamic Acid	23±5	19±6	+ 4±2	23±3	18±6	+ 5±3	
Citrulline	14±1	15±2	- 1±3	12±1	13±3	- 2±2	
Glycine	131±19	149±22	- 18±11	146±16	150±17	- 5±7	
Alanine	149±19	233±29	- 84±18*	87±6	109±12	- 22±8*	<.01
α-Amino-n-butyric Acid	27±4	34±16	- 7±7	14±2	19±5	- 5±4	
Valine	457±99	503±87	- 45±55	221±43	263±51	-42±41	
Half Cystine	83±11	90±6	- 7±7	46±5	51±4	- 5±3	
Methionine	15±3	18±4	- 2±3	10±3	11±4	- 1±1	
Isoleucine	140±47	145±41	- 6±13	41±14	49±15	- 7±20	
Leucine	286±85	287±77	- 1±34	96±21	114±20	-17±17	
Tyrosine	40±6	44±6	- 4±2	34±6	35±5	- 1±1	
Phenylalanine	43±4	46±3	- 3±3	51±2	50±3	+ 2±2	
Ornithine	22±4	30±4	- 7±4	14±1	17±3	- 3±3	<.05
Lysine	139±53	174±65	- 41±20	102±35	111±32	- 9±15	
Histidine	82±10	92±9	- 10±9	63±10	59±9	+ 4±8	<.025
Arginine	24±4	31±7	- 7±4	16±3	14±3	+ 2±3	

*Probability that arterio-venous difference does differ from zero (paired t test), p=.05 or less.

phenylalanine were seen. Glutamine levels did not change significantly.

Plasma

Comparison of the arterial plasma amino acid levels of normal subjects¹¹ with that of untreated diabetics in ketoacidosis (table 1) revealed significant decreases in levels of threonine, serine, proline, citrulline, glycine, alanine, methionine, ornithine, and arginine, and significant increases in levels of valine, leucine, and amino-n-butyrate. Significant arteriovenous amino acid differences (table 1) were noted for serine, glutamine, alanine, and tyrosine by forearm muscle of untreated diabetics in ketoacidosis.

Following initiation of therapy that included insulin-saline-bicarbonate, significant decreases in arterial plasma levels of proline, alanine, α -amino-n-butyrate, valine, cystine, isoleucine, leucine, and ornithine were seen. When compared to admission A-V amino acid differences, significant decreases in A-V differences of serine, glutamine, alanine, ornithine and histidine were observed (figure 2). When total plasma amino acid A-V differences are summed in the two groups, it can be seen that the untreated group's total plasma A-V difference is -404 ± 200 μ moles per liter of plasma and this difference declines significantly ($p < .05$, paired t) to -125 ± 127 μ moles per liter of plasma following four hours of therapy.

Following institution of corrective therapy, plasma glucagon levels declined in five and increased in one patient (see table 2). The normal postabsorptive value for glucagon in this laboratory is 143 ± 9 pg/ml., $\bar{X} \pm S.E.M.$ Plasma free fatty acids also declined ($p < .0025$, paired t) from $1,879 \pm 499$ to 870 ± 49 μ Eq./L. of plasma.

DISCUSSION

It must be appreciated from the onset that the many changes in substrate and hormone levels recorded here and elsewhere^{11,12} during the course of this investigation may have been influenced to varying degrees by the state of hydration, circulatory fluid volume, electrolyte balance, and acid-base status of the patients studied. For these reasons, it was not always possible to clearly distinguish or attribute changes induced by the foregoing from that induced by insulin alone. Nevertheless, the timing and magnitude of a few of the documented changes in blood and cerebrospinal fluid permit, with some security, the drawing of a number of conclusions.

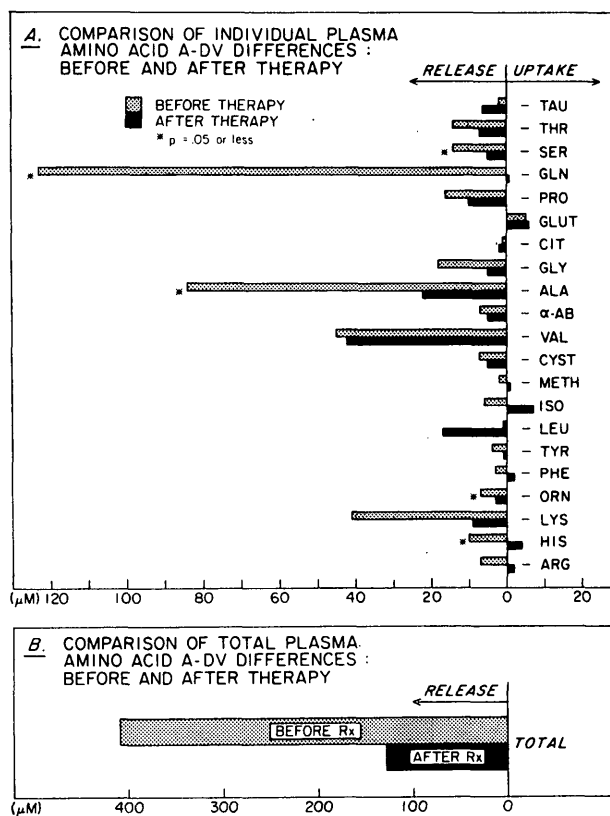


FIG. 2. Comparison of individual (Panel A) and total (Panel B) amino acid differences before and after four hours of therapy. For convenience, when arterial amino acid levels are higher than the corresponding venous levels, the term uptake is used. When the reverse is seen, "release" is used.

Cerebrospinal Fluid

Normal values. CSF amino acid levels in normal adults (see figure 1) obtained in this investigation compare favorably with that reported by Perry et al.¹⁵ with the following exceptions: CSF glutamine levels are much higher in the present study mainly because enzymatic determinations were done on freshly collected CSF. Degradation of glutamine was therefore minimized. In addition, the present CSF glutamine levels agree closely with published data from plasma

TABLE 2
 Plasma glucagon and free fatty acid levels in diabetic ketoacidosis

Patient	Glucagon Pg/ml.		Free Fatty Acids μ Eq./Liter	
	Before	After	Before	After
A.C.	>800	140	1,950	800
F.F.	140	70	1,400	1,025
B.B.	>800	340	2,000	875
B.S.	70	40	2,300	1,000
A.R.	310	360	2,150	725
R.C.	210	115	1,475	800

glutamine levels in normal subjects determined using the same methodology.¹³ Second, threonine and serine levels are much lower in the present study, and the reason for these differences is not known. Third, the eight unknown peaks reported by Perry et al. were inconsistently seen due to the much smaller sample injected (10 ml. vs. 0.375 ml.).

Diabetic ketoacidosis. On admission, CSF amino acid levels in the diabetic patients were comparable to the controls except for those amino acids which were either elevated (α -amino-n-butyrate, valine, isoleucine, leucine) or decreased (glutamine) in plasma. The major exception to this generalization was alanine ($p < .05$, unpaired t , two tailed) which was higher in the CSF of diabetic than in control subjects.

Following four hours of corrective therapy, CSF amino acid levels appeared to mirror changes seen in plasma. Levels of branched-chain amino acids, alanine, and α -amino-n-butyrate declined while phenylalanine increased slightly. Of interest, CSF glutamine levels failed to change despite a rather marked decline in plasma glutamine levels, and after four hours of therapy, there appeared to be a CSF:arterial plasma glutamine gradient ($p < .05$, unpaired t , two tailed) that favored passage of glutamine from cerebrospinal fluid to arterial plasma. The possible importance of this observation will be discussed later.

Plasma Amino Acid Levels and Differences across Forearm Tissue

Comparison of arterial plasma amino acid levels of normal subjects⁹ with that of untreated diabetics in ketoacidosis (unpaired t , two tailed) revealed significantly diminished levels of threonine, serine, proline, citrulline, glycine, alanine, and ornithine and significantly elevated levels of α -amino-n-butyrate and the branched-chain amino acids. However, four hours after initiation of therapy, levels of virtually all amino acids declined. These latter observations, taken together, described what appears to be a pronounced and characteristic hypoaminoacidemia induced by the administration of reasonably large quantities of insulin.¹⁶

Arterio-venous amino acid differences analyses were of interest. On admission, the mean algebraic sum of the individual A-V amino acid differences was $-404 \pm 200 \mu\text{M/L}$. of plasma, with alanine and glutamine being quantitatively the most important. Following institution of corrective therapy with insulin-saline-bicarbonate, this sum (figure 2) was remarkably attenuated ($p < .05$, paired t , two tailed) with the most marked individual change evidenced by glutamine. If

it is assumed that forearm blood flows were comparable during the two sampling periods, the presumed splanchnic (and renal) uptake of these two amino acids together with their presumed diminished release from muscle would favor the gradual decline in levels actually observed.

Most interesting was the observation that admission branched-chain amino acid A-V differences were not reduced or converted to an uptake despite the administration of large amounts of insulin. Since levels of these amino acids usually decline in response to very small doses of insulin, it was anticipated that their A-V differences from muscle would be similarly affected. Indeed precedence for such an expectation can be found in the work of Pozefsky et al.¹¹ who infused small quantities of insulin into normal human forearm muscle bed and found that the release of these amino acids (valine, isoleucine, leucine) was inhibited. Thus, the failure to find a reduction in the arterio-venous difference of valine, isoleucine, and leucine in the present investigation was somewhat puzzling. These data suggest that (1) whole blood should have been analyzed rather than plasma or (2) other tissues besides muscle¹⁷ are capable of removing the branched-chain amino acids from the circulation. With respect to the first possibility, to date plasma levels and plasma arterio-deep venous branched-chain amino acid differences appear to accurately reflect, in both normal and nitrogen-depleted subjects,¹⁴ qualitatively, changes found in whole blood amino acids, with the possible exception of glutamine. Second, inspection of the individual (patient by patient) arterio-venous differences for the branched-chain amino acids failed to reveal a clear pattern, i.e. no clear inhibition or uptake of these amino acids following institution of therapy was seen. The latter data tend to suggest that there may be other tissues such as adipose tissue¹⁷ which actively remove these three amino acids from the circulation under the cited conditions.

Corrective therapy does result in a decline in arterial plasma amino acid levels and, assuming forearm blood flows were comparable during both blood sampling periods, appears to effect a decrease in alanine and glutamine release from forearm tissue, primarily muscle. These changes appear to be closely reflected by complementary changes in CSF with the exception of glutamine. This latter observation not only suggests that the brain is actively synthesizing this amino acid but may be providing glutamine for use elsewhere. The possible importance of this capability is underscored by the realization that glutamine release

from muscle appears to be virtually abolished, as reflected by plasma glutamine determinations.

In summary, substrate availability in the form of alanine and glutamine may be greatly reduced following the institution of insulin-saline-bicarbonate therapy of diabetic ketoacidosis. Thus, indirectly and possibly directly, hepatic gluconeogenesis may be similarly curtailed. CSF amino acid levels appeared to reflect changes in blood with the exception of glutamine.

ACKNOWLEDGMENT

The study was supported in part by Eli Lilly and Company, Indianapolis, Ind., by grants (AM-05077 and AM-15191) from the U.S.P.H.S. and by the Adler Foundation, Inc., Rye, N.Y.

Thomas T. Aoki, M.D., is a Fellow of the Medical Foundation, Boston, Massachusetts.

We would like to express our appreciation of the technical help of Mmes. D. Rumba, P. Hatch, V. Ramolins, and A. Allen.

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