Blood Cell and Plasma Amino Acid Levels Across Forearm Muscle During a Protein Meal

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SUMMARY

To elucidate the role of blood cells in amino acid metabolism, substrate balance across the forearm was studied in a nitrogen-depleted subject fed 200 gm. of meat. After ingestion of the meal, there was the expected outpouring of amino acids from the splanchnic bed into the general circulation. Both cell and plasma levels of most amino acids in arterial blood increased rapidly. Whole blood arterio-deep venous amino acid differences frequently differed from that of plasma. In conclusion, it appears that both blood cells and plasma transport amino acids from the splanchnic bed to the periphery and that both participate actively in the deposition of amino acids in the forearm of the subject studied. DIABETES 22:768-75, October, 1973.

Traditionally, plasma levels of amino acids or α-amino nitrogen have been reported by investigators concerned with amino acid transport and interorgan flux. Such an approach will not reflect tissue production or utilization if the circulating cellular constituents of blood can be shown to have a role in amino acid transport. The publications by Elwyn and associates[1-3] have shown erythrocytes to play an active role in gastrointestinal and hepatic amino acid exchange in dogs and have suggested that a direct tissue-to-blood cell transfer of amino acids may occur without any change in plasma concentrations.

Recently, glutamate metabolism across human forearm muscle has been studied both in the basal state and after infusion of insulin into the brachial artery.[4] It was found that while plasma glutamate arterio-deep venous (A-DV) differences did not change significantly during the infusion, whole blood glutamate A-DV differences demonstrated increased uptake into forearm muscle. Thus, it appeared that the circulating blood cells of man played an active role in amino acid transport and that whole blood amino acid, and not plasma determinations, reflected amino acid flux.

To study possible interactions between the plasma, the circulating blood cell, and the muscle compartments in man with respect to amino acid transport from splanchnic bed to muscle, a 200 gm. ground sirloin meal was given to a single patient who had fasted for six weeks and was, therefore, nitrogen depleted. This choice of subject was predicated on the assumption that a moderately nitrogen-depleted individual would exhibit more marked changes with respect to amino acid levels than one in nitrogen balance. While the findings stemming from this investigation cannot be interpreted as being characteristic of normal man, nevertheless, they may lead to a better understanding of the role of the blood cells in amino acid transport.

With the above reservations in mind, changes in amino acid levels (plasma and whole blood) across forearm muscle were studied for four hours after the patient had ingested the meal. In addition, insulin and glucagon levels, as well as the levels of a number of other substrates, were determined.

MATERIALS AND METHODS

A thirty-nine year old white female weighing 250 lb. was interviewed, examined, and screened biochemically as described elsewhere[5] to exclude abnormalities other than obesity. All procedures and risks were explained to the patient, and her informed consent was obtained. After a therapeutic six week fast, the patient was placed on a 1,200 calorie low salt diet for two days. On the morning of the third postfast day, and after a twelve hour fast, a 20 gauge catheter was inserted percutaneously into the brachial artery. A second 20 gauge catheter was inserted retrograde deep into the ipsilateral antecubital vein, thereby insuring sampling of deep

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venous blood draining forearm muscle. A pediatric blood pressure cuff was applied to the wrist five minutes before sampling and inflated to 300 mm. Hg to exclude blood flow from the hand. Paired A-DV blood sampling was performed before the patient ingested, within a fifteen minute period, 200 gm. of broiled ground sirloin and at one, two, three and four hours after the start of the meal. Flow was not measured and, therefore, no quantitation of substrate exchange per minute per gram of forearm muscle can be made. However, for convenience of presentation, when the venous level of a given substrate is less than the arterial level, the term uptake (+) will be used. When the reverse is found, the term release (—) will be used.

Preparation and analysis of samples for plasma amino acids and free fatty acids, whole blood lactate, pyruvate, and glycerol as well as insulin have been described elsewhere.[5,6] Plasma glucagon levels were determined using 30K antibody kindly supplied by Dr. Roger Unger (Dallas, Texas).[7]

Plasma glutamate determinations were performed using the enzymatic assay of Pagliara and Goodman,[8] in which 0.5 ml. of deproteinized (10 per cent perchloric acid, v:v) filtrate, the pH of which is adjusted to 4.9 with KOH, is added to 1.5 ml. of tris-hydrazine buffer. After initial fluorimetric readings are obtained, 10 μl. of glutamate dehydrogenase (Boehringer No. 15140 EGAG) are added to each tube. The tubes are then mixed and allowed to stand at room temperature for one hour and subsequently reread on the fluorimeter (American Instruments, Model 4-7103). An NADH curve is then run and compared to the glutamate standard curve (5, 10, 15, 20 nmoles per tube). The two curves should be virtually identical.

The glutamine assay is based on the glutamate assay, in that two glutamate determinations are performed on each sample, i.e. before and after incubation with glutaminase. The first value obtained is then subtracted from the second value. In this assay, after the initial aliquot of the processed sample is removed for the first glutamate determination, 0.1 ml. of the same filtrate is added to a 20 ml. penicillin bottle which contains 0.9 ml. of 0.2 M. sodium acetate buffer (pH 4.9). Glutamine standards (250 and 500 μmoles per liter) are carried through all assays in an identical fashion. After the addition of 0.2 ml. (0.05 U.) of glutaminase (glutamate decarboxylase-free, Sigma type V), the bottles are stoppered and incubated at 37° C. in a Dubnoff metabolic shaker for forty-five minutes. At this point, a second glutamate determination is performed as outlined above.

Recovery of known amounts of glutamate added to plasma, whole blood, and their respective acid filtrates is approximately 97 to 100 per cent. Similar recoveries are obtained when known amounts of glutamine are added to plasma, whole blood, and their respective acid filtrates. Whole blood glutamate and glutamine determinations are performed on samples deproteinized (within fifteen seconds of sampling) with chilled 30 per cent perchloric acid, v:v. No difference in glutamate or glutamine recovery has been found when this acid is used rather than 10 per cent perchloric acid; however, a clearer filtrate is obtained with 30 per cent perchloric acid. The glutamate and glutamine contents of processed filtrates (pH 4.9) of plasma and whole blood are stable for four to six weeks at —20° C.

The intra-assay coefficients of variation of plasma and whole blood glutamate are 2.3 per cent (49 ± 1 μmoles per liter of plasma, mean ± S.D.) and 1.5 per cent (119 ± 2 μmoles per liter of whole blood) respectively. The corresponding interassay (n = 7) coefficients of variation are 3.8 per cent (48 ± 2 μmoles per liter of plasma, mean ± S.D.) and 4.5 per cent (115 ± 5 μmoles per liter of whole blood). The intra-assay coefficients of variation of plasma and whole blood glutamine are 3.7 per cent (576 ± 21 μmoles per liter of plasma, mean ± S.D.) and 2.1 per cent (696 ± 15 μmoles per liter of whole blood, mean ± S.D.). The corresponding interassay (n = 7) coefficients of variation are 4.3 per cent (554 ± 24 μmoles per liter of plasma, mean ± S.D.) and 4.1 per cent (718 ± 29 μmoles per liter of whole blood).

A Beckman 120C anion exchange amino acid analyzer was used for determination of both plasma and whole blood amino acids not determined enzymatically. Whole blood samples were deproteinized in 10 per cent sulfosalicylic acid (v:v). Other acids have been tested (6 per cent, 10 per cent, and 30 per cent perchloric acid as well as 20 per cent sulfosalicylic acid) and have been found to give nearly equivalent results. However, since 10 per cent sulfosalicylic acid is used to deproteinize plasma samples in this laboratory and since it yields results indistinguishable from the previously mentioned acids, this acid was used throughout the study. Recovery studies varied between 97 and 104 per cent for the individual amino acids found in whole blood. Glutathione was removed from the filtrates by the addition of 0.2 ml. of 0.5 M. Na_2SO_3 to 4 ml. of filtrate as outlined in the Beckman 120C manual (section: preparation of samples). Cystine was converted to cysteine-S-sulfonate, while methionine was variably affected by this process.

Therefore, the whole blood values of these two amino acids are not reported here. Since duplicate macrohematocrits were obtained on all blood samples, it was possible to determine the amount of any given amino acid in either the plasma or blood cell compartment of a given sample of blood: Cell content = whole blood content—plasma content (μmoles per liter of blood).

RESULTS

As seen in table 1, glucose levels rose only modestly, with maximal uptake present at four hours. Plasma free fatty acids fell to a nadir by two hours, while A-DV differences showed an uptake at one hour. Blood glycerol levels fell one hour after the start of the meal and increased gradually back to baseline. Blood lactate and pyruvate levels did not change noticeably during the investigation.

Serum insulin and plasma glucagon levels both increased rapidly with insulin reaching a peak at two hours (figure 1). The peripheral insulin:glucagon ratio rose to a peak at one hour, reflecting the more rapid rise of insulin relative to glucagon, as found elsewhere.[9]

Duplicate macrohematocrits were virtually identical for all ten blood samples. Plasma and whole blood amino acid levels during the study are shown in table 2. Comparison of individual amino acids (arterial versus deep venous, whole blood versus plasma) underscore the differences that exist between plasma and whole blood amino acid determinations. For example, table 2 data demonstrate that the arterial plasma contribution of proline, citrulline, glycine, alanine, valine, isoleucine, tyrosine, and phenylalanine, if expressed as a percentage of whole blood, declined during the study period. This observation suggests that the blood cell content of these amino acids has increased. Since this increase occurred in the face of rising plasma concentrations, the distribution of these amino acids became biased in favor of the blood cell compartment. The reverse appears to be true for glutamine, glutamate, lysine, and histidine. Serine, α-n-butyric acid, and leucine levels initially increased then decreased, while threonine values exhibited little change.

Detailed compartmental analyses, together with whole blood arterio-deep venous differences, for glutamate, glutamine, lysine, phenylalanine, alanine, glycine, and the branched chain amino acids are shown in figures 2 and 3. It can be seen that the rate and extent of change of the plasma and blood cell compartments varied with time and with the individual amino acids studied. Arterial plasma glutamate levels rose rapidly following the ingestion of the meal while arterial blood cell glutamate levels appeared to decline. Plasma A-DV differences increased from a resting uptake of +23 to +43 μmoles per liter of blood at two hours while whole blood glutamate A-DV differences increased from +17 to +58 μmoles per liter of blood.

Arterial blood cell glutamine appeared to gradually decline, reaching a nadir at three hours, while arterial plasma levels of this amino acid increased over the same period. Plasma and blood cell A-DV differences appear to complement each other over all five time periods; i.e. when a release is indicated by one, the other demonstrated an uptake. However, there was a gradual and steady trend towards uptake of this amino acid by muscle at two hours followed by a gradual return towards basal conditions, as evidenced by whole blood A-DV differences.

While both the arterial plasma and blood cell compartments of lysine and phenylalanine increased during the study, arterial plasma lysine levels increased from 79 to a maximum of 235 μmoles per liter of blood at two hours, while arterial blood cell lysine increased from 77 to a maximum of 129 μmoles per liter of

TABLE 1

Changes in blood levels of various fuels following ingestion of a protein meal

	0 Artery	0 Deep Vein	1° Artery	1° Deep Vein	2° Artery	2° Deep Vein	3° Artery	3° Deep Vein	4° Artery	4° Deep Vein
Glucose mg./100 ml. blood	97	94	102	99	102	100	97	94	99	91
Lactate μM./L. blood	580	880	560	700	650	630	610	690	560	730
Pyruvate μM./L. blood	55	68	59	65	65	45	64	68	62	75
Glycerol μM./L. blood	158	192	99	131	96	114	125	152	122	184
Free fatty acids μM./L. plasma	1,200	1,400	1,150	1,075	750	950	800	900	750	850

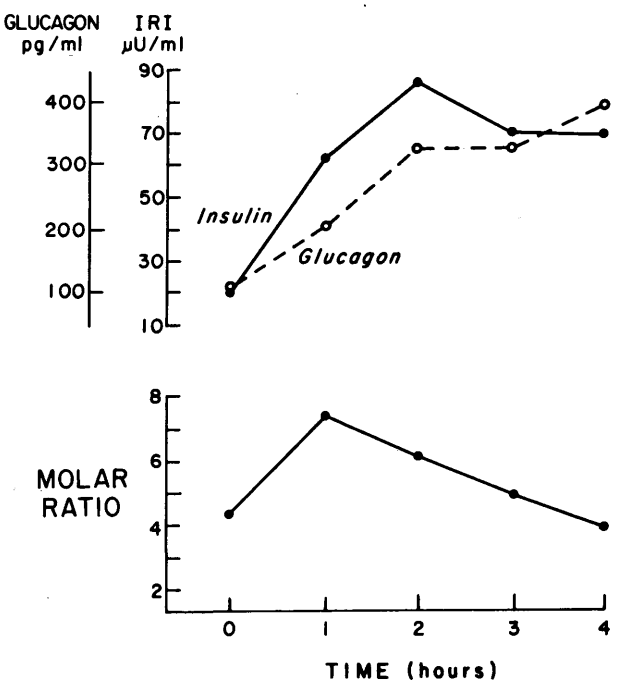

FIG. 1. Insulin and glucagon levels after ingestion of a 200 gm. meat meal by a nitrogen-depleted subject. Note the insulin peak at two hours and the sustained elevation of plasma glucagon. The insulin:glucagon ratio is shown in the lower panel.

blood at three hours. In contrast, arterial plasma phenylalanine levels were increased by a factor of 2 at the second and third hours of the study while blood cell phenylalanine levels increased from 8 to a maximum of 57 μmoles per liter of blood at two hours, with levels declining by one half at three and four hours.

Arterial blood cell and plasma levels of alanine were only modestly increased during the study, and the maximum increase of this amino acid, reached at two hours, was virtually equal to the maximum increment reached in the blood cell compartment at the fourth hour of the study. Again a discrepancy between plasma and whole blood A-DV differences is seen.

Both arterial plasma and blood cell compartments of glycine increased during the study. In contrast to all the other amino acids studied, arterial plasma levels declined below basal values by the third and fourth hours of the investigation. A modest discrepancy between plasma and whole blood A-DV differences is present.

The branched chain amino acids showed remarkable increases in both arterial plasma and blood cell compartments. On either an absolute or relative basis, these increments over baseline were large. In addition, plasma and whole blood A-DV differences were similar except at two hours.

TABLE 2

Changes in blood amino acid content across forearm muscle of a nitrogen-depleted subject during a protein meal (μmoles/liter of whole blood)

Amino acid	0 Artery			0 Deep Vein			1° Artery			1° Deep Vein			2° Artery		
	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*
Taurine	130	31	99	105	39	66	136	39	97	113	38	75	156	33	123
Aspartic acid	137	—	137	133	—	133	151	—	151	136	—	136	155	—	155
Threonine	144	101	43	154	101	53	193	131	62	191	135	56	233	165	68
Serine	203	113	90	194	121	73	228	143	85	217	141	76	231	151	80
Glutamine†	482	88	394	506	161	345	509	65	444	514	161	353	602	273	329
Glutamate†	163	42	121	146	19	127	174	56	118	141	24	117	198	75	123
Proline	177	150	27	201	143	58	246	204	42	243	186	57	283	205	78
Citrulline	18	15	3	19	13	6	19	14	5	26	13	13	24	15	9
Glycine	379	216	163	428	233	195	433	228	205	461	257	204	416	220	196
Alanine	207	128	79	249	161	88	246	160	86	281	193	88	286	172	114
α-Amino-n-butyric acid	31	22	9	32	26	6	34	26	8	40	24	16	38	27	11
Valine	194	139	55	205	144	61	288	201	87	289	190	99	381	260	121
Isoleucine	63	44	19	67	43	24	124	82	42	118	76	42	192	129	63
Leucine	95	65	30	96	66	30	190	131	59	179	124	55	284	203	81
Tyrosine	41	31	10	48	30	18	75	50	25	74	46	28	110	73	37
Phenyl-alanine	35	27	8	44	28	16	63	42	21	61	42	19	111	54	57
Ornithine	82	41	41	80	39	41	102	65	37	98	46	52	99	44	55
Lysine	156	79	77	154	99	55	258	193	65	250	155	95	339	235	104
Histidine	55	27	28	47	27	20	76	52	24	76	43	33	87	56	31
Arginine	23	19	4	22	19	3	69	65	4	63	56	7	113	65	48
Total	2,815	1,378	1,437	2,930	1,512	1,418	3,614	1,947	1,667	3,571	1,950	1,621	4,338	2,455	1,883

* WB = whole blood PL = plasma CELL = blood cell
† Enzymatically determined

(continued on page 772)

TABLE 2 *(continued from page 771)*

Changes in blood amino acid content across forearm muscle of a nitrogen-depleted subject during a protein meal (μmoles/liter of whole blood)

Amino acid	2° Deep Vein			3° Artery			3° Deep Vein			4° Artery			4° Deep Vein		
	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*	WB*	PL*	Cell*
Taurine	106	37	69	152	33	119	137	38	99	205	31	174	160	36	124
Aspartic acid	138	—	138	151	—	151	142	—	142	144	—	144	144	—	144
Threonine	202	145	57	246	162	84	228	149	79	227	160	67	222	146	76
Serine	198	133	65	218	134	84	200	124	76	218	116	102	199	113	86
Glutamine†	581	151	430	489	355	134	477	375	102	527	295	232	559	225	334
Glutamate†	140	32	108	184	73	111	142	34	108	170	69	101	146	40	106
Proline	245	180	65	285	192	93	258	178	80	282	198	84	253	171	82
Citrulline	20	13	7	29	19	10	23	15	8	31	16	15	28	18	10
Glycine	393	223	170	375	183	192	395	193	202	351	152	199	371	174	197
Alanine	284	179	105	281	169	112	304	190	114	266	145	121	306	176	130
α-Amino-n-butyric acid	34	29	5	42	25	17	38	28	10	44	29	15	43	29	14
Valine	312	222	90	438	284	154	403	248	155	479	309	170	442	274	168
Isoleucine	145	105	40	219	147	72	196	107	89	251	150	101	211	135	76
Leucine	228	163	65	337	231	106	289	176	113	362	158	204	318	210	108
Tyrosine	89	62	27	128	82	46	117	70	47	133	83	50	125	78	47
Phenyl-alanine	67	46	21	81	56	25	75	46	29	78	51	27	76	48	28
Ornithine	95	42	53	134	54	80	100	68	32	124	67	57	123	45	78
Lysine	262	195	67	353	224	129	252	228	24	333	226	107	332	158	174
Histidine	83	52	31	82	47	35	63	52	11	72	46	26	74	36	38
Arginine	92	78	14	97	88	9	110	83	27	109	92	17	95	71	24
Total	3,714	2,087	1,627	4,321	2,558	1,763	3,949	2,402	1,547	4,406	2,393	2,013	4,227	2,183	2,044

* WB = whole blood PL = plasma CELL = blood cell
† Enzymatically determined

The most marked changes with respect to all the amino acids occurred at two hours, a time when insulin values have peaked. The changes with time were documented by both the enzymatic assays (glutamine and glutamate) and anion exchange chromatography.

In figure 4, the total A-DV amino acid difference across the forearm before and during the investigation is depicted together with the relative contributions from the plasma and blood cell compartments. It can be seen that the blood cell compartment, despite exhibiting a smaller total increment (in arterial blood) than plasma, accounted for approximately one-half and one-third of the total A-DV amino acid difference at two and three hours, respectively.

DISCUSSION

Although the patient had eaten during the two days before the study, the moderately elevated plasma free fatty acid levels, together with diminished release of plasma amino acids from resting forearm muscle, suggest that she was in a modified fasting state.[10] From table 1, it is apparent that the ingestion of the protein meal initiated a number of changes in the basal hormone-fuel interrelationships. The fall of both arterial and venous plasma free fatty acid levels was most likely due to the rise of serum insulin. However, the failure to demonstrate a sustained uptake of this substrate across the forearm was somewhat surprising, especially when forearm insulinization of normal subjects is known to induce both a pronounced and sustained net uptake of this fuel, due to inhibition of its release from adipose tissue located in the perfused area.[11] This failure may be attributable in part to the persistence of the relative insensitivity to insulin of the adipose tissue after prolonged fasting.[12]

Similarly, muscle uptake of glucose, glycerol, lactate, and pyruvate is either small or not apparent. Thus, from a metabolic point of view, amino acid metabolism, and in particular, the movement of amino acids from the splanchnic bed into blood and out of blood into muscle, is the main focus of activity, as expected after the ingestion of a protein meal by a nitrogen-depleted subject.

The increase in arterial blood cell levels of threonine, proline, citrulline, α-n-butyric acid, valine, isoleucine, leucine, tyrosine, phenylalanine, and ornithine and, to a lesser extent, of glycine, alanine, and lysine appear to corroborate the concept that the blood cells of man are active in amino acid transport. These data also substantiate the major role of the plasma compartment in

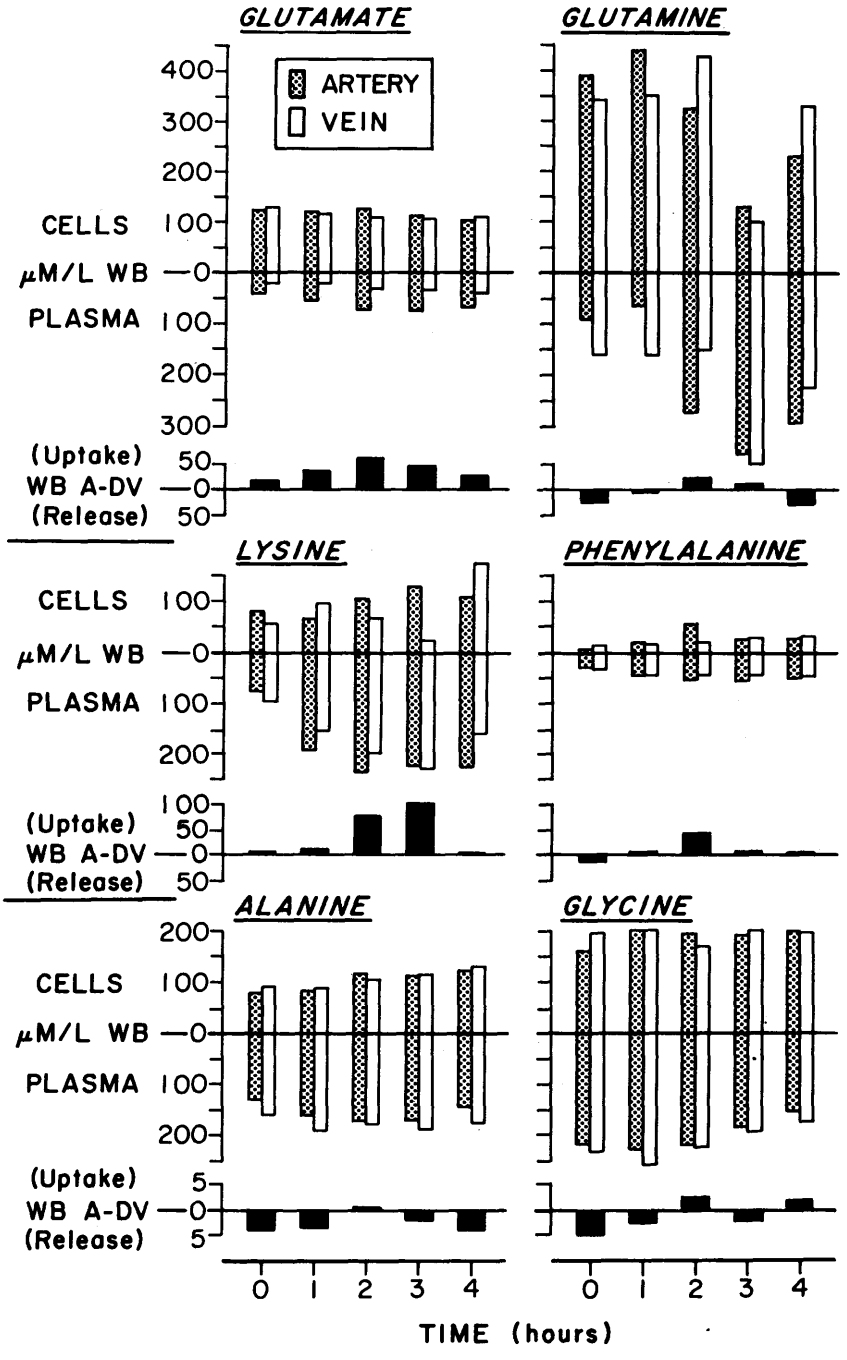

FIGURE 2

Detailed blood cell and plasma compartment amino acid analyses. The vertical bars above the horizontal line represent the amino acid content of the blood cell compartment, while the vertical bars below the line represent the amino acid content of the plasma compartment. See text for discussion.

amino acid transport, for in terms of absolute quantity, this arterial compartment increased by approximately 1.2 mmoles (at two and three hours) while the arterial blood cell compartment increased by 600 μmoles (at four hours).

While the arterial plasma compartment plays a major role in amino acid transport, as evidenced by the absolute increase of this compartment during the study, it is seen that the blood cell A-DV differences, in particular at two and three hours of the investigation, account for a large portion of the total whole blood A-DV changes at these times (figure 4). Thus, despite exhibiting a smaller absolute increment in amino acid content, the arterial blood cell compartment released, either indirectly or directly, to forearm muscle an amount of amino acids nearly equal to that released by the arterial

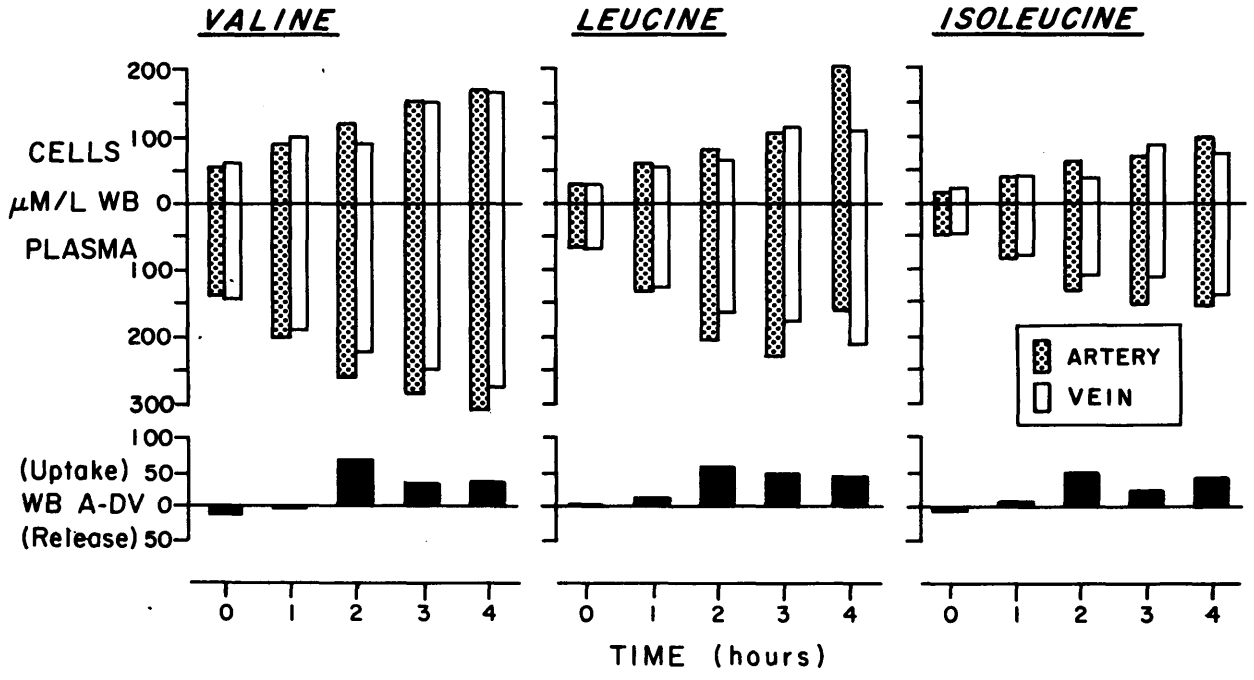

FIG. 3. Blood cell—plasma compartment analysis of the branched chain amino acids. Note the remarkable expansion of the blood cell compartment in particular.

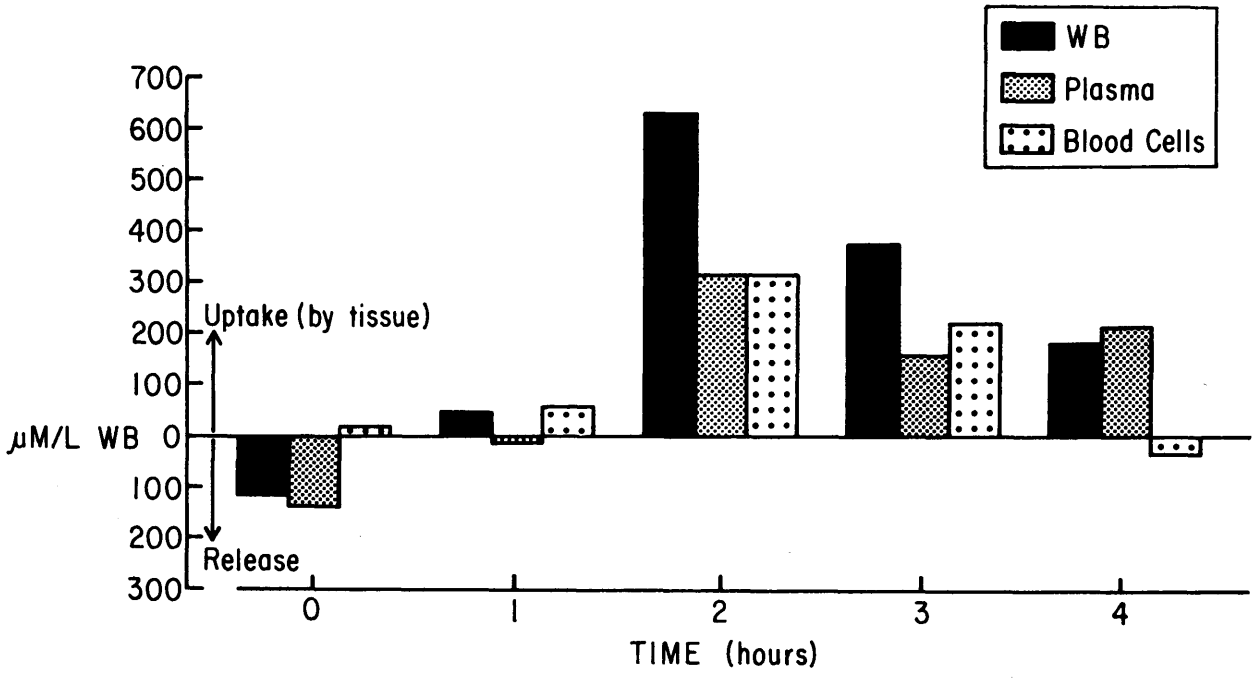

FIG. 4. Summary of total amino acid arterio-deep venous differences (whole blood, plasma, and blood cells) across forearm muscle.

plasma compartment.

The absolute increase in arterial blood cell content of the amino acids enumerated above and the A-DV blood cell differences for these same amino acids suggest that the blood cells of the subject studied are actively involved in amino acid transport. The data, derived from a single study of a patient who had completed a six weeks' fast, may not pertain generally to man in the postabsorptive state. Studies now under way at this laboratory should establish the role of the blood cells in amino acid transport in healthy men after the ingestion of protein and other experimental meals.

In conclusion, combined arterio-deep venous blood cell and plasma compartmental analyses were performed across the forearm muscle of a nitrogen-depleted subject fed 200 gm. of meat. Arterial blood cell and plasma levels of a number of amino acids increased during the course of the study. Compartmental analyses revealed complex interactions between the blood cells, plasma, and forearm tissue and appeared to impute to the blood cell compartment an active role in amino acid transport.

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