Amino Acids and Glucose in Human Blood Plasma after Beef and Nonprotein Meals[1] (34370)

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The regulation of plasma amino acid concentrations in man is not well understood partly because of the meager information on the upper and lower limits of concentration in healthy human subjects. After introduction of their ion exchange column chromatography, Stein and Moore reported amino acid analyses of single samples of plasma from 5 fasting subjects (1). Several investigators have since reported similar analyses but much of this work was either directed toward improvement of analytical technic (2, 3) or a determination of responses to unusual dietary conditions (4, 5). Generally analysis of a single sample from a fasting subject has served as the base line for all subsequent modifications of experimental procedure. We felt the need of more extensive information, as a basis for our own future work, not only of the fasting state but of the postprandial state in the same man at fixed times after ingestion of the test meal. The results described below were obtained from 6 men who ate the same type of test meals 6 times.

Methods and Materials. The 6 subjects were healthy students or staff members (22–43 years, 59–85 kg). They fasted for at least 12 hr before having the first 10 ml of blood sample taken from an arm vein (labeled 0 hr in the tables and figures). In the first series of experiments (21 Oct.–15 Dec.) each subject ate 200 g of lean beef, pan fried without addition of fat, 6 times at intervals of 5–14 days. The meal was salted to taste and eaten with 200 ml of water in about 20 min. Succeeding blood samples were timed from the last swallow of test meal and were taken 1, 2, 4, and 8 hr thereafter. In the second series (26 Jan.–5 March) the same subjects ate a nonprotein meal 6 times at weekly intervals. The nonprotein meal was made as follows: One part by weight of melted margarine, clarified by centrifugation was mixed with 5 parts of brown sugar. The solidified mixture was not unpleasant to taste and 60 g of it, calculated to be isocaloric with 200 g of lean beef, were ingested with 200 ml of water. Oral temperature (08:00), as well as hematocrit on the 0 and 8-hr blood samples, were determined on the day of each experiment.

Amino acids were determined by the method of Spackman *et al.* (6) on 1.0-ml samples of ultrafiltrate prepared promptly by passing plasma through 2 layers of cellophane under about 40 atm pressure of nitrogen. Repeated tests of the ultrafiltrate failed to show any protein precipitate with sulfosalicylic acid. Plasma glucose, amino N and urea N were determined respectively, according to Saifer and Gerstenfeld (7), Frame *et al.* (8), and Karr (9).

Results. The amino acid data obtained with the beef test meal are presented in Table I. Glucose, amino N and urea N results are displayed in Fig. 1, 2, and 3. To save space the amino acid data obtained with the nonprotein meal are omitted but the molar ratios obtained in both series are given in Table II. In the nonprotein series aspartic acid and tryptophan were often not detectable by our methods and the scattered results are therefore omitted from Table II. The amino acid total of the 0 hr values in the nonprotein series was 11% lower than the corresponding values in the beef series. Since the experimental work extended from Octo-

[1] The experimental work was completed in the Department of Physiology School of Medicine and Dentistry, University of Rochester just before the authors left that institution in January 1966. Supported by NIH grant AM 02837.

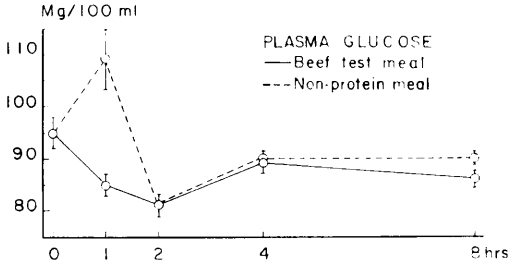

FIG. 1. Plasma glucose: (——), beef test meal; (- - -), non-protein meal.

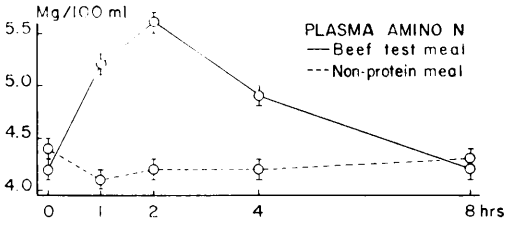

FIG. 2. Plasma amino nitrogen.

ber to March the small difference in total 0-hr values may be a seasonal one. The leucines and methionine fell to about half their 0-hr values in the first 2 hr after ingestion of the nonprotein meal. The maximal fall of the essential amino acids occurred within 4 hr after ingestion but for alanine, glutamic acid, glycine, proline, and serine it occurred at 8 hr. In the nonprotein series, mean hematocrit values of all 0 and 8-hr samples were 46 and

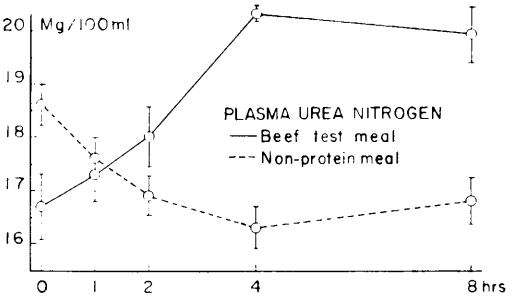

FIG. 3. Plasma urea nitrogen.

TABLE I. Free Amino Acids in Human Blood Plasma (μmoles/liter).[a]

Amino acid	Fasting[b] (0 hr)	After test meal[c] (hr): 1	2	4	8
Ala	476 ± 35	602 ± 43[d]	588 ± 30[d]	425 ± 26	375 ± 20[d]
Arg	69 ± 2	99 ± 12[d]	127 ± 17[d]	75 ± 10	42 ± 7[d]
Asp	21 ± 2	25 ± 5	30 ± 6	23 ± 5	13 ± 4[d]
½ Cys	69 ± 11	67 ± 11	64 ± 8	56 ± 6	67 ± 8
Glu	145 ± 14	169 ± 13	153 ± 14	139 ± 12	132 ± 11
Gly	288 ± 21	371 ± 30[d]	352 ± 18[d]	265 ± 15	265 ± 15
His	119 ± 9	207 ± 14[d]	187 ± 19[d]	134 ± 8	97 ± 11
Iso	75 ± 6	119 ± 9[d]	146 ± 8[d]	107 ± 7[d]	66 ± 6
Leu	134 ± 7	208 ± 15[d]	238 ± 12[d]	187 ± 12[d]	119 ± 10
Lys	309 ± 17	488 ± 30[d]	586 ± 45[d]	418 ± 29[d]	310 ± 20
Met	20 ± 3	34 ± 5[d]	34 ± 5[d]	18 ± 3	11 ± 2[d]
Phe	59 ± 7	61 ± 6	58 ± 5	46 ± 5	39 ± 5[d]
Pro	225 ± 17	241 ± 22	250 ± 22	226 ± 19	152 ± 17[d]
Ser	327 ± 22	386 ± 32	341 ± 24	263 ± 20[d]	211 ± 23[d]
Thr	194 ± 20	259 ± 27[e]	243 ± 21[e]	219 ± 19	173 ± 16
Try	1.9 ± 0.2	5.0 ± 1.8[e]	6.1 ± 1.7[d]	2.8 ± 1.2	1.5 ± 1.3
Tyr	68 ± 5	75 ± 6	88 ± 5[d]	66 ± 5	38 ± 5[d]
Val	240 ± 12	335 ± 27[d]	381 ± 20[d]	314 ± 16[d]	236 ± 16
Totals	2840	3751	3872	2984	2348

[a] Mean values ± SEM.

[b] Six samples from each of 6 adult males.

[c] Thirty-five samples at each interval after ingestion of 200 g of beef muscle.

[d] $p < 0.02$.

[e] $p < 0.05$.

TABLE II. Molar Ratios of Amino Acids in Human Blood Plasma and in Beef Muscle Protein (moles/1000 amino acid residues).

	Fasting (0 hr)		After meal of beef muscle (hr): 1		2		4		8		Beef muscle protein[b]
Amino acid	B[a]	NP	B	NP	B	NP	B	NP	B	NP	
Ala	169	154	162	200	154	168	144	173	161	154	106
Arg	25	16	27	13	33	15	25	17	18	16	55
½ Cys	25	13	18	16	17	11	19	15	29	15	4
Glu	52	82	45	82	40	75	47	85	57	78	144
Gly	104	102	100	111	92	124	89	113	114	110	95
His	42	46	56	38	49	47	45	53	42	51	26
Iso	27	26	32	16	38	14	36	19	28	19	52
Leu	48	49	56	29	62	29	63	36	51	41	88
Lys	110	91	131	91	153	109	141	112	133	117	83
Met	7	4	9	2	9	5	6	1	5	3	34
Phe	21	14	16	13	15	11	16	11	17	12	39
Pro	80	80	65	72	65	67	76	58	65	52	63
Ser	116	141	104	137	90	149	89	131	91	135	70
Thr	69	68	70	71	64	74	74	67	74	73	56
Tyr	24	18	20	15	23	14	22	12	16	12	25
Val	85	96	90	94	99	85	106	96	101	110	62

[a] B = beef meal series; NP = nonprotein meal series.

[b] Computed from values in "Amino Acid Handbook," R. J. Block and K. W. Weiss, Thomas, Springfield, Illinois (1956).

45%. Oral temperatures at 0 hr were between 36 and 37°. The fall in plasma glucose occurred in all subjects after ingestion of beef and the maximal drop occurred in the second hour (Fig. 1). Ingestion of the nonprotein meal caused a first hour rise and a second hour fall that was identical with that caused by the beef meal. Plasma amino N (Fig. 2) confirms grossly what was demonstrated by the amino acid analyses. We have no explanation for the difference between the 0-hr values for plasma urea N (Fig. 3) in the second series of experiments. Apparently urea formation continued at a high rate for many hours after ingestion of beef but it appeared to be significantly slowed after ingestion of the nonprotein meal.

Discussion. The mean values in Table I show that the maxima for all amino acids occur in the first or second hour after the meal and that some significant increases continue to the fourth hour. At the eighth hour about half of the amino acids are significantly lower than in the fasting state. The sum of the increases is 32% in the first hour and 37% in the second. At 4 hr the total is not different from the 0-hr value and at 8 hr it is 17% less than the 0-hr value. Since all of the values in Table I are means of 35 or 36 analyses they are probably fairly representative of the type of experiment reported here. The inter- and intraindividual variations were large even in the fasting state. For example, the greatest change occurred in lysine concentration which on the average rose from 309 μm/liter at 0 hr to 586 μm/liter at the second hour (Table I). Only 4 subjects showed mean maxima at 2 hr. None of the subjects exhibited consistently high or low values at fixed time intervals. Subject A, for instance, showed 2 lysine maxima in the first hour, 1 in the second hour and 3 in the fourth hour. The concentrations of lysine ranged from 327–565 μm/liter in the first hour, 342–737 in the second hour, and 140–950 in the fourth hour. The mean lysine 0-hr value for subject A was 323 μm/liter (range 203–488). The evidence indicates that a single amino acid analysis of blood from a fasting subject is not a valid base line for

repeated experimntal procedures on the same or different subjects.

One objective of these experiments was to determine whether the amino acid pattern of the beef muscle meal would be reflected in the plasma amino acid pattern. Molar ratios (Table II) provide some useful clues. A comparison of amino acid molar ratios of beef muscle with either the first or second hour values after ingestion of beef muscle shows very little agreement and many discrepancies. The best agreement is found for glycine, proline, and tyrosine and the poorest for arginine, cystine, glutamic acid, histidine, the leucines, methionine, phenylalanine, and valine. If only the rise above 0-hr values is used for the comparison the discrepancies are much greater, especially for valine, lysine, and histidine. The discrepancy in alanine values is doubtless the result of gut wall transamination of aspartic and glutamic acids. There may be some methodological errors involved in the sulfur amino acid comparisons but in any case it would be impossible to tell, from these data, that the subjects had eaten a meal of beef. Earlier work demonstrated that large quantities of endogenous protein are delivered to and recovered from lumen of the small gut during digestion (10). The amino acids available for absorption from the gut are derived from digestion of the mixture of exogenous and endogenous protein that is always found there after ingestion of any meal that contains protein. It is not surprising, therefore, that the amino acid pattern of ingested protein is not found in blood plasma.

The hypoglycemic effect of protein ingestion remains unexplained but our results confirm its existence. Experiments *in vitro* indicate that leucine, in the absence of glucose, is the only essential amino acid that can stimulate the β cells of the pancreas to produce insulin, but if glucose is present, arginine, isoleucine, and lysine are also effective (11). All of these amino acids were significantly increased in our subjects after ingestion of beef. Amino N as determined in plasma corresponds reasonably well with the total computed from the amino acid analyses (Table I).

Summary. Amino acid analysis of human blood plasma showed considerable intra- and terindividual variation in fasting as well as in the postprandial state. The mean maximal postprandial rise of all amino acids usually occurred in the first or second hour after test meal ingestion but in any individual it could be delayed as long as 4 hr. The plasma amino acid molar ratios were quite different from those calculated for beef muscle protein and we conclude that the amino acid pattern of the test meal was not reflected in the plasma. Ingestion of a nonprotein meal resulted in reduced plasma amino acid concentrations that persisted for 8 hr. Ingestion of beef resulted in a significant drop in plasma glucose concentration in the first 2 hr and the expected rise in amino N and urea N.

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