

EFFECT OF A 24-h FAST ON THE AMINO ACID CONCENTRATIONS OF RAT BLOOD, LIVER AND STRIATED MUSCLE

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Summary

A 24-h fast induced different patterns of change in the amino acid concentrations of liver, muscle, plasma and blood cells. Starvation produced generalized increases in blood amino acids despite decreases in plasma, thus increasing the blood cells amino acid pool. Muscle increased amino acid levels with fasting, while the changes were much more buffered in liver. The fraction of essential amino acids carried by the blood was considerably greater than that of muscle and liver. The size of muscle pool in the whole rat was much greater than that of liver and more than two orders of magnitude higher than that of the whole blood. Fasting-induced changes agree with the known transport of amino acids from muscle and other peripheral tissues towards the liver and other splanchnic organs.

Introduction

Free amino acids are a small fraction of all mammalian organism amino acids and consequently small changes in protein turnover are known to affect their concentration intensely (Munro, 1970). Free amino acid concentration is much higher in tissue than in plasma (Christophe et al., 1971; Bergström et al., 1974; Soley and Alemany, 1980b). Tissue amino acid concentration may be altered with food deprivation because of non-essential amino acid release by peripheral tissue and utilization by the splanchnic bed for gluconeogenesis and energy purposes (Aikawa et al., 1973; Felig, 1973a). Plasma-free amino acid changes have been found to reflect the balance between their release from

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muscle and their uptake by liver (Adibi, 1971). However plasma is probably not the only compartment of blood related with amino acid transport (Elwyn, 1966; Elwyn et al., 1968, 1972; Felig et al., 1973a). In the present work we have studied the effects of 24 h of starvation in the rat on the levels of free amino acids in plasma, whole blood, liver and muscle in an effort to understand the extent of their interchanges among these compartments.

Materials and Methods

Adult female Wistar rats were kept in a temperature- ($23 \pm 1^\circ\text{C}$) and light- (12 h on/12 off) controlled animal room; they were fed rat chow pellets (Panlab, Barcelona, Spain) and had free access to tap water. Two groups were randomly selected: the first was left as control and the second had all food removed during 24 h. At the beginning of the lit cycle all animals were beheaded with a guillotine with as little stress as possible (Arola et al., 1980). Blood was collected in dry heparinized beakers and used for hematocrit estimation and plasma separation. Aliquots of whole blood and plasma were deproteinized with cold acetone (Arola et al., 1977) and used for the individual amino acid concentration estimation (Arola et al., 1976). The amino acid molality of plasma and blood cells was calculated as previously described (Soley and Alemany, 1980a).

Immediately after decapitation, pieces of liver and hind leg striated muscle were rapidly dissected, blotted and frozen in liquid nitrogen. Tissue samples were used for their individual amino acid content estimation as previously described (Soley and Alemany, 1980b).

Results

After a 24 h fast the hematocrit value in the rat did not differ from that of fed animals (44.7 ± 1.1 and 46.6 ± 1.4 in the fed and fasted rats, respectively).

In Table 1 the blood, plasma, liver and muscle amino acid concentrations of fed and fasted rats are shown. Fasting induced significantly different patterns of change in blood and in plasma amino acid concentrations.

In blood there was a significant rise in the values of Glu+Gln, Ser, Gly, Leu+Ile, Val, Cit, Tyr and Phe as well as the total amino acid concentration, and only a significant reduction in His and Cys+cysteate. In plasma, however, fasting produced a significant reduction in most amino acids (Ala, Lys, Arg, His, Cit, Cys+cysteate, Met, taurine, Trp, Phe and the composite total amino acids) with only a significant increase in Glu+Gln and Pro.

Blood amino acids transporting ability (or content) did not change dramatically with fasting, while the plasma/cell distribution was considerably altered as can be seen in Fig. 1, this shows the variation in percentage of amino acid content of blood in both main compartments, cells and plasma. As a general trend, it can be said that blood cells amino acid content increased with fasting despite decreases or small changes in plasma content and concentration.

Free amino acid concentrations were much higher in liver and muscle than in either blood or plasma (Table 1), although the percentage of essential amino acids as compared to the totals was much lower in both liver and muscle than in blood or plasma.

TABLE 1

AMINO ACID CONCENTRATIONS IN THE BLOOD, PLASMA, LIVER AND MUSCLE OF FED AND 24-H FASTED RATS

Amino acid concentrations are given in $\mu\text{mol/l}$ blood or plasma and $\mu\text{mol/kg}$ tissue. The values are the mean \pm S.D. of six to seven different animals. Significance of the differences versus the fed state: (a) increases: $\uparrow = P < 0.05$; (b) decreases: $\downarrow = P < 0.05$.

Amino acid	Blood fed	Blood fasted	Plasma fed	Plasma fasted	Liver fed	Liver fasted	Muscle fed	Muscle fasted
Alanine	590 \pm 120	511 \pm 51	660 \pm 132	345 \pm 96 \downarrow	1789 \pm 918	1064 \pm 642	2160 \pm 644	2049 \pm 181
Glutamate+glutamine	1163 \pm 181	1583 \pm 113 \uparrow	960 \pm 46	1232 \pm 249 \uparrow	5409 \pm 1494	7817 \pm 1839 \uparrow	5832 \pm 1132	6602 \pm 1729
Aspartate+asparagine	147 \pm 34	179 \pm 49	162 \pm 29	181 \pm 30	4597 \pm 879	8283 \pm 1742 \uparrow	2481 \pm 1134	3648 \pm 977
Threonine	247 \pm 64	260 \pm 69	180 \pm 68	151 \pm 34	1268 \pm 607	1365 \pm 446	1289 \pm 372	1043 \pm 539
Serine	332 \pm 100	544 \pm 171 \uparrow	382 \pm 98	287 \pm 61	3336 \pm 1154	1510 \pm 306 \downarrow	1421 \pm 603	1652 \pm 289
Glycine	284 \pm 86	439 \pm 78 \uparrow	308 \pm 103	308 \pm 78	1180 \pm 343	2243 \pm 149 \uparrow	1378 \pm 274	2333 \pm 370 \uparrow
Proline	325 \pm 108	442 \pm 98	301 \pm 64	434 \pm 51 \uparrow	127 \pm 95	114 \pm 24	111 \pm 37	124 \pm 39
Hydroxyproline	23 \pm 7	14 \pm 2	18 \pm 7	23 \pm 7	78 \pm 32	66 \pm 51	106 \pm 24	349 \pm 191 \uparrow
Leucine+isoleucine	209 \pm 66	490 \pm 54 \uparrow	304 \pm 91	360 \pm 88	441 \pm 223	330 \pm 277	313 \pm 108	740 \pm 32 \uparrow
Valine	120 \pm 24	197 \pm 32 \uparrow	162 \pm 49	180 \pm 22	175 \pm 83	148 \pm 29	175 \pm 69	271 \pm 73 \uparrow
Lysine	405 \pm 120	309 \pm 32	567 \pm 100	345 \pm 110 \downarrow	218 \pm 120	400 \pm 81 \uparrow	313 \pm 73	503 \pm 191
Arginine	288 \pm 113	186 \pm 56	295 \pm 88	148 \pm 19 \downarrow	90 \pm 46	68 \pm 17	466 \pm 96	254 \pm 46
Histidine	64 \pm 22	22 \pm 12 \downarrow	59 \pm 46	16 \pm 2 \downarrow	471 \pm 375	329 \pm 130	1844 \pm 321	3551 \pm 1655
Ornithine	32 \pm 17	27 \pm 7	40 \pm 22	19 \pm 10	252 \pm 309	112 \pm 78	23 \pm 17	65 \pm 2 \uparrow
Citrulline	108 \pm 47	346 \pm 17 \uparrow	136 \pm 32	50 \pm 10 \downarrow	1806 \pm 590	1731 \pm 140	379 \pm 191	383 \pm 142
Cysteine+cysteate	189 \pm 56	81 \pm 37 \downarrow	40 \pm 5	26 \pm 7 \downarrow	392 \pm 83	405 \pm 193	309 \pm 122	325 \pm 93
Methionine	44 \pm 29	22 \pm 5	44 \pm 15	23 \pm 12 \downarrow	123 \pm 85	99 \pm 27	77 \pm 64	118 \pm 68
Taurine	533 \pm 81	564 \pm 117	417 \pm 68	314 \pm 59 \downarrow	12090 \pm 3850	9050 \pm 2964	20580 \pm 4090	26190 \pm 8426
Tyrosine	100 \pm 29	247 \pm 51 \uparrow	113 \pm 46	93 \pm 20	157 \pm 44	81 \pm 19 \downarrow	197 \pm 103	306 \pm 64
Tryptophan	97 \pm 27	96 \pm 27	191 \pm 51	122 \pm 20 \downarrow	101 \pm 91	95 \pm 66	109 \pm 32	86 \pm 29
Phenylalanine	60 \pm 34	133 \pm 42 \uparrow	107 \pm 37	50 \pm 17 \downarrow	95 \pm 18	88 \pm 29	155 \pm 88	97 \pm 83
β -Alanine	—	—	—	—	1178 \pm 710	459 \pm 63 \downarrow	1845 \pm 578	2310 \pm 487
DOPA	—	—	—	—	195 \pm 85	73 \pm 12 \downarrow	—	—
Total	5178 \pm 578	6789 \pm 598 \uparrow	5328 \pm 230	4615 \pm 566 \downarrow	36000 \pm 5220	35720 \pm 4190	42150 \pm 5730	53410 \pm 8132 \uparrow
Essential (%)	24.1	22.5	30.0	27.0	9.0	8.0	10.1	12.0

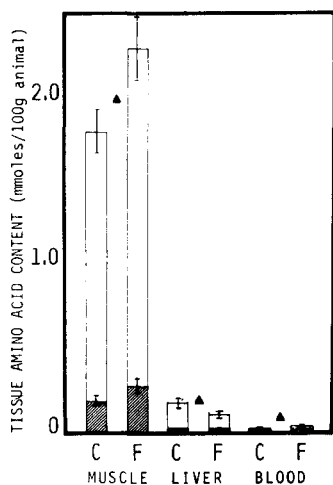


Fig. 1. Amino acid distribution in the plasma and blood cells of fed (C) and 24-h fasted (F) rats. The white areas of the columns correspond to the plasma and the shaded areas to the blood cells. The values presented are the mean \pm S.D. of six to seven different animals. The data were calculated from the hematocrit value and the results presented in Table 1. Significance of the differences between fed and fasted groups: $\blacktriangle = P < 0.05$ for total blood amino acid content comparisons; $\bullet = P < 0.05$ for plasma amino acid content comparisons; $\circ = P < 0.05$ for blood cells amino acid content comparisons.

In liver there was a significant decrease with fasting in Ser, Tyr, β -alanine and DOPA and increases in Glu+Gln, Asp+Asn, Lys and Gly but no change in the composite total amino acids. In muscle there were significant increases with a 24 h fast in Gly, Hyp, Leu+Ile, Val, Orn and composite total amino acids (Table 1).

The differences became enhanced in the tissues when the whole tissue amino acid content was taken into account instead of the concentration.

Muscle mass does not change with a 24 h fast (Li et al., 1979; Remesar and Alemany, 1980a). Using the data from Arola et al. (1979) as mean muscle mass in the rat, as well as the actual sizes of muscle, liver and whole circulating blood estimated from the data of Everett et al. (1956) The actual free amino acid pool sizes of the tissues studied were calculated and are presented in Fig. 2.

The main compartment where amino acids can be found is the striated muscle, that of liver being only a fraction of it and blood an even smaller fraction. Most of these pools are due to accumulation of non-essential amino acids, except in blood, where they are comparatively less abundant. Fasting induced a growth of muscle pools and a decrease of those of liver, with little changes in blood (Fig. 2).

Discussion

Comparative concentrations of the different free amino acids found in the present study in plasma, liver and muscle of fed rats agree with those previously reported (Tallan et al., 1954; Schimassek and Gerok, 1965; Herbert et al., 1966; Ferris and Clark, 1972; Gressner, 1974; Soley and Alemany, 1980a; 1980b).

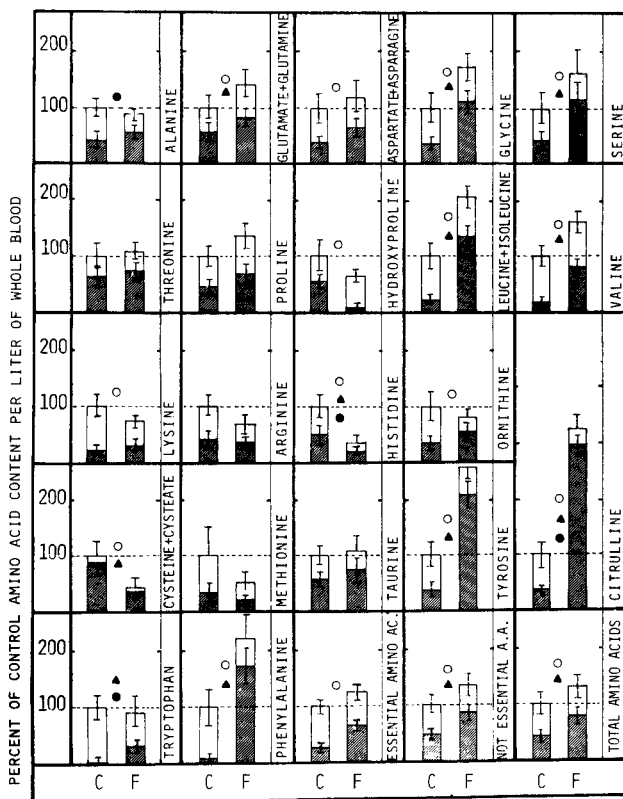


Fig. 2. Muscle, liver and blood free amino acid pool sizes in fed (C) and 24-h fasted (F) rats. All values presented are the mean \pm S.D. of six to seven different animals. The shaded areas correspond to the combined essential amino acid fraction of the tissue pool. The muscle size was calculated from Arola et al. (1979); blood volume calculated from the data of Everett et al. (1966). Significance of the differences between the fed and fasted states: $\blacktriangle = P < 0.05$.

In the liver, taurine, Glu+Gln, Asp+Asn, Ser, Ala and Gly constitute about 80% of the total free amino acids. This is in accordance with the central role of the liver as the site of gluconeogenesis (Exton, 1972) and main controller of amino acid metabolism (Gressner, 1974), as most amino acids are metabolized (nitrogen interchanged and excreted) through Glu, Gln, Asp and Ala metabolism.

Liver taurine is high, in accordance with Bloxan and Fisher (1974) but our values were higher than those of Garvin (1960); Ferris and Clark (1972) and Buttery and Rowsell (1974).

The free amino acid levels were of the same order of magnitude in liver and muscle but were higher in muscle. The distribution patterns of amino acids were also similar in both tissues, with some differences as in the case of His and Arg, lower in both cases in liver due to the relative absence of His derivatives in the liver proteins and the high arginase levels in the liver (Remesar et al., 1980b). This tissue amino acid pattern was considerably different from that of plasma and that of whole blood, the proportion of essential amino acids being much higher in plasma and blood cells.

In muscle, essential amino acids constituted a small fraction of total amino acids, in agreement with Bergström et al. (1974) with considerable levels of taurine, practically 50% of the whole amino acid pool (Scharff and Wool, 1964; Sturman, 1973). The meaning of this huge pool is obscure, its relationship with muscle concentration having been put forward (Spaeth and Schneider, 1974). The same amino acids as in liver, but now with the inclusion of His (our data probably include 3-methyl-histidine and other 'rare' histidine derivatives difficult to metabolize), also constitute about 80% of the muscle free amino acid pool, this data is in agreement with several authors (Scharff and Wool, 1964; Adibi, 1971; Bergström et al., 1974).

Muscle mass contains the largest amino acid pool of the entire rat, some 10-fold higher than that of liver and about 60-fold higher than that of the blood.

Starvation produced a different response in muscle and liver free amino acids concentrations being enhanced in the former and reduced in the latter.

Increased muscle free amino acid levels with fasting was mainly due to proteolysis and protein synthesis arrest (Wannemacher and Cooper, 1970; Goldberg and Chang, 1978; Goldberg, 1980a; Goldberg et al., 1980b). Most amino acids are released into the bloodstream by muscle, especially Ala and Gln (Cahill et al, 1966; Felig, 1973a; Garber et al, 1976a; 1976b) as part of the glucose-alanine cycle; other amino acids are not liberated so speedily, as are the His derivatives, Hyp and Gly. Thus, their muscle concentrations rose, and so did the essential amino acid proportion in muscle, that rose slightly, especially by branched chain amino acids (Adibi, 1971). It has recently been proposed that these amino acids may not only regulate the release of ammonia and gluconeogenic precursors for metabolism by the liver (Odessey et al., 1974; Snell, 1979) but may be used as metabolic fuels by the muscle itself (Adibi, 1976).

Fasting induced a significant decrease in the actual content of amino acids in liver with a marked Ser decrease (due to the extraordinary increase in serine dehydratase with fasting (Palou et al., 1980) and increases in Asp+Asn and Glu+Gln, in a situation in which the general amino acid catabolism is increased as indicated by increased aspartate transaminase activities (Remesar et al., 1980c).

Plasma amino acid level changes induced by fasting were in agreement with those of other authors (Adibi and Drash, 1970; Felig et al., 1970; Scriver and Rosenberg, 1973; Palou et al., 1981). In general there was a significant decrease, slightly more marked in essential amino acid levels.

In the blood, however, there was a significant increase in total amino acids due to a more marked increase in the amino acid content of the blood cells compartment that compensated the decrease in plasma. The cell/plasma ratio increased for all amino acids with a mean 2-fold increase.

The meaning of these changes is far from clear, but a general outline of the amino acid homeostasis changes with fasting. The results presented are in accordance with the known increased flux of amino acids from peripheral tissues to the splanchnic bed organs (Marliss et al., 1971; Felig, 1973a; Aikawa et al., 1973; Blackshear et al., 1974) with increased proteolysis in muscle and liver (Wannemacher and Cooper, 1970) and active uptake of Ala and other amino acids by the liver (Nallathambi et al., 1972). They are in accordance too with the obvious transporting role of blood; however, the significance of the blood cell amino acid concentration changes seem to attribute to this compartment

a more dynamic role than the one usually assigned to it, in accordance with previous work by Felig et al. (1973b) and especially Elwyn (1966), Elwyn et al. (1968; 1972).

The definitive interpretation of these findings must, however wait until the dynamics of the amino acid exchange by blood cells and plasma or tissues in the fasting state are established. In any event they stress the need to carry out parallel determinations of amino acid concentrations both in whole blood and plasma to obtain a more complete picture of the inter-organ amino acid relationship under different physiological situations.

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