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ESTROGEN EFFECTS ON BLOOD AMINO ACID COMPARTMENTATION

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Summary

The present paper focuses on the study of blood amino acid compartmentation in healthy men (lean and obese) and women, with special emphasis on the estimation of the recently described blood-cell adsorbed amino acid pool. The wide range of changes found in this pool on comparing different physiological situations may be attributable to its proposed characteristic high dynamism on the one hand, but also to the influence of other factors such as hormones. Along these lines, the sex- and obesity-linked variations found here in human blood led to the speculation as to whether these differences could be related to the influence of estrogens. This hypothesis was further tested by chronically treating a group of male rats with estrone and checking their subsequent blood amino acid compartment changes (which yielded a greater difference in the adsorbed pool). From the overall results obtained it may be concluded that the higher production of estrogens in women and obese men affects amino acid availability to the tissues by modulating the blood-cell adsorbed amino acid pool through a mechanism that is, at present, unknown.

Key Words: amino acid compartmentation, erythrocyte membrane, adsorption, estrone

Blood amino acid compartments are known to play an important role in the availability and inter-organ distribution of amino acids and in their metabolism (1-6), plasma amino acids being commonly considered the more readily available portion of blood amino acids (7) –since they are in constant equilibrium with tissue amino acids (8)– whereas the role and relevance of the blood-cell amino acid pool has been the subject of speculation due to the discrepancies observed in the very slow rates of exchange between intracellular and plasma amino acids found in *in vitro* studies (9-11) compared to *in vivo* models showing profound, fast changes in blood cell amino acids after their passage through certain key organs (3, 4, 13). In fact, blood cells carry a significant portion of blood amino acids (14-16) whose concentration is subject to changes under different conditions such as starvation –when their concentration increases in spite of marked, generalized decreases in plasma concentrations (17, 18)– and also pregnancy (19).

Recent findings of a particular division of blood-cell amino acids into two well-defined, differentiated compartments –amino acids dissolved in the interior and those adsorbed onto the surface of the blood cells and thus carried by them (20)– have enabled a better understanding of

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the dynamic role of some of these amino acids during blood-tissue exchange (20-22). Moreover, the adsorbed pool has been found to undergo deep noticeable changes under differing physiological conditions (21, 22).

Since the role of the membrane-adsorbed blood-cell amino acid pool is assumed to be directly related to the ease of amino acid exchange with tissue pools (20), its size and composition may be useful as an indicator of possible differences in overall nitrogen handling induced by hormones. Preliminary results have shown a higher variability in the blood amino acid compartmentation in men compared with women (unpublished data). In fact, estrogens and androgens deeply alter the rates of protein extraction and accrual from the diet as well as those of nitrogen disposal through urinary excretion (23).

In the present investigation, we have studied the distribution of amino acids in the blood of healthy men –lean and obese– and women, with special emphasis on the estimation of the blood cell-adsorbed amino acid pool. Hence, the sex- and obesity-linked variation in size and composition of this pool described in the present paper, may be a consequence of the postulated high dynamism of the pool together with the different effect of sexual development hormones. Thus, it was speculated as to whether the differences observed in blood amino acid compartmentation could be related to the influence of estrogens. This hypothesis was further tested and confirmed by chronically treating a group of male rats with estrone and then checking their blood amino acid compartment changes.

It may be concluded, then, that the higher production of estrogen in women and obese men affects amino acid availability to the tissues by modulating the blood-cell adsorbed amino acid pool through a mechanism that is, at present, unknown.

Methods

Subjects and sampling

Human blood samples were obtained from consenting healthy subjects undergoing a routine blood test. Three groups were selected: a) control men, n=16; age: 22-**44**-65 yrs. (minimum-**mean**-maximum), BMI (body mass index) lower than 25 (mean ± SEM: 23.5 ± 0.3 kg/m^2); b) control women, n=12; age: 20-**37**-61 yrs., BMI lower than 25 (mean ± SEM: 20.8 ± 0.5 kg/m^2); and c) obese men, n=17; age: 23-**41**-61 yrs., BMI higher than 25 (mean ± SEM: 27.6 ± 0.4 kg/m^2). After overnight fasting, 5 ml blood samples were extracted and kept in heparinized glass tubes at 4°C. The samples were immediately processed.

Male Wistar rats weighing 280-300 g were randomly distributed in two experimental groups (n=9 each). The animals were maintained in individual metabolic cages in a controlled environment: 12-hour light cycle; 21-22°C, 60-70% relative humidity; pellet diet (type A04, from Panlab, Barcelona, Spain) and tap water available *ad libitum*. After two days of adaptation to the cages, the rats in the estrone-treated group had an Alzet type 2002 osmotic minipump (Alza Res., Palo Alto CA, USA) inserted in the peritoneal cavity whilst under ether anaesthesia. The minipump was filled with 5.6 mM estrone (Sigma, St. Louis, MO, USA) in propyleneglycol, providing a constant flow of 0.5 µl/h (i.e. 3 nmol estrone per hour) for 2 weeks. The control rats were inserted with minipumps –containing only propyleneglycol– under the same conditions.

The rats recovered rapidly, resuming their eating pattern in one day. The food eaten and water drunk were recorded, as were the weight of droppings and volume of urine, which were saved for analysis and kept frozen until processing. On day 14 after introduction of the minipumps and after 12 h food privation, the rats were anaesthetized at the beginning of the light cycle with an i.p. injection of sodium pentobarbital (Sigma, 40 mg/kg body weight), and blood was drawn from the aorta. Blood samples were heparinized and immediately processed as in human samples under the same conditions.

Blood fraction amino acid analysis

Blood amino acid pool evaluation was carried out following a previously developed protocol (20) outlined in Figure 1. All blood fraction samples were deproteinized with cold acetone (24) and the clear supernatants were used for individual amino acid analysis using the PICO-TAG™ method (25) (Waters, Milford, MA USA) by separating the phenyltiocarbamil amino acids obtained after derivatization with phenyl-isothiocyanate by means of HPLC (Waters) using a PICO-TAG™ column for free amino acids (Waters).

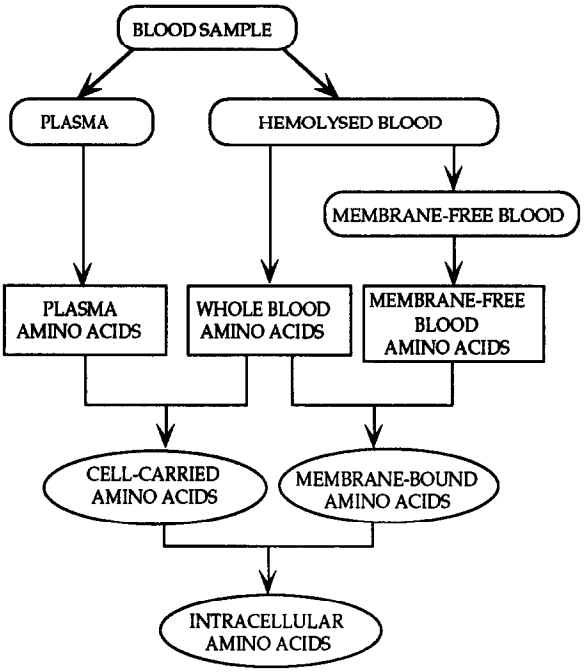

Fig. 1

Diagram of the procedure used for the estimation of plasma, cell, intracellular and membrane-bound amino acids from blood samples.

Amino acid concentrations were calculated from the peak areas using the MAXIMA™ 820 programme (Waters). All samples included L-methionine sulfone (Sigma) 400 nmol/ml as the internal standard. The packed cell volume value for each sample was determined after centrifugation at 15,000 g for 5 minutes; plasma trapped in blood cell fractions was estimated (26), and the amino acid values in the cell fractions corrected accordingly.

In vitro verification of the binding of [^{3}H]-labelled amino acids onto erythrocyte membranes

In order to corroborate *in vitro* the experimental data obtained *in vivo* for the blood-cell membrane-adsorbed amino acid pools, an experiment was carried out in which the binding of

[^{3}H]-labelled amino acids to isolated erythrocyte membranes was determined using erythrocyte ghosts (27) incubated in the presence of labelled glutamine (Amersham, Amersham, UK) 0.5 mM (74 kBq/μmol) for 15 s at 37°C. Glutamine was selected for this binding assay because it is the most abundant amino acid detected in the blood-cell adsorbed pool *in vivo* (see Table I). [^{14}C]-sucrose was used for non specific binding measurement purposes. The radioactivity fixed in the membrane fraction was later compared with that remaining in the medium in order to establish the relationship between the bound and free amino acid concentrations. Membrane phosphate was measured (28) and used as an index of membrane mass for comparative purposes.

Nitrogen balance in rats

Rat chow diet pellet samples as well as the droppings of the rats were frozen and powdered using liquid nitrogen and the powder sampled and used –as were the urine samples– for the estimation of total nitrogen using a Carlo Erba NA-1500 elemental nitrogen analyser. The computed amount of food ingested and its nitrogen content were used to calculate the nitrogen intake. Nitrogen losses for the whole period were estimated from the pooled amount of urine and stools excreted and their nitrogen content. The differences between ingested and excreted nitrogen were taken as nitrogen accrued. This figure includes the unaccounted for *nitrogen gap* (29), and is, thus, an overestimation of the nitrogen actually accrued.

Statistical significance of the differences between individual groups and statistically different from zero values by means of 95% confidence limits were established using Student's *t* test.

Results

In the experiment devised to determine the binding of labelled amino acids to membranes, the amount of glutamine bound to erythrocyte membranes was 0.12 ± 0.03 μmol per mg of membrane inorganic phosphate in the case of men, and 0.25 ± 0.02 for women (n=7 and 4 respectively); these data were the equivalent of 20.2 ± 4.3 nmol per ml of blood (men) and 42.8 ± 3.5 (women); the results obtained from *in vivo* measurements in human blood samples (see Table I) processed as described under Methods were, respectively, 21.5 ± 5.6 for men and 38.1 ± 7.4 for women.

Table I shows the individual amino acid content of human blood compartments. There were statistically significant differences between men and women for plasma essential amino acids (EAA) as well as for branched-chain amino acids, methionine and glutamic acid, with women presenting lower concentrations for all of them. When the whole blood content is taken into account, women presented significantly lower concentrations of these same amino acids –except glutamic acid –in addition to phenylalanine, tryptophan and alanine compared with control men. The concentrations in the cell compartment were not different between women and lean men, the latter presenting higher leucine concentrations than women as the sole significant difference observed.

Obese men presented lower plasma concentrations of isoleucine, glutamine and serine and higher concentrations of glutamic acid and aspartic acid than lean men whereas whole blood isoleucine, valine, tyrosine, glutamic acid, aspartic acid and proline concentrations where higher in obese than in lean men. The intracellular blood compartment also showed considerable changes produced in men as a consequence of obesity, with an increase in the overall pool size involving both essential and non-essential amino acids (NEAA); of the former, the branched chain amino acids and tyrosine as well as the total essential amino acids had significantly higher concentrations in obese than both control men and women. The non-essential amino acids, particularly glutamine, aspartic acid, glycine, alanine and proline, as well as the total were higher in obese men than in women (except for aspartic acid) and lean men alike.

Lean men showed only a small proportion of membrane-adsorbed amino acids: the branched chain amino acids and glutamine were the only ones attached at concentrations significantly different from zero. On the other hand, the profile found in women was different, the

TABLE I

Amino acid levels in whole blood (B), plasma (P) and blood cells –intracellular (I) and adsorbed (AD)– in men (lean and obese) and lean women

	MEN				OBESE MEN				WOMEN			
	B	P	AD	I	B	P	AD	I	B	P	AD	I
Ile	44.8±2.1	31.7±1.9	2.66±0.9	9.97±1.2	59.4±3.5*•	39.0±2.7*•	3.69±1.0	16.6±1.1*•	36.3±2.0*	26.3±1.4*	ndz	7.78±0.9
Leu	122±3.7	87.2±4.7	5.10±1.8	28.5±3.7	129±4.0•	84.0±2.7•	5.71±1.8	38.8±2.4*•	92.5±4.1*	69.4±3.9*	ndz	19.1±1.8*
Val	207±6.5	147±6.3	11.9±3.0	47.2±5.4	231±5.5*•	151±4.2•	11.5±2.8	68.0±3.5*•	171±8.2*	124±6.8*	8.63±2.0	37.7±4.0
His	55.3±2.6	35.1±1.7	ndz	18.0±2.1	61.7±2.6•	38.8±1.8	4.35±1.5	18.0±1.3	51.6±3.7	34.5±3.1	2.54±1.1	14.0±1.9
Lys	81.7±5.6	77.0±5.0	ndz	1.29±3.8	87.1±4.8	75.2±3.2	ndz	9.03±2.2•	73.9±3.7	71.7±3.7	ndz	0.28±2.6
Phe	48.5±1.6	34.1±1.2	ndz	12.6±1.6	49.9±1.8•	32.7±1.0	ndz	15.5±0.9•	41.8±1.9*	31.3±1.5	ndz	9.74±1.2
Tyr	60.4±2.2	38.7±1.3	ndz	18.1±1.4	71.4±4.0*•	43.0±2.1•	3.74±1.1	24.3±2.1*•	53.9±3.1	35.8±1.6	ndz	16.1±1.7
Trp	35.9±1.7	42.8±3.7	ndz	ndz	34.2±2.7	40.1±2.1	ndz	ndz	29.9±1.9*	37.8±2.5	ndz	ndz
Thr	107±7.3	71.8±3.6	ndz	28.6±3.7	113±6.9	69.9±4.1	7.21±2.1	35.5±2.0•	109±9.0	75.3±6.7	4.55±1.6	28.5±2.7
Met	19.3±1.1	14.4±0.5	ndz	4.06±1.1	20.4±1.1•	14.7±0.8•	ndz	5.40±2.8	16.1±0.9*	12.1±0.8*	ndz	3.7±0.5
Cys-S	90.2±13	58.6±13	ndz	20.3±7.6	60.1±8.4	38.3±4.7	5.79±2.6	19.1±2.7	86.2±24	34.4±5.7	ndz	32.8±15
EAA	872±23	638±30	ndz	180±25	918± 26•	630±18•	36.5±11	240±12*•	762±32*	553±17*	28.6±9.2	161±16
Gln	538±17	370±11	24.4±10	123±19	543±14	335±11*	33.9±5.0	175±9.3*•	539±20	364±14	38.1±7.4	136±12
Glu	172±11	27.1±2.5	ndz	123±8.7	204±10*	37.1±4.1*•	29.6±5.5	135±9.2	177±11	19.3±1.5*	23.1±6.4	133±13
Asn	61.7±7.2	18.6±2.9	ndz	38.0±4.1	60.4±6.3	16.4±2.4	3.49±1.6	39.6±3.8	67.9±7.8	22.7±4.0	5.9±2.6	38.4±3.8
Asp	96.8±7.4	5.18±0.6	ndz	79.1±5.2	142±13*	7.29±0.8*•	19.6±3.4	115±12*	106±13	4.14±0.6	15.0±2.7	87.3±12
Gly	233±9.6	109±3.3	ndz	105±13	254±12	101±7.0	ndz	144±11*•	241±12	118±9.0	9.5±3.0	112±5.0
Ser	119±8.7	63.2±3.2	ndz	42.5±3.4	112±4.1•	53.4±3.0*•	ndz	44.6±1.7	122±8.1	70.3±5.3	ndz	45.9±3.4
Ala	321±20	214±17	ndz	88.7±8.4	355±20•	211±10•	19.9±4.9	123±11*•	265±16*	177±12	ndz	74.8±6.0
Pro	166±14	123±13	ndz	31.3±5.4	206±21*•	133±12•	9.67±2.6	62.9±8.4*•	135±7.3	99.0±4.6	5.00±2.1	30.5±3.7
Hyp	14.9±1.8	10.6±1.3	ndz	3.49±0.8	15.8±1.6	9.92±1.0	1.09±0.5	4.48±0.6	14.0±1.9	9.63±1.5	ndz	3.46±0.3
NEAA	1723±56	939±35	ndz	634±49	1893±41*•	903±19	132±24	843±32*•	1667±41	884±35	108±20	662±27
TOTAL	2595±71	1578±63	ndz	814±66	2810±55*•	1528±32	174±35	1078±41*•	2429±61	1437±47	136±25	824±37

Data are the mean ± SEM of 16, 17 and 12 individuals respectively. Blood values are the μM concentrations in whole blood. Plasma, cell and adsorbed columns represent the μmoles of each amino acid in that fraction present in one liter of blood. Statistical significance of the differences with respect to lean men: * = $p<0.05$, or with respect to women; • = $p<0.05$; ndz = value statistically not different from zero.

adsorbed amino acids being: valine, histidine, threonine and the total amount of essential amino acids, along with glutamine, glutamic acid, asparagine, aspartic acid, glycine, proline and the total amount of non-essential amino acids, as well as the sum of amino acids as a whole. However, obese men showed a pattern partly superimposable to that of lean men and women, with statistically significant amounts of branched chain amino acids, histidine, threonine and combined essential and non-essential amino acids, plus glutamine, glutamic acid, asparagine, aspartic acid and proline besides tyrosine, cystine, alanine and hydroxyproline. Total values for this compartment were higher in both obese men and women than in lean men (which were not different from zero).

Table II displays the blood amino acid compartments of control and estrone-treated male rats. There were no differences between the two groups in whole blood amino acid concentrations. Although, estrone-treated rats showed higher plasma valine than controls. There was a definite trend for all cell amino acid concentrations to decrease when compared to controls although significantly different concentrations were only attained for asparagine and proline. Adsorbed amino acid concentrations were higher in estrone-treated rats than in controls in the case of valine, tyrosine, glutamic acid, alanine and combined non-essential and total amino acids.

TABLE II

Amino acid concentrations in whole blood (B), plasma (P) and blood cells – intracellular (I) and adsorbed (AD)– in control and estrone-treated male rats

	CONTROL RATS				ESTRONE RATS			
	B	P	AD	I	B	P	AD	I
Ile	70.1±5.0	46.4±3.7	ndz	20.2±3.7	75.0±5.8	50.2±4.3	ndz	11.9±4.4
Leu	123±10	74.9±4.0	ndz	44.7±7.3	140±12	88.6±6.8	ndz	39.8±4.0
Val	164±6.8	105±3.2	ndz	51.2±6.9	186±10	123±6.2*	24.9±11*	34.8±6.3
His	49.6±3.1	33.7±2.1	ndz	13.7±2.5	51.8±3.8	35.7±1.9	ndz	11.1±2.3
Lys	249±25	192±16	ndz	33.5±11	286±19	228±11	ndz	27.5±14
Phe	61.1±3.5	37.9±1.5	ndz	20.7±3.2	63.7±4.5	40.5±2.6	ndz	16.0±2.1
Tyr	74.6±5.0	45.8±3.6	ndz	22.6±2.8	72.9±5.2	45.2±2.9	10.3±3.7	16.0±2.7
Trp	59.8±3.0	61.3±2.5	ndz	ndz	62.7±3.9	66.3±4.2	ndz	ndz
Thr	248±14	165±14	26.2±8.7	56.9±8.5	236±20	161±11	46.3±11	25.3±22
Met	44.1±2.6	27.7±1.8	ndz	14.7±2.0	49.5±2.4	31.8±1.4	ndz	10.6±1.3
Cys-S	33.7±11	18.0±5.8	9.57±3.2	ndz	41.2±11	22.0±6.1	15.3±4.6	ndz
EAA	1182±53	808±33	ndz	273±40	1249±51	892±39	ndz	185±44
Gln	650±35	427±28	108±26	114±19	600±21	402±23	118±19	79.2±23
Glu	296±11	63.9±4.0	ndz	202±20	270±15	61.5±3.7	48.2±14	157±19
Asn	57.7±3.5	30.2±3.3	ndz	24.9±2.6	56.4±3.0	31.0±3.1	ndz	17.3±1.8*
Asp	18.8±2.0	8.42±0.84	ndz	9.60±2.0	19.4±1.8	10.3±1.0	ndz	6.46±1.7
Gly	501±31	304±26	65.6±19	131±17	470±27	293±22	83.0±15	92.4±17
Ser	252±10	157±8.2	ndz	83.6±8.6	247±6.8	155±6.7	ndz	67.8±6.1
Ala	321±25	204±19	ndz	91.0±11	362±23	251±15	48.0±17	56.4±18
Pro	140±6.1	91.2±3.1	ndz	42.7±4.4	143±5.0	97.8±3.9	ndz	26.6±3.7*
Hyp	50.5±3.7	32.6±2.3	10.1±2.2	7.54±1.8	45.3±1.4	30.9±1.4	9.33±1.2	5.02±1.1
NEAA	2287±83	1318±67	ndz	706±81	2212±75	1333±63	340±87	508±75
TOTAL	3433±151	2126±95	ndz	979±120	3447±109	2225±89	482±153	694±117

Data are the mean ± SEM of 9 rats per group. Blood values are the μM concentrations in whole blood. Plasma, cell and adsorbed columns represent the μmoles of each amino acid in that fraction present in one liter of blood. Statistical significance of the differences between both groups: *=P<0.05; ndz = value statistically not different from zero.

Nitrogen balance in control and estrone-treated rats is presented in Table III. The data refer to the whole 2-week period. Rats treated with estrone ingested less food and lost less nitrogen,

both total and percentage, in faeces which is the consequence of a more efficient uptake of nitrogen from food. The percentage of nitrogen lost in the urine was also lower in this group, the result being a higher share of nitrogen accrued compared with the untreated controls.

TABLE III

Nitrogen balances of male rats subjected to chronic treatment with estrone

PARAMETER	CONTROLS		ESTRONE
Body weight increase (g)	68.8±3.4		63.0±5.4
Food ingested (g)	357.8±2.3	*	320.7±2.2
Weight of faeces (g)	115.3±2.4		112.9±8.9
Water drunk (ml)	493.1±35.2		408.5±25.2
Urine production (ml)	178.9±33.2		116.7±19.4
N ingested (g)	9.55±0.06		8.55±0.05
N accrued (g)	3.70±0.20		4.18±0.10
% N accrued	39.6±1.9	*	47.5±1.7
N in faeces (g)	2.37±0.11	*	1.91±0.04
% N in faeces	25.4±1.1	*	21.8±0.8
N in urine (g)	3.27±0.21		2.94±0.22
% N in urine	35.0±1.2	*	31.0±0.9

Data are the mean±sem of 6 different animals per group. Statistical significance of the differences between groups: *=P<0.05.

Discussion

The physiological significance of the different blood amino acid compartments has been previously discussed (3, 4, 13), the picture being widened when including the role of the blood membrane-bound amino acid pool, which is probably instrumental in the transfer of amino acids from blood to tissues because of its fast turnover (20). However, very little is known of the factors governing the extension, size and dynamics of this fraction, especially in relation to the well known amino acid-carrying functions of the other blood compartments.

Focusing on the study of the factors affecting amino acid compartmentation, obese men showed a pattern of amino acid distribution in blood compartments which was very different to that of lean men and which was therefore fully attributable to obesity. Even more apparent differences were found when comparing obese men with lean women. Whole blood amino acid concentrations were higher in obese men than in both the other two groups –lean men and women–, although the cell and plasma compartments did not reflect changes of the same order: whereas blood cell amino acids showed the most striking diferences between groups –affecting most of the non-essential and several essential amino acids–, the increase in plasma amino acids concentrations was of a lesser extent.

The increased concentrations of blood cell amino acids shown by obese men are in agreement with the high availability of energy described in obesity, with the general depression of the a-amino nitrogen metabolism –protein turnover, urea cycle and activity of the enzymes involved– reported in different tissues which enables amino acids to be kept for protein synthesis; and also with increased circulating concentrations of amino acids –as found in hyperphagic experimental animal models of obesity (18, 20, 30, 31). However, this ample availability of amino acids produced by obesity does not explain the roughly similar increase in the adsorbed pool found in women which was also quite different from that found in lean men, in which this pool was practically negligible as a whole. This difference in blood amino acid compartmentation

could, thus, be directly related to a certain characteristic of obesity necessarily shared by lean women, since obesity as a whole should present differences both with respect to men and to women (as is the case with the intracellular amino acid pool).

Knowing that obese men produce higher amounts of estrogens –predominantly estrone (32)– in their enlarged white adipose tissue masses (33) compared with lean men, and that women also show higher concentrations of estrogens because of ovarian, mammary gland and adipose tissue production, it was, thus, hypothesized that the differences in blood amino acid compartmentation could be directly or indirectly related to the increased secretion of estrogen found in women and obese men. As a consequence, the experiment in which male rats were infused with large amounts of estrone was devised. The results obtained indicated that estrone treatment indeed influences blood amino acid compartmentation, essentially by affecting the membrane-bound amino acid compartment.

Prolonged estrogen treatment is known to lower body weight (34) because of a decrease in fat stores (35), although ß-estradiol and estrone in fact have slightly different physiological effects, the latter affecting fat mobilisation less than ß-estradiol (23). Prolonged estrone treatment has also been found to alter the nitrogen balance in the rat by enhancing protein deposition (23). The results presented here agree with this effect of estrone, bringing about a positive nitrogen balance in the rat after only two weeks' treatment.

The effects of estrone on nitrogen balance may be related to a higher ability to absorb dietary amino acids as observed in estrone-treated rats, but obesity- and sex-linked alterations in the blood amino acid compartmentation suggest that estrone action could also be partly mediated by significant changes in the transporting/releasing capability of blood in relation with inter-organ amino acid distribution. The data presented here hint at some type of modification of the ability of the erythrocyte membrane to bind amino acids. It can only be speculated as to whether the effect is a local transformation involving changes in the pre-formed red blood cell or, perhaps, it is just the consequence of changes in the structure of the newly formed erythrocyte membranes.

Direct action of estrone on the preformed red blood cell can be considered, firstly, since the direct binding of similar structural substrates –e.g. cortisol– have been previously described (36). However, the fact that our results of [^{3}H]-Glutamine binding *in vitro* in the absence of estrone produce a level of binding similar to that found *in vivo* seems to exclude this direct action. It can also be thought that the action of estrogens may be exerted in cell types other than erythrocytes. Thus, it is possible to consider an action on gene expression in other blood cells or tissues regulating the production of factors affecting binding. Despite no further data, this seems to be the more likely mechanism.

We have presented here evidence linking the compartmentation of free amino acid pools in blood (hinting at their different metabolic roles) with varying *in vivo* exposure to estrone. The distribution of amino acids may help explain the effects of estrone on nitrogen metabolism with enhanced protein accrual, but it may also help link sexual differences in amino acid metabolism to the secretion of a single steroid hormone.

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References

1. D.H. ELWYN, Fed. Proc. 25 854-861 (1966).
2. D.H. ELWYN, H.C. PARIKH and W.C. SHOEMAKER, Am. J. Physiol. 215 1260-1275 (1968).
3. D.H. ELWYN, W.J. LAUNDER, H.C. PARIKH and E.M. WISE, Am. J. Physiol. 222 1333-1342 (1972).
4. T.T. AOKI, M.F. BRENNAN, W.A. MÜLLER, F.D. MOORE and G.F. CAHILL, J. Clin. Invest. 51 2889-2894 (1972).
5. T.T. AOKI, W.A. MÜLLER, M.F. BRENNAN and G.F. CAHILL, Diabetes 22 768-775 (1973).
6. T.T. AOKI, M.F. BRENNAN, W.A. MÜLLER, J.S. SOELDNER, J.S. ALPERT, S.B. SALTZ, R.L. KAUFMANN, M.H. TAN and G.F. CAHILL, Am. J. Clin. Nutr. 29 340-350 (1976).
7. H.N. MUNRO, Mammalian Protein Metabolism H.N. Munro (Ed) Vol IV., 299-375, Academic Press, New York (1970).
8. P. FELIG, Ann. Rev. Biochem. 44 933-955 (1975).
9. C.G. WINTER and H.N. CHRISTENSEN, J. Biol. Chem. 239 872-878 (1964).
10. H.N. CHRISTENSEN, Physiol. Rev. 62 1193-1233 (1982).
11. J.D. YOUNG, J.C. ELLORY and E.M. TUCKER, Biochem. J. 154 43-48 (1976).
12. J.D. YOUNG, M.W. WOLOWYK, S.E.M. JONES and J.C. ELLORY, Biochem. J. 216 349-357 (1983).
13. P. FELIG, J. WAHREN and L. RÄF, Proc. Natl. Acad. Sci. USA 70 1775-1779 (1973).
14. L. HAGENFELDT and A. ARVIDSSON, Clin. Chim. Acta. 100 133-141 (1980).
15. R. JONTOFSOHN, G. TRIVISAS, N. KATZ and R. KLUTHE, Am. J. Nutr. 31 1956-1960 (1978).
16. M. SOLEY and M. ALEMANY, ICRS Med. Sci. 8 159-160 (1980).
17. M. SOLEY, E. HERRERA and M. ALEMANY, Mol. Physiol. 2 89-97 (1982).
18. M. GIANOTTI, P. ROCA and A. PALOU, Arch. Physiol. Biochim. 98 155-161 (1990).
19. M. GIANOTTI, P. ROCA and A. PALOU, Int. Feto-Maternal Med. 4 37-44 (1991).
20. C. PICÓ, A. PONS and A. PALOU, Biosci. Rep. 11 223-230 (1991).
21. A.M. PROENZA, P. ROCA and A. PALOU, Plügers Arch. 427 R39 (1994a).
22. A.M. PROENZA, A. PALOU and P. ROCA, Biochem. Molec. Biol. Int. 34 971-982 (1994b).
23. M.J. LOBO, X. REMESAR and M. ALEMANY, Biochem. Mol. Biol. Int. 29 349-358 (1993).
24. LL. AROLA, E. HERRERA and M. ALEMANY, Anal. Biochem. 82 236-239 (1977).
25. B.A. BIDLINGMEYER, S.A. COHEN and T.L. TARVIN, J. Chromatogr. 336 93-104 (1984).
26. A. FERRANDO, I.G. BOBADILLA, A. BOBADILLA, A. PALOU and M. ALEMANY, Comp. Biochem. Physiol. 70A 611-613 (1981).
27. D.J. HANAHAN and J.E. EKHOLM, Methods Enzymol. 31 168-172 (1974).
28. E.J. KING, Biochem. J. 26 292-297 (1932).
29. M. ESTEVE, I. RAFECAS, X. REMESAR and M. ALEMANY, Int. J. Obesity 16 237-244 (1992).
30. F. SERRA, M. GIANOTTI, A. PALOU and A. PONS, Biochem. Int. 24 769-776 (1991).
31. M. GIANOTTI, P. ROCA and A. PALOU, Horm. Metabol. Res. 20 208-212 (1988).
32. K.L. BULTON, D.U. HUDSON, S.W. COPPACK and K.N. FRAYN, Metabolism 41 556-559 (1992).
33. A.E. SCHINDLER, A. ALBERT and E.J. FREDERICK, Clin. Endocrinol. Metab. 35 627-630 (1972).
34. R.D. GAMBELL, Med. Times 9/89 35-43 (1989).
35. B. GUYARD, J. FRICKER, L. BRIGANT, D. BETOULLE and M. APFELBAUM, Metabolism 40 529-533 (1991).
36. R. HIRAMATSU and B.C. NISULA, J. Clin. Endocrinol. Metab. 64 1224-1232 (1987).