ELSEVIER

Journal of Nutritional Biochemistry 12 (2001) 431–440

THE JOURNAL OF Nutritional Biochemistry

Gender related differences in the effect of aging on blood amino acid compartmentation☆

Ana M. Proenza, Catalina Crespí, Pilar Roca*, Andreu Palou

Laboratori de Bioquímica i Biologia Molecular, Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Cra. Valldemossa Km 7.5, 07071 Palma de Mallorca, Spain

Received 21 September 2000; received in revised form 7 February 2001; accepted 23 February 2001

Abstract

This work has been focused on the study of the variations in blood amino acid compartmentation (plasma and blood cells) with aging, both in men and women. Aging is a situation which, under the influence of gender, involves a decline in body weight functions and variations in energy metabolism with a deterioration of muscular metabolism leading to changes in amino acid handling. We determined the blood levels of individual amino acids in whole blood, plasma compartment and blood cell compartment of 51 men and 51 women. Subjects were classified in three age groups—AG1 (18 to 35 y), AG2 (35–50 y) and AG3 (more than 50 y). Aging was accompanied by significant changes in blood levels of amino acids showing gender-linked differences which were distinct for both blood compartments (plasma and blood cells). In men, aging was accompanied by a drop in blood levels of several amino acids, due mainly to the plasma compartment, whereas in women aging brought about a rise in blood levels of various amino acids mainly in blood cell compartment. This paper contributes to enhancing the physiological importance of the blood cell pool in the handling of amino acids.

Keywords: Blood amino acid compartmentation; Age; Gender effect; Humans

1. Introduction

Aging is associated with a decline in the body functions and with a drop in the ability to maintain the homeostatic and integrated functions, a lower energy metabolism, a deterioration of muscular metabolism and a decreased respiratory muscle capacity [1–4]. Body composition also changes with aging: lean body mass decreases whereas fat mass increases [5]. Moreover, there are significant alterations in the serum total lipids [6,7] which are related possibly to changes in the hormonal environment [8–10] and variations in the availability of amino acids are also described in parallel with a reduction in the amount of muscle mass [11–13].

In particular, young men have higher levels of amino acids in the plasmatic compartment than young women, differences that tended to disappear with age [11,14,15]. These higher levels have been attributed to their relatively greater muscle mass compared to women [16,17].

However, the changes in the blood cell pool of amino acids with age have not been previously studied, despite the fact that the importance of blood cells as a carrier of circulating amino acids for interorgan exchange has received recent support in humans where the changes in amino acid handling can be better understood when blood cell compartmentation is taken into account [17,18]. Previous works focused on the role of the blood cell compartment in amino acid handling have contributed to show the importance of this pool of amino acids in the whole blood context. Its importance has been postulated due to the profound changes in amino acid red cell concentration during the fast flow of blood through the tissues, in spite of the slow rates of amino acid plasma-erythrocyte exchange found in *in vitro* studies [19–22].

To go further into the role of the human blood cell compartment we focused this paper on the study of blood amino acid compartmentation, in both men and women, with aging, a situation that is accompanied by impairment of energy and protein metabolism thus involving changes in amino acid handling.

☆ Work supported by grant PB97–0094 from the "Dirección General de Enseñanza Superior e Investigación Científica (DGESEIC)" of the Government of Spain.

* Corresponding author. Tel.: +34-971-17-31-72; fax: +34-971-17-31-84.

E-mail address: dbsprs0@ps.uib.es (P. Roca).

0955-2863/01/$ – see front matter
PII: S0955-2863(01)00157-7

Table 1
Anthropometric data of men and women grouped according to their ages

		AG1	AG2	AG3[a,b]
Age, y	M	27.9 ± 0.8	43.8 ± 0.9	56.4 ± 1.5
	W	26.8 ± 1.1	46.5 ± 1.0	62.4 ± 1.8***
Weight, kg	M	89.0 ± 4.1	80.9 ± 2.2	72.3 ± 2.4*
	W	61.0 ± 2.8***	67.2 ± 4.4***	63.1 ± 2.9***
Height, m	M	1.77 ± 0.02	1.71 ± 0.02*	1.65 ± 0.02*
	W	1.61 ± 0.01***	1.59 ± 0.01***	1.55 ± 0.02*,***
BMI, kg/m^2	M	28.4 ± 1.2	27.7 ± 0.7	26.5 ± 1.0
	W	23.4 ± 1.0***	26.7 ± 1.7	26.3 ± 1.1

[a] One way ANOVA (for each gender) significance ($p < 0.05$): *A* effect of age—* AG1 *vs* AG2, AG3, ** AG2 *vs* AG3-, *SxA* interaction of sex and age.
[b] *t*-test significances ($p < 0.05$): ***men *vs* women.

2. Materials and methods

2.1. Subjects and sampling

Consistent with current guidelines in Spain, human blood samples were obtained from consenting healthy subjects, all of them Caucasian of Spanish origin, undergoing a routine blood test, which were grouped according to gender and then to age in three groups: AG1 (18 to 35 years old), AG2 (between 35 and 50 years old) and AG3 (50 to 80 years old). Table 1 shows the anthropometric features of each group. After overnight fasting, 5 ml blood samples were obtained by venipuncture from the forearm, were kept in heparinized glass tubes at 4°C, and processed in the following 2 h.

2.2. Blood fraction amino acid analysis

Blood amino acid pool evaluation was carried out following a previously developed protocol [23]. In brief, two blood fractions were studied: plasma—obtained by centrifuging an aliquot of whole blood—and hemolysed whole blood—obtained by diluting (1:1) an aliquot of whole blood with the internal standard solution (L-methionine sulfone [Sigma, St. Louis, MO USA] 400 mmol/L). The plasma was also diluted with an equal volume of the internal standard solution and subsequently all blood fraction samples were deproteinized with cold acetone [24]. 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). Amino acid levels were calculated from the peak areas using the MAXIMA™ 820 program (Waters).

The plasma levels of amino acids (P), expressed as μmoles of amino acids per liter of plasma, and the amino acids in hemolysed whole blood (HWB), expressed as μmoles of amino acids per liter of whole blood were obtained from both fractions. The packed cell volume value for each sample—apparent hematocrit value—was determined after centrifugation at 15,000 g for 5 min; plasma trapped in blood cell fractions was estimated [26], and the amino acid values in the cell fractions corrected accordingly—true hematocrit value (Ht). Thus, the μmoles of amino acids in both fractions referred to whole blood volume were calculated in the following way:

$Pc = P(1\text{-}Ht/100)$ Pc: μmoles of amino acids in plasma per litre of whole blood

$Cc = HWB\text{-}Pc$ Cc: μmoles of amino acids in cells per litre of whole blood

2.3. Additional plasma parameter analysis

Plasma samples, deproteinized with 120 g/L perchloric acid (1:1) and neutralized with 2 mol/L KHCO3–2 mol/L KOH (2:0.35), were used for the enzymatic measurement of glucose [27], urea [28] and β-hydroxybutyrate [29]. Besides, levels of free fatty acids (FFA) and esterified fatty acids (EFA) were measured by Gas Chromatography [30]. Insulin levels were measured by an immunoenzymetric assay kit (DRG-Instruments GmbH, Marburg, Germany).

2.4. Statistics

The gender and age effects on amino acid levels were determined by two-way analysis of variance (ANOVA), once the main effects were determined (age and/or sex), data were analyzed independently for each sex by one way ANOVA with post-hoc comparison to determine differences between age groups whereas *t*-test was performed to assess gender differences within each age group. The influence of obesity on changes observed with age and gender was determined by introducing the body mass index (BMI) value as a covariate in analyses of covariance (ANCOVA). A similar analysis was performed when studying the age effect for each gender. To analyze the correlation between age and amino acid concentration in the different blood compartments simple linear regression was performed. All statistics were performed with DBASE IV and SPSS-WIN packages for PC.

Table 2
Blood levels of amino acids in men and women grouped according to their age—AG1, AG2 and AG3[a]

		AG1	AG2	AGE3				
		μmol/L of blood[f]			I[b]	II[c]	III[d]	IV[e]
Ile	M	62.4 ± 4.0	53.5 ± 2.2	48.8 ± 6.3	*S SxA*	*O S SxA*		*O*
	W	40.4 ± 1.8***	46.8 ± 3.9	41.5 ± 3.4				*O*
Leu	M	133 ± 4	129 ± 4	127 ± 5	*S*	*O S*		*O*
	W	94.9 ± 3.6***	108 ± 4***	105 ± 8***				*O*
Val	M	234 ± 8	227 ± 6	198 ± 7*,**	*S*	*O S*	*A*	*O*
	W	191 ± 9***	212 ± 13	202 ± 16				*O*
His	M	59.1 ± 2.2	56.8 ± 2.7	54.3 ± 6.0		*O*		
	W	56.0 ± 2.2	55.8 ± 2.2	53.6 ± 3.2				
Lys	M	77.2 ± 3.6	89.9 ± 3.2*	84.9 ± 5.7	*Aab*	*O S A*	*A*	
	W	80.2 ± 6.4	92.0 ± 7.1	94.3 ± 8.6				*O*
Phe	M	50.0 ± 1.4	47.7 ± 1.2	54.5 ± 2.2**	*S Abc*	*O S A*	*A*	
	W	44.7 ± 1.6***	45.8 ± 1.8	50.2 ± 2.2				*O*
Tyr	M	69.5 ± 2.8	64.5 ± 2.6	67.0 ± 2.1	*SxA*	*O A*		
	W	56.4 ± 3.7***	66.3 ± 4.2	74.1 ± 4.9*			*A*	
Thr	M	117 ± 5	106 ± 5	100 ± 15	*Aa*			
	W	131 ± 8	108 ± 9	112 ± 10				
Met	M	20.1 ± 0.9	18.1 ± 0.8	20.7 ± 1.4		*O*		
	W	18.7 ± 1.0	19.0 ± 0.8	21.2 ± 1.2				
E	M	823 ± 23	791 ± 18	755 ± 40	*S*	*O S*		*O*
	W	713 ± 29***	752 ± 36	754 ± 44				*O*
Gln	M	536 ± 16	525 ± 12	543 ± 30				
	W	520 ± 20	532 ± 13	544 ± 22				
Glu	M	217 ± 17	192 ± 12	178 ± 9		*O*		*O*
	W	199 ± 7	212 ± 14	208 ± 14				*O*
Asn	M	46.3 ± 4.8	56.1 ± 4.1	76.4 ± 10*,**	*Abc*	*O A*	*A*	*O A*
	W	50.6 ± 3.5	44.9 ± 5.9	64.0 ± 7.9				
Asp	M	135 ± 9	117 ± 12	101 ± 18		*O*		
	W	122 ± 9	110 ± 11	133 ± 10				
Gly	M	238 ± 6	229 ± 11	253 ± 14	*Ab SxA*			
	W	229 ± 11	289 ± 16*,***	275 ± 20*			*A*	
Ser	M	111 ± 6	108 ± 6	123 ± 14				
	W	124 ± 5	113 ± 6	120 ± 7				
Ala	M	335 ± 15	346 ± 19	327 ± 24		*O A*		
	W	315 ± 15	394 ± 23*	354 ± 25			*A*	*O*
Pro	M	210 ± 16	204 ± 20	143 ± 16				*O*
	W	158 ± 9***	194 ± 14	166 ± 11				*O*
Hyp	M	17.2 ± 1.4	16.5 ± 1.6	16.6 ± 2.9				*O*
	W	14.8 ± 1.3	15.9 ± 2.1	12.4 ± 1.2				
Orn	M	64.7 ± 3.4	73.1 ± 3.3	70.4 ± 8.2	*Aa*			
	W	57.4 ± 2.7	68.3 ± 5.3	65.6 ± 5.5				*O*
NE	M	1911 ± 37	1866 ± 46	1831 ± 79	*SxA*	*O*		
	W	1790 ± 56	1974 ± 51	1943 ± 66				*O*
T	M	2733 ± 50	2658 ± 55	2586 ± 115	*SxA*	*O*		*O*
	W	2503 ± 74***	2726 ± 80	2697 ± 100				*O*

[a] Values are means ± SEM of 25, 18 and 8 male individuals respectively, and 22, 13, and 16 female individuals.
[b] Two way ANOVA significances ($p < 0.05$): *S* effect of sex, *A* effect of age—*a* AG1 *vs* AG2, *b* AG1 *vs* AG3, *c* AG2 *vs* AG3-, *SxA* interaction of sex and age.
[c] ANCOVA significances ($p < 0.05$): *O* effect of obesity, *S* effect of sex, *A* effect of age.
[d] One way ANOVA (for each gender -male and female-) significances ($p < 0.05$): *A* effect of age—* AG1 *vs* AG2, AG3, ** AG2 *vs* AG3-, *SxA* interaction of sex and age.
[e] ANCOVA (for each gender -male and female-) significances ($p < 0.05$): *O* effect of obesity, *A* effect of age.
[f] *t*-test significance ($p < 0.05$): *** men *vs* women.

3. Results

Tables 2, 3 and 4 show the individual amino acid concentrations of blood compartments (whole blood, plasma and cellular, respectively) expressed as μmol of each amino acid in the fraction present in 1L of blood. Considering first the essential amino acids, a gender effect in blood levels of Ile, Leu, Val, Phe and total essential amino acids was found. This effect was more patent in the youngest group, since the differences between sexes tended to disappear as the age of the subjects increased due to the decrease undergone by men and the increase observed in women. This effect of

Table 3
Plasma contributions of amino acids in men and women grouped according to their ages—AG1, AG2, and AG3[a]

		AG1	AG2	AG3	I[b]	II[c]	III[d]	IV[e]
		μmol/L of blood[f]						
Ile	M	42.5 ± 2.6	37.8 ± 1.8	30.4 ± 3.5*	*S Abc*	*O S*	*A*	*O A*
	W	30.8 ± 2.3***	32.5 ± 2.8	27.7 ± 2.1				*O*
Leu	M	91.0 ± 2.4	90.1 ± 3.0	78.2 ± 3.7*·**	*S*	*O S*	*A*	*O A*
	W	69.9 ± 3.3***	74.5 ± 3.2***	70.5 ± 4.9				*O*
Val	M	157 ± 4	158 ± 5	127 ± 5*·**	*S*	*O*	*A*	*O A*
	W	135 ± 7***	145 ± 8	135 ± 9				*O*
His	M	37.4 ± 1.5	36.6 ± 1.5	35.9 ± 2.4		*O S*		
	W	42.4 ± 2.2	41.6 ± 1.5***	38.4 ± 1.9				
Lys	M	67.8 ± 2.4	79.2 ± 2.1*	70.9 ± 3.8	*Aab*		*A*	
	W	70.2 ± 3.1	80.4 ± 5.1	83.0 ± 4.4				*O*
Phe	M	34.1 ± 0.8	33.6 ± 0.8	34.0 ± 2.1				
	W	33.2 ± 1.3	33.4 ± 1.5	33.7 ± 1.4				
Tyr	M	43.1 ± 1.8	43.7 ± 1.7	40.5 ± 2.4		*O*		*O*
	W	38.1 ± 2.5	49.4 ± 8.3	46.0 ± 2.4				*O*
Thr	M	73.3 ± 3.1	69.5 ± 2.6	66.5 ± 8.3	*Aab*			
	W	90.4 ± 5.1***	70.9 ± 5.2*	72.2 ± 6.6*			*A*	
Met	M	15.3 ± 0.6	14.4 ± 0.6	14.1 ± 1.0		*O*		
	W	14.0 ± 0.8	13.9 ± 0.7	14.4 ± 0.9				
E	M	562 ± 13	563 ± 11	497 ± 25*·**		*O*	*A*	*O A*
	W	523 ± 11***	542 ± 24	520 ± 25				*O*
Gln	M	339 ± 14	339 ± 11	337 ± 15				
	W	349 ± 13	355 ± 8	357 ± 15				
Glu	M	46.0 ± 12	40.6 ± 6.2	23.8 ± 1.4		*O S*		
	W	24.4 ± 1.8	31.9 ± 4.7	32.5 ± 3.1***				*O*
Asn	M	13.1 ± 1.8	15.8 ± 2.0	23.0 ± 4.2*	*Abc*	*O A*	*A*	
	W	17.6 ± 1.9	16.1 ± 2.8	23.5 ± 3.3				
Asp	M	7.3 ± 0.8	6.2 ± 0.6	4.8 ± 1.2				
	W	6.4 ± 0.7	7.8 ± 0.8	6.0 ± 0.8				*O*
Gly	M	102 ± 3	96.9 ± 3.4	116 ± 8*·**	*S Ab SxA*		*A*	
	W	108 ± 6	145 ± 12*·***	135 ± 15			*A*	
Ser	M	57.2 ± 2.5	54.5 ± 2.8	60.7 ± 4.8	*S*			
	W	71.5 ± 3.0***	64.2 ± 3.8***	65.8 ± 4.4				
Ala	M	206 ± 10	221 ± 13	201 ± 20		*O*		
	W	209 ± 11	247 ± 13	225 ± 15				*O*
Pro	M	143 ± 11	143 ± 15	98.0 ± 11	*Abc*			
	W	116 ± 7***	132 ± 9	112 ± 7				*O*
Hyp	M	10.7 ± 0.8	10.7 ± 1.0	11.7 ± 1.8				
	W	10.3 ± 0.8	10.6 ± 1.4	8.03 ± 0.6				
Orn	M	25.0 ± 1.4	28.7 ± 1.5	28.4 ± 3.7	*Ab*			
	W	27.0 ± 1.9	31.2 ± 2.5	33.6 ± 2.7				
NE	M	950 ± 20	956 ± 30	904 ± 35				
	W	939 ± 35	1041 ± 23***	1001 ± 44				*O*
T	M	1512 ± 27	1519 ± 37	1401 ± 56		*O*		
	W	1463 ± 46***	1583 ± 42	1521 ± 60				*O*

[a] Values are means ± SEM of 25, 18 and 8 male individuals respectively, and 22, 13 and 16 female individuals.
[b] Two way ANOVA significances ($p < 0.05$); *S* effect of sex, *A* effect of age—*a* AG1 *vs* AG2, *b* AG1 vs AG3, *c* AG2 *vs* AG3-, *SxA* interaction of sex and age.
[c] ANCOVA significances ($p < 0.05$): *O* effect of obesity, *S* effect of sex, *A* effect of age.
[d] One way ANOVA (for each gender -male and female-) significances ($p < 0.05$): *A* effect of age—* AG1 *vs* AG2, AG3, ** AG2 *vs* AG3-, *SxA* interaction of sex and age.
[e] ANCOVA (for each gender -male and female-) significances ($p < 0.05$): *O* effect of obesity, *A* effect of age.
[f] *t*-test significances ($p < 0.05$): *** men *vs* women.

aging is significant for Lys, Phe and Thr. All blood compartments contributed to this pattern, although the cellular compartment played a more important role than plasma in the case of women (age effect on cell contributions of 4 amino acids), while in men it was the plasma that was more affected with age (age effect on plasma contributions of 4 amino acids).

The non-essential amino acids showed a similar but less marked pattern: they dropped with age in men and rose with age in women, due to changes in different compartments.

Table 4
Cellular contributions of amino acids in men and women grouped according to their ages—AG1, AG2 and AG3[a]

		AG1	AG2	AG3	I[b]	II[c]	III[d]	IV[e]
		μmol/L of blood[f]						
Ile	M	19.8 ± 1.6	15.7 ± 1.2	18.4 ± 3.2	*S SxA*	*O S SxA*		*O*
	W	9.5 ± 1.4***	14.3 ± 1.4*	13.8 ± 1.6*			*A*	*O*
Leu	M	41.8 ± 2.4	38.4 ± 2.9	48.4 ± 4.9	*S A*	*O S*		*O*
	W	25.0 ± 1.4***	33.0 ± 2.4*	34.7 ± 3.6*·***			*A*	*O*
Val	M	77.1 ± 4.5	69.1 ± 4.5	71.4 ± 8.6	*S*	*O S*		*O*
	W	56.7 ± 4.4***	66.2 ± 7.5	67.0 ± 7.7				*O*
His	M	21.6 ± 1.3	20.2 ± 2.0	18.5 ± 4.9	*S*			
	W	13.6 ± 1.7***	14.2 ± 2.5	15.3 ± 2.4				
Lys	M	9.3 ± 2.8	10.7 ± 3.1	14.0 ± 6.2		*O*		
	W	9.9 ± 6.5	11.6 ± 4.9	11.3 ± 5.5				
Phe	M	15.9 ± 1.0	14.1 ± 1.2	20.6 ± 2.6*·**	*S Abc*	*O S A*	*A*	
	W	11.5 ± 0.9***	12.4 ± 1.6	16.5 ± 1.5*·**			*A*	
Tyr	M	26.4 ± 1.4	20.7 ± 1.5*	26.5 ± 2.7	*A Ac*	*O S A*	*A*	*O A*
	W	18.3 ± 1.5***	16.9 ± 7.0	28.1 ± 2.7*·**				
Thr	M	43.7 ± 2.4	36.0 ± 2.8	34.0 ± 7.0				
	W	40.5 ± 3.0	36.6 ± 4.4	40.0 ± 3.7				*O*
Met	M	4.8 ± 0.6	3.7 ± 0.5	6.7 ± 1.4	*Abc*	*O*		
	W	4.6 ± 0.6	5.1 ± 0.3***	6.8 ± 0.5*·**·***			*A*	
E	M	260 ± 14	229 ± 16	258 ± 30	*S*	*O S*		
	W	190 ± 13***	210 ± 21	233 ± 23				*O*
Gln	M	197 ± 8	186 ± 11	206 ± 17				
	W	171 ± 9***	177 ± 11	186 ± 11				
Glu	M	171 ± 8	151 ± 9	154 ± 8	*S*	*O*		*O*
	W	175 ± 6	180 ± 12	176 ± 12				
Asn	M	34.5 ± 3.2	40.3 ± 2.7	53.4 ± 7.1*·***	*Abc*	*O S A*	*A*	
	W	33.0 ± 2.2	28.8 ± 3.8***	40.5 ± 4.9				
Asp	M	128 ± 8	111 ± 11	95.8 ± 18		*O*		
	W	115 ± 9	102 ± 11	127 ± 10				
Gly	M	136 ± 6	132 ± 12	137 ± 9		*O*		
	W	121 ± 6	144 ± 7*	140 ± 7*			*A*	
Ser	M	54.2 ± 4.4	53.8 ± 6.9	62.1 ± 12		*O*		
	W	52.4 ± 2.5	48.9 ± 3.9	54.6 ± 3.0				
Ala	M	129 ± 7	125 ± 12	127 ± 10		*O*		
	W	106 ± 5***	147 ± 13*	129 ± 10			*A*	*O*
Pro	M	66.7 ± 5.4	60.8 ± 9.0	44.9 ± 6.6	*SxA*	*O*		*O*
	W	42.9 ± 3.3***	62.1 ± 7.1*	53.2 ± 4.4			*A*	*O*
Hyp	M	6.5 ± 0.8	5.8 ± 0.8	4.9 ± 1.6		*O*		*O*
	W	4.5 ± 0.6	5.3 ± 0.7	4.4 ± 0.8				
Orn	M	39.7 ± 2.5	44.4 ± 2.9	42.1 ± 7.4	*S*	*O*		
	W	30.5 ± 2.3***	37.1 ± 4.8	32.0 ± 4.3				*O*
NE	M	961 ± 29	910 ± 47	927 ± 57		*O*		
	W	851 ± 29***	933 ± 41	942 ± 35				*O*
T	M	1221 ± 40	1139 ± 60	1185 ± 81	*S*	*O*		
	W	1040 ± 37***	1143 ± 57	1175 ± 56				*O*

[a] Values are means ± S.E.M. of 25, 18 and 8 male individuals respectively, and 22, 13 and 16 female individuals.

[b] Two way ANOVA significances ($p < 0.05$): *S* effect of sex, *A* effect of age—*a* AG1 *vs* AG2, *b* AG1 *vs* AG3, *c* AG2 *vs* AG3-, *SxA* interaction of sex and age.

[c] ANCOVA significances ($p < 0.05$): *O* effect of obesity, *S* effect of sex, *A* effect of age.

[d] One way ANOVA (for each gender -male and female-) significances ($p < 0.05$): *A* effect of age—* AG1 *vs* AG2, AG3, ** AG2 *vs* AG3-, *SxA* interaction of sex and age.

[e] ANCOVA (for each gender -male and female-) significances ($p < 0.05$): *O* effect of obesity, *A* effect of age.

[f] *t*-test significances ($p < 0.05$): *** men *vs* women.

Again, it was the plasma compartment which was more altered in men, while in women it was the cellular compartment: plasma contributions of Asn and Gly showed an age effect in the former and cell contributions of Gly, Ala and Pro showed it in the latter.

Taking into account the BMI, ANCOVA analysis, additional differences can be observed. Thus in women 1 out of 2 (in blood) and 4 out of 7 (in blood cell compartment) amino acids showing age-related differences disappeared when BMI was introduced as covariante. Whereas for men in no compartment did the effect of BMI bring about the disappearance of age related differences. Besides,

Table 5
Significant correlations between blood, plasma and cell levels of amino acids and age in men[a]

	Blood[b]				Plasma				Cells			
	A	B	r	p	A	B	r	p	A	B	r	p
Ile	79.0	−0.576	−0.382	0.006	101	−0.817	−0.450	0.001	52.6	−0.297	−0.203	*NS*
Leu	140	−0.249	−0.173	*NS*	186	−0.722	−0.330	0.018	84.8	0.267	0.108	*NS*
Val	266	−1.04	−0.347	0.013	331	−1.52	−0.412	0.003	189	−0.583	−0.141	*NS*
Lys	64.8	0.477	0.323	0.021	117	0.325	0.177	*NS*	3.69	0.540	0.195	*NS*
Thr	138	−0.730	−0.310	0.027	149	−0.582	−0.245	*NS*	127	−0.977	−0.355	0.011
E	894	−2.45	−0.274	*NS*	1135	−3.93	−0.367	0.008	610	−1.11	−0.079	*NS*
Asn	20.2	0.904	0.422	*NS*	7.41	0.533	0.372	0.008	38.2	1.39	0.416	0.003
Asp	164	−1.08	−0.261	*NS*	18.5	−0.179	−0.336	0.016	341	−1.96	−0.212	*NS*

[a] Values correspond to the adjustment of 51 concentration data to a linear regression.
[b] Origin (A)—μmol/L blood, μmol/L plasma, μmol/L cells respectively-, slope (B), correlation (r) and probability (p) values.

ANCOVA analysis using the BMI value as a covariate showed that plasma and cell compartments were affected in a different way which is linked to the gender of the subjects. Thus, 4 out of 4 age-affected plasma contributions showing an obesity effect, maintained the aging effect when corrected for degree of obesity in men (those corresponding to Ile, Leu, Val and total essential amino acids), whereas none out of 4 blood cell contributions maintained the aging effect in the same situation in women. Moreover, plasma and cell compartments had a similar number of amino acids, 5 and 4 respectively, undergoing age effects independently of obesity degree for men and women taken together.

To further analyze the gender-related differences in amino acid compartmentation with aging, the data were considered individually. Tables 5 and 6 show the statistically significant linear correlations of the blood, plasma and blood cell concentrations of individual and sets of amino acids with age found in our study according to gender.

In men (Table 5), blood concentrations of Ile, Val and Thr decreased as age increased, whereas Lys followed the opposite pattern. On the contrary, aging in women brought about a linear increase of Lys, Phe, Tyr, Met, Gly and Orn blood concentrations, as well as non essential and total amino acids (Table 6).

In men, the concentration in the plasma compartment of a higher number of amino acids than in the cell compartment rose lineally with aging (Table 5). Thus, Ile, Leu, Val, essential, Asp and total amino acid plasma concentration decreased lineally as age increased whereas Asn rose lineally, whereas in blood cell compartment only Thr and Asn underwent variations—decreasing the former and increasing the latter.

In the case of women (Table 6), changes in plasma concentrations affected a lower number of amino acids (Lys, Glu, Gly, Orn and non essential) and in all cases plasma concentrations increased lineally with age. Whereas, in blood cell compartment Ile, Leu, Lys, Phe, Tyr, Met, Gly, non essential and total amino acids increased lineally with age.

To obtain a better reference of the metabolic context for the amino acid changes with age, levels of insulin, glucose, urea, β-hydroxybutyrate, free fatty acids (FFA) and esterified fatty acids (EFA) in plasma were also determined in a

Table 6
Significant correlations between blood, plasma and cell levels of amino acids and age in women[a]

	Blood[b]				Plasma				Cells			
	A	B	r	p	A	B	r	p	A	B	r	p
Ile	40.9	0.035	0.048	*NS*	55.9	−0.118	−0.116	*NS*	18.0	0.274	0.297	0.035
Leu	90.1	0.261	0.192	*NS*	115	0.104	0.064	*NS*	51.5	0.529	0.343	0.014
Lys	65.0	0.526	0.283	0.044	97.7	0.731	0.393	0.004	13.3	0.297	0.081	*NS*
Phe	40.4	0.148	0.305	0.030	52.5	0.082	0.135	*NS*	21.8	0.257	0.334	0.017
Tyr	41.3	0.538	0.468	0.001	55.3	0.420	0.211	*NS*	21.3	0.724	0.332	0.017
Met	16.1	0.080	0.293	0.037	21.3	0.056	0.151	*NS*	8.00	0.126	0.342	0.014
Glu	188	0.411	0.150	*NS*	32.6	0.365	0.286	0.042	433	0.143	0.024	*NS*
Gly	190	1.61	0.394	0.004	138	1.71	0.355	0.011	270	1.36	0.336	0.016
Orn	48.2	0.339	0.310	0.027	34.6	0.375	0.360	0.009	70.4	0.231	0.108	*NS*
NE	1651	5.45	0.351	0.011	1440	4.99	0.296	0.035	1986	5.69	0.298	0.034
TOT	2325	6.88	0.315	0.024	2298	5.61	0.240	*NS*	2376	8.62	0.312	0.026

[a] Values correspond to the adjustment of 51 concentration data to a linear regression.
[b] Origin (A)—μmol/L blood, μmol/L plasma, μmol/L cells respectively-, slope (B), correlation (r) and probability (p) values.

Table 7
Plasma levels of β-hydroxybutyrate, urea, glucose, free fatty acids (FFA), esterified fatty acids (EFA) and insulin in men and women grouped according to their ages—AG1, AG2 and AG3[a]

		AG1	AG2	AG3	I[b]	II[c]	III[d]	IV[e]
β-Hydroxybutyrate[f]	M	36.3 ± 6.1	48.7 ± 8.5	66.3 ± 14.8				
(μmol/L)	W	37.2 ± 3.9	34.1 ± 4.7	44.5 ± 7.9				
Urea (mmol/L)	M	5.82 ± 0.33	5.45 ± 0.44	5.99 ± 0.46				
	W	4.50 ± 0.39[f,***]	5.28 ± 0.29	6.05 ± 0.48*				
Glucose (mmol/L)	M	4.71 ± 0.18	4.86 ± 0.21	5.52 ± 0.23*,**	*S Ab*		*A*	
	W	4.11 ± 0.31	4.55 ± 0.15	4.81 ± 0.37				
FFA (μmol/L)	M	624 ± 75	804 ± 80	748 ± 68	*Abc*			
	W	712 ± 63	662 ± 67	979 ± 85*,**			*A*	
EFA (mmol/L)	M	7.07 ± 0.97	6.70 ± 0.97	9.70 ± 1.7	*Abc*			
	W	5.61 ± 0.66	7.44 ± 1.2	11.8 ± 1.1*,**			*A*	
Insulin (pIU/L)	M	14.8 ± 21.6	18.9 ± 6.1	22.5 ± 2.9				
	W	14.0 ± 1.9	14.6 ± 2.9	16.3 ± 1.3				

[a] Values are means ± S.E.M. of 10, 11 and 6 male individuals respectively, and 15, 12 and 14 female individuals.
[b] Two way ANOVA significances ($p < 0.05$): *S* effect of sex, *A* effect of age—*a* AG1 *vs* AG2, *b* AG1 *vs* AG3, *c* AG2 *vs* AG3-, *SxA* interaction of sex and age.
[c] ANCOVA significances ($p < 0.05$): *O* effect of obesity, *S* effect of sex, *A* effect of age.
[d] One way ANOVA (for each gender -male and female-) significances ($p < 0.05$): *A* effect of age—* AG1 *vs* AG2, AG3, ** AG2 *vs* AG3-, *SxA* interaction of sex and age.
[e] ANCOVA (for each gender -male and female-) significances ($p < 0.05$): *O* effect of obesity, *A* effect of age.
[f] *t*-test significances ($p < 0.05$): *** men *vs* women.

representative set of individuals in the three groups studied (AG1, AG2 and AG3) as shown in Table 7. Aging produced a rise in glucose, FFA and EFA levels, with significant differences between the first and third groups. Women in the first group had lower levels of urea than men while, in the eldest they showed higher levels than men. Mean values of β-hydroxybutyrate and insulin showed the same tendency to increase as age rose in both men and women although differences did not reach statistical significance.

4. Discussion

Aging was accompanied by significant changes in blood levels of amino acids which showed gender-linked differences and were different for both blood compartments—plasma and blood cells. Thus, in men amino acid blood levels decreased as age increased, whereas in women they increased, as has been previously reported for the plasma compartment [11,14,15]. This fact led to propose that it is possible to discriminate between young men and young women according to their free amino acid profile (especially essential amino acids), but this is not so when age increases [11].

The greater concentration of amino acids, particularly essential amino acids, in young men compared to young women would be in agreement with their potential greater utilization by the former, as suggested by the higher production of urea observed. Although little is known about the possible effects of gender on protein metabolism, a higher leucine oxidation rate of men compared to women has been recently reported [31] and this has been considered to be one of the factors contributing to the higher resting metabolic rate in men.

Aging is accompanied by a drop in resting metabolic rate [32]. Besides, aging affects body composition and fuel metabolism differently in each gender, leading to reduced fat oxidation and a loss of striated muscle in men, and to an increased ratio of upper- to lower-body fat in women [5]. Thus, in men, the drop in the levels of the blood amino acids seen when comparing the first and the second age groups, and the continuous drop to the eldest group would be correlated with the general profile of loss of muscle mass. Whereas in women the increase in blood levels of amino acids observed between the first and second age group, could be attributed mainly to differences in the BMI, because women in the AG1 group were leaner than those of AG2 (BMI = 23.4 ± 1.0 kg/m^2 vs BMI = 26.7 ± 1.7 kg/m^2) since an increase of blood amino acid levels linked to obesity has been previously described [17] and has been associated to the insulin resistance characteristic of this obese situation [33,34]. In women, the greater concentration of blood amino acids in the second age group as well as the slight drop of FFA compared to the first group could suggest a lower metabolization of fats. Likewise, an increase of body weight does not only involve a gain in fat but also in muscle mass [12] and it has been reported that protein oxidation is negatively related to lipid oxidation in obese women [35]. In women over 50 years (AG3) levels of blood amino acids remained practically unchanged, as well as BMI values, thus suggesting a close relationship between amino acid concentration and degree of obesity in women.

The blood compartments may play a role in the blood

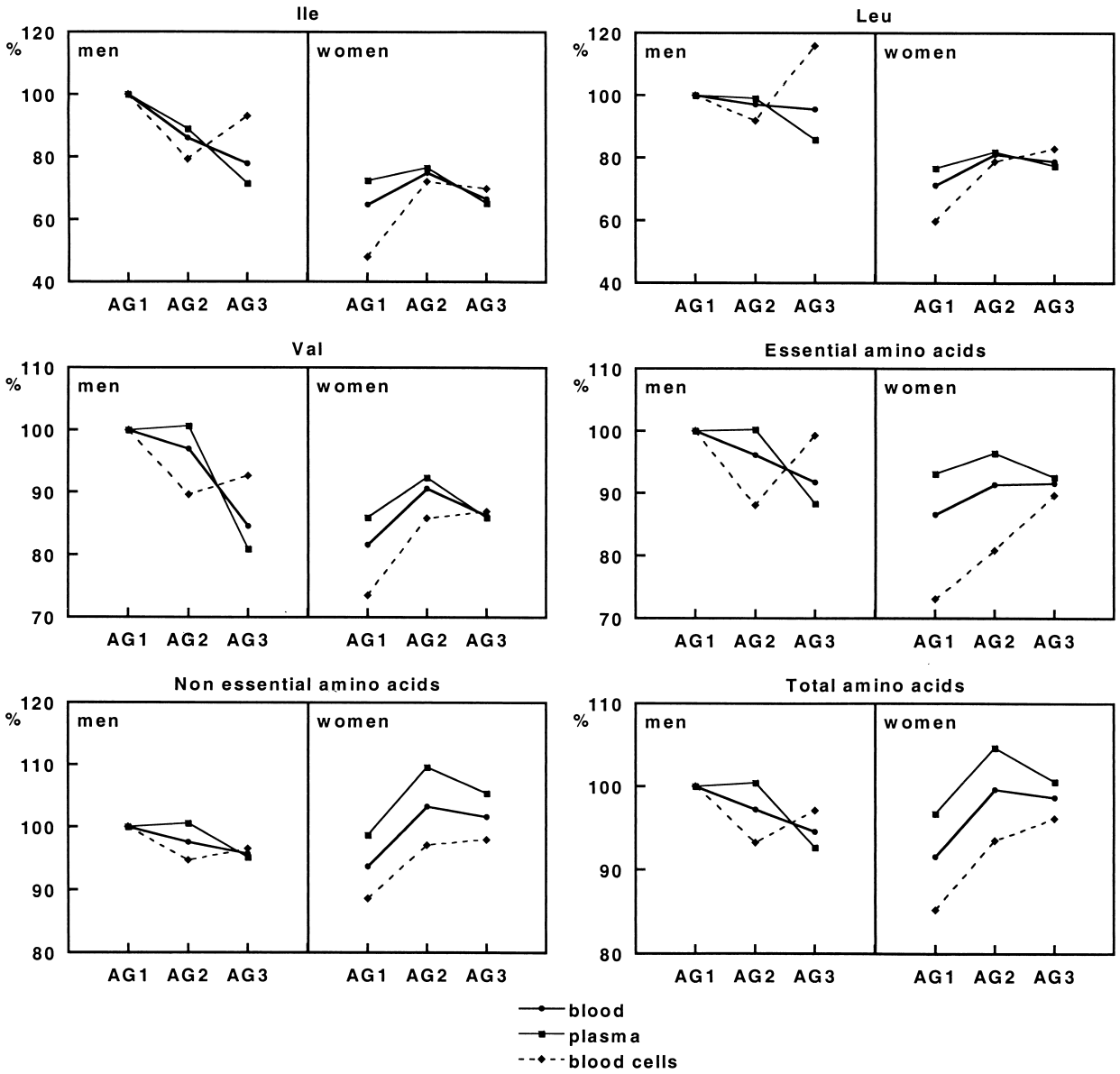

Fig. 1. Age-related changes in blood concentrations and blood cell and plasma contributions of selected amino acids in men and women. Values are the percentage respect to the respective values in AG1. AG1: 18 to 35 years old. AG2: 35 to 50 years old AG3: 50 to 80 years old.

free amino acid pool differential behavior between women and men with aging. In women, aging-related changes in the levels of amino acids on blood were hardly reflected in plasma, whereas the blood cell compartment underwent more important changes. That is, all the significant age-related changes undergone by blood amino acids were reflected in the blood cell pool—except Orn—whereas changes in plasma concentration involved a lower number of amino acids. On the other hand, in men, the blood amino acid concentration age-related changes observed were more reflected by the plasma compartment (see Fig. 1). The difference in the blood compartment affected in both sexes could also be associated with the degree of obesity (BMI value) since obesity-linked changes in the blood amino acid levels are mainly due to the blood cell compartment [17]. However, including BMI value as a covariate in the ANCOVA analysis (see Tables 2, 3 and 4) allowed us to state that the degree of obesity did not account for the changes observed with aging as a whole, but provoked the aging effect wearing off for several amino acids in women's cell compartment (Ile, Leu, Ala and Pro). On the other hand, in men, the existence of an obesity effect on plasma levels of amino acids did not imply the disappearance of the age effect in any case.

Studies *in vitro* have shown the rate of flux between plasma and blood cell pools to be slow, whereas *in vivo* studies have shown the changes in the blood cell amino acids levels observed when blood passes through the tissues

to be rapid [20]. Hence this compartment could play an important role in the interorgan transport of amino acids. These aging-arising differences between men and women in the blood concentration of amino acids, which also involves the identity of which blood compartment is more affected, would be accompanied by a set of alterations involved in this physiological process which also seems to be affected by gender. Thus, gender dimorphism has been shown in aged rats to favor females in terms of maintenance of muscle protein content [36]. In humans, total fat oxidation and fat oxidation per kilogram of lean soft-tissue mass decrease with age in men but not in women [5] and a sex dimorphism in post-absorptive fat metabolism has been reported in the elderly [37].

As far as we know this is the first report on age-related changes in amino acid compartmentation in humans. These results contribute to enhancing the physiological importance of the blood cell pool in the handling of blood amino acids. Thus, changes in blood amino acid concentration in women could not be observed by studying only the plasma compartment, whereas in men the study of the plasma compartment would highlight the drop in blood amino acid concentration with aging. For this reason the blood cell compartment should be taken into account to understand the handling of blood amino acids as a whole.

Acknowledgments

Thanks are given to the Hospital General de Mallorca (Consell Insular de Mallorca), Department of Medicine of EMAYA (Palma de Mallorca Town Hall), Hospital de Son Dureta (INSALUD), Old People's Home (Consell Insular de Mallorca) and Camp Redó Health Center (INSALUD), for kindly providing the blood samples that made this work possible. We also thank Jordi Pericás for essential technical assistance.

References

[1] Meites, J. (1990). Alterations in hypothalamic-pituitary function with age. In *Endocrine function and ageing*, (Armbrecht, H.J., Coe, R.M., and Wongsurawat, N., eds.), pp. 1–12, Springer-Verlag, New York.

[2] Fukagawa, N.K., Bandini, L.G., Young, J.B, Effect of age on body composition and resting metabolic rate, Am J Physiol, 259 (1990) E233–238.

[3] Sial, S., Coggan, A.R., Carroll, R., Goodwin, J., and Klein, S, Fat and carbohydrate metabolism during exercise in elderly and young subjects, Am J Physiol, 271 (1996) E983–989.

[4] Schunk, K., Pitton, M., Duber, C., Kersjes, W., Schadmand-Fisher, S., and Thelen, M, Dynamic phosphorus-31 magnetic resonance spectroscopy of the quadriceps muscle: effects of age and sex on spectroscopic results, Invest Radiol, 34 (1999) 116–125.

[5] Horber, F.F., Gruber, B., Thomi, F., Jensen, E.X., Jaeger, P, Effect of sex and age on bone mass, body composition and fuel metabolism in humans, Nutrition, 13 (1997) 524–534.

[6] Murawski, U., and Egge, H, Longevity study of age changes in serum lipids in rats, Interdiscipl Topics Geront, 13 (1978) 75–80.

[7] Toth, M.J., Arciero, P.J., Gardner, A.W., Calles-Escandon, J., and Poehlman, E.T, Rates of free fatty acid appearance and fat oxidation in healthy younger and older men, J Appl Physiol, 80 (1996) 506–511.

[8] Olefsky, J.M, Insensivity of large rat adipocytes to the antilipolytic effects of insulin, J Lipid Res, 18 (1977) 459–464.

[9] Duckworth, W.C. (1990) Pathogenesis and management of non-insulin-dependent diabetes mellitus. In *Endocrine function and ageing* (Armbrecht, H.J., Coe, R.M., and Wongsurawat, N., eds.), pp. 35–47, Springer-Verlag, New York.

[10] Vermeulen, A., Goemaere, S., and Kaufman, J.M, Testosterone, body composition and ageing, J Endocrinol Invest, 22 (1999) 110–116.

[11] Bertolini, A.M., Santagostino, A., and Barbiano di Belgioioso, G, The behavior of free aminoacids in the plasma of the aged, Gerontol Clin, 14 (1972) 43–55.

[12] Forbes, G.B. (1987) The Adult. In *Human body composition, growth, ageing, nutrition, and activity*, pp 169–195, Springer-Verlag, New York.

[13] Welle, S., Thornton, C., Jozefowicz, MR, and Statt, M, Myofibrillar protein synthesis in young and old men, Am J Physiol, 264 (1993) E693–698.

[14] Galante, A., Angelico, F., Crocchioni, G., and Pennetti, V, Intersexual differences in the serum-free amino acid pattern of young adults, normal obese aged subjects, Nutr Metab, 22 (1978) 119–126.

[15] Caballero, B., Gleason, R.E., and Wurtman, R.J, Plasma amino acid concentrations in healthy elderly men and women, Am J Clin Nutr, 53 (1991) 1249–1252.

[16] Armstrong, M.D., and Stave, U, A study of plasma free amino acid levels. III. Variations during growth and ageing, Metabolism, 22 (1973) 571–578.

[17] Proenza, A.M., Roca, P., Crespí, C. Lladó, I., and Palou, A, Blood amino acid compartmentation in men and women with different degrees of obesity, J Nutr Biochem, 9 (1998) 697–704.

[18] Proenza, A.M., Roca, P., Virgili, J., Remesar, X., Alemany, M., and Palou, A, Estrogen effects on blood amino acid compartmentation, Life Sci, 57 (1995) 1589–1597.

[19] Hagenfeldt, L., and Arvidsson, A, The distribution of amino acids between plasma and erythrocytes, Clin Chim Acta, 100 (1980) 131–141.

[20] Christensen, H.N, Interorgan amino acid nutrition, Physiological Reviews, 62 (1982) 1193–1233.

[21] Gianotti, M., Roca, P., and Palou, A, Patterns of blood plasma and cells amino acid pools in 20-day pregnant rats, Int Foeto-Maternal Med, 4 (1991) 37–44.

[22] Picó, C., Lladó I., Pons, A., and Palou, A, Blood cell to plasma gradients of amino acids in arterial and venous blood in fed and fasted rats, Comp Biochem Physiol, 107A (1994) 589–595.

[23] Picó, C., Pons, A., and Palou, A, A significant pool of amino acids is adsorbed on blood cell membranes, Biosci Rep, 11 (1991) 223–230.

[24] Arola, Ll., Herrera, E., and Alemany, M, A new method for deproteinization of small samples of blood plasma for amino acids determination, Anal Biochem, 82 (1977) 236–239.

[25] Bidlingmeyer, B.A., Cohen, S.A., and Tarvin, T.L, Rapid analysis of amino acids using pre-column derivatization, J Chromatogr, 336 (1984) 93–104.

[26] Ferrando, A., Bobadilla, I.G., Bobadilla, A., Palou, A., and Alemany, M, Comparative estimation of haematocrit and trapped plasma in the packed cell volume in man, rabbit and chicken blood, Comp Biochem Physiol, 70A (1981) 611–613.

[27] Lowry, O.H., and Passoneau, J.P., A flexible system of enzymatic analysis. Academic Press, New York, 1972.

[28] Talke, H., and Schubert, G.E, Enzymatische harnstoffbestimmung im blut und serum im ooptischen test nach warburg, Klin Wochschr, 43 (1965) 174.

[29] Williamson, D.H., and Mellanby, J. (1974) In *Methods of enzymatic analysis*, (Bergmeyer, H.U., eds), Vol. 4, pp. 1836–1839, Academic Press, New York, USA.

[30] Borra, C., Cosmi, G., Corcia, A.D., and Samperi, R, Reliable measurement of non-esterified long-chain fatty acid pattern in blood plasma, J Chromatogr, 311 (1984) 9–15.
[31] Volpi, E., Lucidi, P., Bolli, G.B., Santeusanio, F., and De Feo, P., Gender differences in basal protein kinetics in young adults, J Clin Endocrinol Metab, 83 (1998) 4363–4367.
[32] Visser, M., Deurenberg, P., van Staveren, W.A., and Hautvast, J.G, Resting metabolic rate and diet-induced thermogenesis in young and elderly subjects: relationship with body composition, fat distribution and physical activity level, Am J Clin Nutr, 61 (1995) 772–778.
[33] Felig, P., Marliss, E., and Cahill, G.F, Plasma amino acid levels and insulin secretion in obesity, N England J Med, 281 (1969) 811–816.
[34] Forlani, G., Vannini, P., Marchesini, G., Zoli, M., Ciavarella, A., and Pisi, E, Insulin-dependent metabolism of branched-chain amino acids in obesity, Metabolism, 33 (1984) 147–150.
[35] Solini, A., Bonora, E., Bonadonna, R., Castellino, P., and DeFronzo, R.A, Protein metabolism in human obesity: relationship with glucose and lipid metabolism and with visceral adipose tissue, J Clin Endocrinol Metab, 82 (1997) 2552–2558.
[36] Lawler, J.M., Powers, S.K., and Criswell, D.S, Gender differences in diaphragmatic metabolic properties of the adult Sprague-Dawley rat, Respir Physiol, 97 (1994) 263–273.
[37] Toth, M.J., Gardner, A.W., Arciero, P.J., Calles-Escandon, J., and Poehlman, E.T, Gender differences in fat oxidation and sympathetic nervous system activity at rest and during submaximal exercise in older individuals, Clin Sci, 95 (1998) 59–66.