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Somatomedin C (IGF-1), Dehydroepiandrosterone Sulphate (DHEA-S) and Hcy Metabolism in Postmenopausal African Women

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Biological aging implies a progressive decrease of endocrine and metabolic resources, which correspond to a reduction of working activity and caloric intake. The aim of this study was to measure DHEA-S and IGF-1 and products of methionine metabolism in 76 postmenopausal African women (50 to 100 years of age) compared to 22 adult African fertile women (30 to 45 years of age). Determination of plasma DHEA-S in elderly women gave a markedly low mean concentration and the same was observed for plasma IGF-1 (450.1±263.2 and 64.2±38.1 ng mL^{-1}, respectively). The correlation between DHEA-S and IGF-1 was significantly positive only in 71-100 years women ($r = 0.44$, $p<0.05$). There was a significant decrease of plasma glutathione in all elderly women (3.5±0.4 μMol L^{-1}), while the plasma homocysteine (Hcy) was markedly elevated (18.7±0.5 μMol L^{-1}), whilst that of folic acid, Vit B12 and Vit B 6 levels were found in the normal range. A significant positive correlation between DHEA-S and plasma glutathione levels ($r = 0.56$, $p<0.025$) was found only in the 50-60 old years women group, while the correlation between IGF-1 and plasma glutathione was negative but not significant ($r = -0.36$, $p = NS$) in the same age group. In this study a low level of IGF-1 and DHEA-S in old humans is firstly considered as consequence of hypo-caloric alimentation, moderate physical activity and absence of psychophysics stress. Moreover a positive correlation between IGF-1 and DHEA-S in oldest age populations, ranging from 71 to 100 years, suggests that endocrine and metabolic systems must be maintained in a lower equilibrium to compensate the physiological reduction of the oxidant/antioxidant balance (low level of plasma glutathione).

Key words: IGF-I, DHEA-S, Hcy, Thioles, postmenopausal women, Burkina Faso, Africa

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INTRODUCTION

Biological aging implies a progressive decrease of endocrine and metabolic resources, which correspond to a reduction of working activity and caloric intake. However, an equilibrium between the residual endocrine and metabolic function guarantees what we could define the best living model of successful aging.

Somatomedin C (IGF-1) is the major member of a protein family synthesized by the liver in response to growth hormone stimuli and the main effector of the growth hormone activity (Merimee, 1979). The secretion of this hormone declines with age concomitantly with the fall of IGF-1 from 240 ng mL^{-1} at 30 years, 200 ng mL^{-1} at 40 years, up to 40 ng mL^{-1} at 80 years (Hertoghe, 1996; Bouillanne *et al*., 1996). The variation of the IGF-1 level is often accompanied by increase in atherosclerosis process and cardiovascular mortality (Bengt-Ake, 1996). IGF-1 is the major messenger molecule stimulated by growth hormone. Therefore the measurement of IGF-1 could be an indirect indicator of growth hormone reduction occurring during aging. The IGF-1 level also falls during low caloric intake which is accompanied by loss of both muscle and fat mass, while its increase is related to insulin secretion (Boonen *et al*., 1996). Since IGF-1 has been reported to be a mutagen for breast and prostate epithelial cells in older persons (Shi *et al*., 2004; Stattin *et al*., 2004), a relationship between cancer of these organs and blood IGF-1 level should not be excluded. Dehydroepiandrosterone sulphate (DHEA-S) is the sulphated metabolite of adrenal androgen and steroid hormones, which have received attention regarding aging and aging-related diseases (Parker, 1999; Barrou *et al*., 1997). DHEA-S blood concentration shows little or no diurnal variation, so its measurement corresponds to a valid measure of endocrine activity. Plasma DHEA-S level peaks by the second decade of life and then declines steadily by an average of about 10% per decade (Araghiniknam *et al*., 1996): after the age of 80 DEAH-S level drops up to 10-20% of the initial peak level. DHEA-S promotes the mitochondrial and cellular energy production via the effect on fatty acid metabolism.

Several beneficial effects have been attributed to DHEA-S such as anti obesity, anti cancer, anti oxidant, anti bone fragility (Labrie, 1998), enhancing muscle strength and immunological parameters (Casson *et al*., 1993). Recently it has been demonstrated that the positive effects of DHEA-S are mediated by the IGF-1 and, more intriguing, that DHEA-S replacement induces an increase of IGF-1 level in blood (Solerte *et al*., 1999). However we do not know whether the increased availability of IGF-1 in response to DHEA-S has a metabolic effect on anabolism or attenuates ongoing catabolism. The biological effects of IGF-1 and DHEA-S influence skeletal muscle mass and strength (Orentreich *et al*., 1984). Therefore, these two systems must cooperate in synchrony to prevent the metabolic unbalance responsible for vascular problems, cancer and other conditions which are contrary to successful aging.

Another important aspect of aging is the influence on methionine metabolism, which has in the Hcy the most important product. In fact, Hcy can be converted to glutathione through the transulfuration pathway and since glutathione is a potent scavenger of free radicals, it may be considered an important factor in biological aging (Wu *et al*., 2004). An increase of Hcy has been considered a new risk factor for coronary and other vascular diseases (Refsum *et al*., 1998; Neufeld, 1998; Prasad, 1999). This has been sustained by the evidence that a disrupted sulfur amino acid metabolism not only determines an increase of plasma Hcy, affecting the vascular wall structure through an oxidant damage mediated by LDL and superoxide production (Van der Griend *et al*., 2000) but that it also interferes with coagulation and fibrinolytic systems (Selhub and D'Angelo, 1998). However, the exact role of this sulfured amino acid in the old age is not clear since the plasma Hcy level has been found enormously elevated in European centenarians (Malaguarnera *et al*., 2004).

Consequently there is an increasing interest in considering endocrine and metabolic systems as focal points of old age, where anabolism and catabolism seem to converge. In fact, DHEA-S and IGF-1 deficiencies are associated with reduction of muscle and bone mass and consequently with reduced muscle strength and increased bone fragility (Martin, 2003), while hyperhomocysteinemia is an expression of altered methionine metabolism (Van der Griend *et al*., 2000) with effects on the cardiovascular system. There is a strict relationship among Hcy level and aging, which support the role of hyperhomocysteinemia as a marker of altered antioxidant mechanism. Moreover the connections between DHEA and IGF-1 hormones and other metabolic factors (glutathione, homocysteine, albumin) are mediated by nutritional status, age and antioxidant factors, together with creatinine, Cistatin C and vitamin status. Aim of this study is to measure DHEA-S, IGF-1 and products of methionine metabolism (Hcy and thioles) in a group of postmenopausal African women, compared with a group of young fertile woman.

MATERIALS AND METHODS

Inclusion and exclusion criteria: Four hundred and fifty postmenopausal African women, accommodated in the *Centre Delwende* di Tanghin (Ouagadougou, Africa), were submitted to clinical and laboratory examination in a period July-October, 2004. All examined subjects were

Fig. 1: Old African women spin during the day or clean the millet collected in the road

from Ouagadougou town or nearby villages and they kept alimentation habits proper of their home area: millet flour with vegetable sauce and cereals (maize, rice, sorghum), sheep meat rarely or no more than once a week and local seasonable fruits when possible. As a whole, their diet was poor, averaging about 1200-1400 calories per day. No vitamin supplement was given and they spent their time in the open areas near the *Centre Delwende*, performing simple works such as vegetable cultivation, wool spinning and maintaining the dormitory (Fig. 1).

Preliminarily, we controlled all the subjects determining weight and height, blood pressure in a seated position using a standard protocol. Other measures included heart beat rate (at the radial artery) and ventilation rate. The Body Mass Index (BMI) was calculated in all subjects with the formula weight/height^2.

Some of them refused to be visited, others were ruled out from the study because their weight exceeded the 97° percentile, systolic pressure was over 2 SD for the age in antihypertensive treatment, for clinical evidence of previous atherothrombotic cardiovascular disease with neurological implications, evidence of renal or hepatic diseases, thyroid disease cardiomyopathy under continuous therapy.

In the end, 76 African subjects, selected according the criteria of Euroage Seniour Protocol (Ligthart *et al*., 1984), were included in this study. They were subdivided in three groups: (i) 17 healthy postmenopausal adult women (age range 50-60 years); (ii) 42 healthy elderly women (age range 61-70 years and (iii) 17 additional healthy elderly women with age ranging between 71 and 100 years. Another group of 22 adult African fertile women (age range 30-45 years) were enrolled among the workers of the Centre Medical St Camille (CMSC) and served as a younger control group. All subjects included in this study gave a short demographic and medical history before undergoing a physical examination. In particular, all were mentally competent to give oral informed consent and the study was approved by the Ethic Committee of Centre Medical St Camille (CMSC) of Ouagadougou, Burkina Faso.

Collection, process and storage of blood samples: Blood samples were collected at the end of the interview. Ten milliliter of peripheral blood (5 mL in plain tubes and 5 mL in EDTA) were collected. EDTA-containing blood tubes were centrifuged at 1500 g for 10 min at 4°C, whilst tubes containing blood without additive were left to stand at room temperature for 30 min. Plasma and serum were then separated and stored at -80°(in 250 μL aliquots). The remaining leukocytes and packed red cells were stored at -80°C for DNA analysis. Plasma and serum samples were shipped to the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy, kept at -80°C on dry ice.

The Clinical Biochemistry Laboratory of Catholic University, Rome, Italy gave the Istitutional Review Board (IRB) approval for human subject's research before starting the measurements.

Routine hematological study: Clinical chemistry tests were performed at the Laboratory of Centre Medical St Camille of Ouagadougou, by using standard methods.

Other determinations: Folate, vitamin B6, vitamin B12 and Cistatin C were determined in plasma samples at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy.

Plasma Homocysteine measurement: The determination of circulating plasma Hcy and other thioles was performed by HPLC method (Araki and Sako, 1987) at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy.

IGF-1 and DHEA-S determination: IGF-1 was assayed by Chemiluminescence's IGF-1 Immunoassay (Nichols Advantage, San Juan Capistrano, CA, USA) on Nichols Liaison Advantage instrument, DHEA-S was assayed by Chemiluminescence's Immunoassay (Roche Diagnostics, Mannheim,Germany) by Modular Analytics E 170 (Roche Diagnostics, Mannheim,Germany).

Statistical methods: Data were presented as mean±standard deviation. Statistical comparison of differences were performed using paired and unpaired Student's T test or the Bonferroni test when appropriate, considering statistically significant $p<0.05$. All computations were made using SPSS-10 program for Windows.

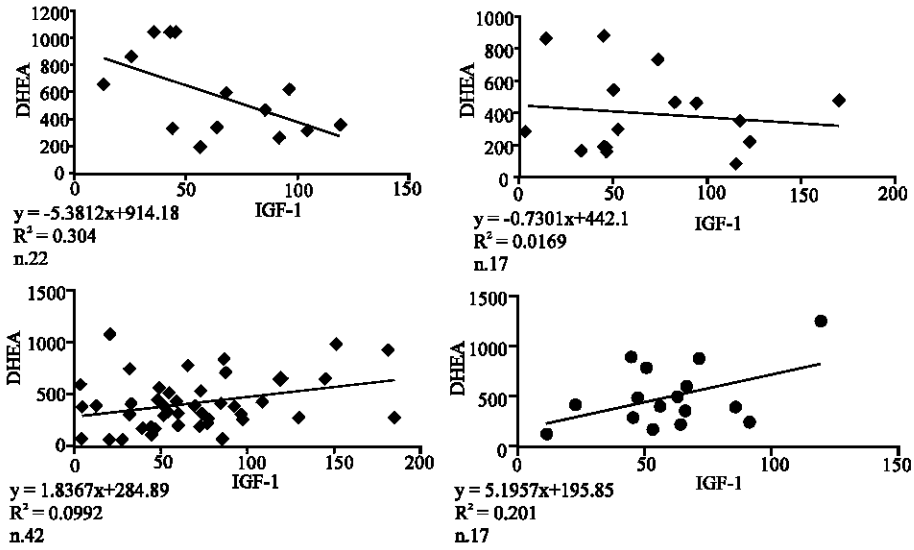

Fig. 2: Correlation between IGF-1 (ng mL^{-1}) and DHEA-S (ng mL^{-1}) in: (A) African adult women (30-45 years old), (B) African postmenopausal women (50-60 years old), (C) African postmenopausal women (61-70 years old), (D) African postmenopausal women (71-100 years old)

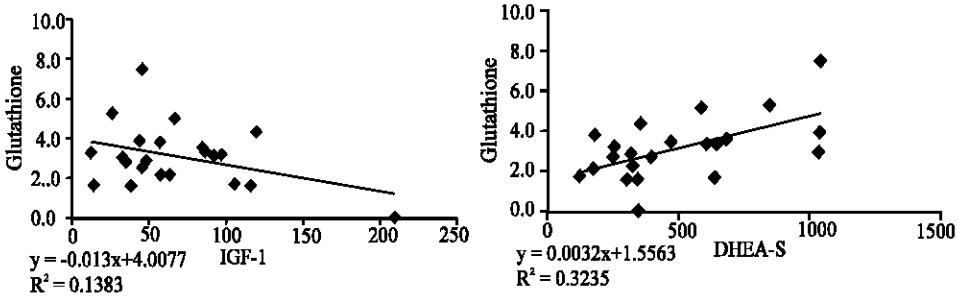

Fig. 3: Correlation between IGF-1 (ng mL^{-1}) and plasma glutathione (μMol L^{-1}) (A) and between DHEA-S (ng mL^{-1}) and plasma glutathione (μMol L^{-1}) in African postmenopausal women (50-60 years old)

Table 1: Clinical and laboratory parameters of African adult women and postmenopausal women

Parameters	African adult women (30-45 years old) (n. 22) A	African postmenopausal women (50-60 years old) (n. 17) B	African postmenopausal women (61-70 years old) (n. 42) C	African postmenopausal women (71-100 years old) (n. 17) D
Age (years)	35.9 (30-45)	53.4 (50-60)	64.2 (61-70)	74.5 (71-100)
BMI (kg m^{-2})	25.2±0.4	24.4±2.3	23.5±3.2	24.7±2.4
Hb (g/100 mL)	14.2±1.4	13.3±2.3	13.6±2.5	12.4±2.4
Blood glucose (mg/100 mL)	84.0±2.3	80.4±11.4 $	83.6±10.2	90.6±14.3
Blood nitrogen (mg/100 mL)	28.0±0.3 % ?	27.0±5.6 $	32.0±4.5	35.0±4.3
Serum cholesterol (mg/100 mL)	176±4.4	168.5±25.7	183.4±36.9	170.4±32.4
Serum triglycerides (mg/100 mL)	95.7±.0.4£ %	87.4±8.3 *	86.3±9.5 &	85.3±8.7
Serum creatinine (mg/100 mL)	0.72±0.11%	0.70±0.12 $	0.79±0.14	0.88±0.23
Cystatin C (mg L)	0.69± 0.08 £%	0.87±0.18	0.84±0.27	0.88±0.27
Serum iron (ug/100 mL)	83.0±2.3	75.0±19.4	78.0±20.4	69.0±15.6
Serum ASP transaminase (mU mL^{-1})	20.0±0.4 £ % ?	22.5±2.1$	23.3±3.2 &	25.4±2.6
Serum ALT transaminase (mU mL^{-1})	22.0±0.1	23.4±9.3	25.3±8.4	22.5±8.1
Serum folate (ng mL^{-1})	5.98±2.0	6.01±2.2	6.05±2.2	5.88±2.7
Serum Vit B6 (μm L^{-1})	6.6±.0.98	6.3±3.1	6.2±3.3	5.95±3.2
Serum Vit B12 (pg mL^{-1})	833.0±41.4	763.6±360.5	716.5±307.9	665.4±461.2
Systolic blood pressure (mm Hg)	132.0±4.2	138.1±20.1	142.7±23.3	139.0±24.1
Diastolic blood pressure (mm Hg)	82.0±3.4	79.4±13.0	80.3±16.1	79.0±11.8

Bonferroni Test ($p<0.05$) A vs B £; B vs C *; B vs D $; A vs D %; C vs D & A vs C ?

Table 2: Values of homocysteine and other thioles in African adult women and African postmenopausal women

Population	IGF-1 (ng mL^{-1})	DHEA (ng mL^{-1})	Homocysteine (μMol L^{-1})	Cysteine (μMol L^{-1})	Cysteinylglycine (μMol L^{-1})	Glutathione (μMol L^{-1})
African adult women n. 22 30-45 years (A)	160.5±24.0 £ ? %	850.3±263.0	6.88±1.33 ? %	96.25±13.0 £ ? %	37.12±10.0	7.32±1.75 £ ? %
African Postmenopausal women n. 17 >50<60 years (B)	69.1±31.8	572.2±310.5	11.0±5.1 * $	154.5±50.7 $	29.3±9.5	3.2±1.6
African postmenopausal women n. 42 >61<70 years (C)	63.5±31.8	504.2±281.8	18.2±5.7	163.0±38.5	29.3±9.6	3.5±2.0
African postmenopausal women n.17 >71<100 years (D)	59.4±24.4	391.5±250.1	20.2±9.1	191.3±43.9	31.9±8.5	4.5±3.2

Bonferroni Test ($p<0.05$) A vs B £; B vs C *; B vs D $; A vs D %; C vs D &; A vs C ?

RESULTS

Clinical and laboratory parameters of old African postmenopausal women enrolled in this study are reported in Table 1. The median systolic blood pressure was 140 mm Hg range 190-90 and median diastolic 80 range 120-50. The BMI of all women was normal for the local standard. The laboratory parameters did not differ substantially in the four groups with the exception of blood glucose, blood nitrogen, serum creatinine, Cystatine C and ASP transaminase, which were all significantly higher in the 71-100 year-old group ($p<0.05$) and serum triglycerides which were significantly higher ($p<0.05$) in the 30-45 year-old group. The determination of plasma DHEA-S in elderly women gave markedly low concentrations ($p<0.05$) and the same was observed for the IGF-1 concentration ($p<0.05$) (Table 2). The correlation between DHEA-S and IGF-1 was significant only in 71-100 years women ($r = 0.44$, $p<0.05$) (Fig. 2).

The levels of plasma Hcy and other thioles are reported in Table 2. There was a significant decrease of glutathione in all postmenopausal women (3.5±0.4 μMol L^{-1}), while the plasma Hcy was markedly elevated (18.7±0.5 μMol L^{-1}). Plasma cysteine level was 168.7±43.7 μMol L^{-1} and plasma cysteinylglycine 30.4±0.5 μMol L^{-1}. A significant correlation was found between plasma Hcy and plasma cysteine ($r = 0.52$, $p>0.0005$) indicating an efficient trans sulfuration and between plasma cysteine and plasma steinylglycine ($r = 0.61$, $p<0.0005$). The correlation between plasma glutathione and plasma cysteinylglycine was less significant. Hcy was elevated whilst folic acid, Vit B12 and Vit B 6 levels were found in the normal range and the normal renal function was guaranteed by creatinine and Cistatin C values in the normal range for the age. Significant positive correlation was found between DHEA-S and plasma glutathione ($r = 0.56$, $p<0.025$) and negative but not significant ($r = -0.36$, p = not significant) between IGF-1 and plasma glutathione only in the 50-60 years group (Fig. 3).

DISCUSSION

The study of IGF-1 levels in postmenopausal African women has demonstrated that this hormone is maintained at lower level with respect to the European women of the same age (Pinzani *et al*., 2002; Paolisso *et al*., 1997), where low levels of IGF-1 correlate with plasma leptin and lipid concentrations, insulin secretion and cognitive function during old age. Moreover, in European women a reduction of the anabolic processes mediated by IGF-I may account for the slow and progressive loss of bone mass that takes place inexorably after the age of 40-50 years. In this population the nutritional caloric or protein deficit may add to the effects of GH, age and other factors in decreasing IGF-I synthesis and therefore further contribute to the development of primary osteoporosis (Calo *et al*., 2000). However the IGF-1 reduction in African postmenopausal women is not associated with increased bone fragility, since the bone density peak in African people is reached early with respect to the European population, due to the their particular life style, nutrition physical activity and to genetic factors (Opotowsky *et al*., 2003). It is also known that a low level of IGF-1 protects from the appearance of breast cancer in female and of prostate cancer in males, being both important determinants for a long life (Pollak, 2000). Moreover high levels of IGF-1 in the follicular phase significantly correlated with breast cancer in pre menopausal women (Jernstrom *et al*., 1997), suggesting

that *in vitro* IGF-1 is a powerful stimulator and it is expressed in high levels by cancer cells (Sugumar *et al*., 2004). Therefore, there is no doubt that in Western countries every hormonal supplement during menopausal period involving growth hormone stimulation must be performed with caution and carefully monitored, with IGF-1, estradiol and estrone measurements and perhaps mammography (Stoll, 1999).

In our African postmenopausal women, DHEA-S hormone concentration was also found to be at lower level, which is expression of a progressive reduction of endocrine resources characteristic of old age (Labrie *et al*., 1997). The reason for the age related decline of DHEA-S and how the sex may affect this reduction are unclear. In women it may be related to menopausal status and to declining adrenal function with age (Hornsby *et al*., 1984, 1987). If recent reports demonstrated that DHEA-S has an anti obesity, anticancer, antioxidant effect, a DHEA-S supplement, inducing a consistent changes in IGF-1 levels, do not seem to be advantageous for women (Willliams, 2000). The present seems to demonstrate that an equilibrium between these two components of the endocrine system (IGF-1 and DHEA-S) is essential when the subjects are candidates to live longer (Fig. 2). In fact, among factors determining a low level of IGF-1 and DHEA-S in human, the hypocaloric alimentation must be considered firstly, as well as the physical activity, the absence of psychophysics stress, which are a characteristic of lifestyle in our African postmenopausal women (Tissandier *et al*., 2001). In such life conditions African postmenopausal women showed metabolic parameters such as glucose, urea nitrogen, cholesterol total, HDL and LDL within the normal range as well as their renal function, documented by creatinine and Cistatin C values. Thus, the low levels of DHEA-S and IGF-1, found in our postmenopausal women, seem to be a characteristic of old age. These two hormones decrease synchronously with the reduction of oxidant/antioxidant systems, typical of old age, as demonstrated by the reduced level of plasma glutathione, constantly found in our postmenopausal women. In fact the levels of glutathione, lower than in the control group, show that an elevated consumption of antioxidant substances is characteristic of old age, while the levels of cysteinylglycine, a product of the glutathione degradation, did not show significant variation. The positive correlation between DHEA-S and glutathione in the younger (50-60 years) group demonstrates that a reduction of antioxidant status is synchronous with a constitutive decline of endocrine activity reaching a stable equilibrium when DHEA-S and IGF-1 become together low. The existence of a strict correlation between DHEA-S and glutathione is clearly demonstrated in polycystic ovary syndrome where low levels of DHEA-S are associated with an elevated risk for cardiovascular diseases, hypertension, hyperlipemia and insulin resistance (Cattrall and Healy, 2004). On the contrary IGF-1 influence negatively the expression of antioxidative molecules through key components of systems that counter the oxidative stress. The reductions in GH and IGF-1 signalling contribute to extended life spans in a variety of species and may be partially explained by an increased antioxidant ability which neutralizes deleterious products of metabolism (Brown-Borg and Rakoczy, 2003; Brown-Brog *et al*., 2004). The correlation between IGF-1 and DEHA was negative in the control African group (aged 31-50 years) because this group must be considered etherogeneous, since it includes woman with different life expectance. On the contrary this correlation was positive in oldest African woman (age 71-100), where DHEA and IGF-1 are both low balancing the pro-oxidant effect of DHEA decline.

As a direct consequence of antioxidant consumption (low glutathione) the plasma Hcy values were found higher in our old African postmenopausal than in the younger women, but lower than that found in European old people of the same age (Malaguarnera *et al*., 2004). This observation is not surprising since in another study on the Hcy level in African population living in Burkina Faso we demonstrated that the levels of Hcy are lower in adult Africans which corresponds to a reduced incidence of vascular pathologies in Burkina Faso (Simpore *et al*., 2000). The lower levels of Hcy in African populations with respect to European populations could be a consequence of a continuous selection operated by *Plasmodium falciparum* malaria in this population (Chillemi *et al*., 2004). In fact the lower Hcy values obtained in African postmenopausal women are in accordance with the lower frequency of C677T mutated alleles in Burkina Faso (Sadewa *et al*., 2002; Botto and Yang, 2000; Amouzou *et al*., 2004), which represent a disadvantage for the population in regions where malaria is endemic (Chillemi *et al*., 2004).

In African as well as in European old postmenopausal women, the elevated level of Hcy does not represent a risk factor for cardiovascular diseases, since the role of higher Hcy in the pathogenesis of vascular damage in old people appeared reduced when endocrine and metabolic systems involved in growth and development process place at minimum

demand (Spotila *et al*., 2003; Chillemi *et al*., 2005). In our study clearly a negative correlation was demonstrated in all groups of age between Hcy, DHEA and IGF-1 supporting the importance of endocrine and metabolic system in the oxidant/antioxidant balance (data not shown). However the increased levels of Hcy in our African postmenopausal women appear prevalently due to reduced availability of glutathione and consequently of NADHP, since normal levels of folate, Vit B 12 and Vit B6 exclude the interference of vitamin deficiency in the pathogenesis of hyper Hcy (Chillemi *et al*., 2005). These considerations correlate perfectly with the reduced level of IGF-1 and DHEA-S typical of old age, when African postmenopausal women are oriented to a longer survival. Moreover the lower level of Hcy in our African postmenopausal women compared to Europeans of the same age, due to genetic or environmental factors adds another point of advantage for the successful aging. In fact, Mutus *et al*. (2000) studying the platelet resistance to a NO-inhibition induced by Hcy in healthy European centenarians, demonstrated the existence of a lower degree of oxidative stress (Paolisso *et al*., 1998) against higher Hcy levels found in old Europeans. In conclusion, the low level of IGF-1 and DHEA-S suggests that in oldest age populations (71-100 years), the endocrine and metabolic systems must be maintained in equilibrium to compensate the physiological reduction of oxidant/ antioxidant balance, if they are candidate to a long life.

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