Hormonal determinants and effect of ER22/23EK glucocorticoid receptor gene polymorphism on health status deterioration in the participants of the Mataró Ageing Study

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Received: 27 December 2010 / Accepted: 17 April 2011 / Published online: 5 May 2011
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Abstract The purpose of this study is to assess the potential relationships of circulating IGF-I, adrenal and gonadal steroids, and polymorphism ER22/23EK of the glucocorticoid receptor (GC-R) gene with nutritional, functional and cognitive deterioration in a group of elderly people living independently. This is a population-based prospective study with 313 individuals (160 women and 153 men, 76.7 ± 7 years) who participated. A physical exam, evaluation of functional capacity (Barthel scale), cognitive function (minimal state examination-MMSE), geriatric depression scale (GDS), mininutritional assessment (MNA-SF) and cardiometabolic status were performed at basal time point and at 2 years of follow-up. Biological measurements included cortisol, dehydroepiandrosterone (DHEA), DHEA sulphate, testosterone, estradiol, IGF-I and polymorphism ER22/23EK of the GC-R gene.

Estradiol was associated with MNA-SF decrease over time ($p < 0.01$, adjusted for age and gender, $\beta = -0.17$, $p = 0.03$). Weight loss was related to testosterone in men (8.6 vs 12.1 pg/ml in no losers; $p = 0.03$), and in women with GDS (13.0% with depression vs 3.3% with no depression; $p = 0.05$) and MMSE (22.2% with cognitive deterioration vs 4.8% with no cognitive deterioration; $p = 0.049$). Barthel decrease was associated with testosterone ($p = 0.02$, after adjusting for age and gender, $\beta = -0.520$, $p < 0.001$), and SHBG ($p < 0.01$, adjusted for age and gender, $\beta = 0.18$, $p < 0.01$). DHEA was associated with deterioration in the MMSE ($p = 0.01$, after adjusting for age, gender, GDS scale and academic status, $\beta = -0.26$, $p = 0.01$). Frailty development was related only in men with testosterone levels at the beginning of the study ($p = 0.017$). ER22/23EK was found in 3% of the subjects and carriers had

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a lower prevalence of hypertension. Adrenal and gonadal steroids are associated to impairment of the ageing health condition in elderly individuals living independently in Spain. ER22/23EK polymorphism of the GC-R gene has a low prevalence in our population.

Keywords Ageing · Steroidal hormones · Frailty · Functional capacity · Nutritional status · Cognitive function · Depression

Introduction

Among healthy elderly individuals there is a considerable variation in the effects of age on physiological functions, with some people showing an important and accelerated decline whilst others exhibit a high resistance to this process. The continuous decline of certain hormonal systems throughout the lifespan has been related to the loss of function that characterizes the ageing process; in particular, diminution of adrenal steroids, specifically dehydroepiandrosterone (DHEA) and its sulphate (DHEAs)/cortisol ratios, circulating sexual steroids and GH, have been claimed to be associated with poor cognitive status, depression and decreased functional performance in elderly people (Wolf 2003; Barrett-Connor et al. 1999a; Cappola et al. 2001). However, heterogeneous results have been reported by different studies (De Bruin et al. 2002; Janssen et al. 1998) leading difficult understanding of the ageing process and its associated diseases in relation to the endocrine system. Furthermore, the associated diseases of the elderly and their treatments do not facilitate how to define the frontier between physiology and pathophysiology whilst ageing, and can themselves influence hormonal status (Lamberts et al. 1997). Moreover, normative hormonal data in elderly people are just beginning to become available (Mohr et al. 2005) and the diagnosis and treatment of certain endocrine defects in aged people are still a matter of debate, i.e. late-onset male hypogonadism (Nieschlag et al. 2005). Also, some gene polymorphisms involving hormonal action, such as the ER22/23EK (rs6189 and rs6190) of the glucocorticoid receptor (GC-R) gene have been linked to a particular protective situation in elders, related to a less pronounced cortisol effect on target tissues (Van

Rossum and Lamberts 2004; Van Rossum et al. 2004; Van Rossum et al. 2005).

This paper reports the results of the Mataró Ageing Study in which a cohort of 313 old people have been enrolled and followed for 2 years. Investigations were carried out to clarify the potential relationship between sexual, adrenal and somatotrophic hormones and ER22/23EK polymorphic variant of the GC-R gene and the development of impaired health condition in this cohort.

Subjects and methods

Individuals of both sexes, aged more than 70 years, living in the neighbourhood of Cirera-Molins in Mataró and the nearby village of Argentona, north of Barcelona, were invited to participate in this longitudinal study aiming to identify factors influencing a frail or robust condition whilst ageing. The recruitment phase was performed between May 2002 and June 2003, and participants were re-evaluated in 2005. The initial cohort included 313 individuals (160 women and 153 men; mean age was 76.7 ± 7 years for the total group, with no differences between sexes) and was evaluated at the basal time point and after a follow-up of 2 years. The general characteristics of the participants have been described elsewhere (Puig-Domingo et al. 2008). Cardiovascular risk factors were highly prevalent in both women and men and most of them were taking medication for these conditions (Puig-Domingo et al. 2008); however, none of these treatments influenced the relationships described in this paper. The study was approved by the Ethics Committee of the Hospital de Mataró.

Measurements

Hormonal measurements

Blood samples were drawn in the morning after overnight fasting. Hormonal measurements were performed by commercial validated kits with low CVs, and included: free testosterone (men, 9–41 pg/ml; women, 0.2–3.2 pg/ml. pg/ml; Immunotech, Marseille Cedex, France), estrone (men, 30–90 pg/ml; women, 20–40 pg/ml; DSL, Webster, Texas, USA), dehydroepian-

drosterone (DHEA; men, 1.51–9.0 ng/ml; women, 0.7–2.1 ng/ml; Immunotech, Marseille Cedex, France) and DHEA sulphate (DHEAs; men, 50–560 mcg/dl; women, 35–430 mcg/dl; Immunotech, Marseille Cedex, France), IGF-1 (men, 49–250 ng/ml; women, 49–250 ng/ml; Nichols Institute, San Clemente, CA, USA); estradiol (pg/ml), LH (men, 2.0–12.0 mUI/ml; women, 10.0–62.0 mUI/ml) and FSH (men, 2.0–12.0 mUI/ml; women, 5.0–60.0 mUI/ml; Advia Centaur, Bayer Diagnostics, Spain), cortisol (5.0–25.0 mcg/dl) and GH (0.1–5 ng/ml; Immulite 2000, Dipeasa, Spain).

ER22/23EK polymorphism of the glucocorticoid receptor gene

Genomic DNA was extracted from blood samples as the serum for the hormonal studies according to standard procedures; sample availability for genotyping was possible in 309 out of 313 individuals. Genotypes were determined using the Taqman allelic discrimination assay. The Assay-by-Design service (<http://www.appliedbio-systems.com>) was used to set up a Taqman allelic discrimination assay for ER22/23EK. Primer and probe sequences are available on request. To confirm the accuracy of genotyping results, 80 randomly selected samples were re-genotyped using the same method and showed similar results. The polymerase chain reaction (PCR) mixture included 20 ng of genomic DNA in a 25 µl volume and the following reagents: FAM and VIC probes (250 nM), primers (900 nM), 2× Taqman PCR master mix (ABgene). PCR cycling reactions were performed in 96-well PCR plates in an ABI 7900 PCR system (Applied Biosystems Inc) and consisted of initial denaturation for 15 min at 95°C, 40 cycles with denaturation for 15 s at 95°C, and annealing and extension for 60 s at 60°C. The results were analyzed using the sequence detection system 2.3 software (Applied Biosystems Inc.).

Functional capacity and nutritional status

Functional capacity was assessed by calculation of Barthel (Mahoney and Barthel 1965) and Lawton scores (Lawton and Brody 1969). Nutritional status evaluated by performing the reduced form of Mini Nutritional Assessment test (MNA-SF; Rubenstein et al. 2001).

Mental status and depression

Mental status was measured by the Mini Mental State Examination (MMSE; Folstein et al. 1975) and depressive status by performing the Geriatric Depression Scale (GDS; Hoyle et al. 1999).

Evaluation of frailty

Frailty was evaluated by using Fried criteria (Fried et al. 2001).

Metabolic syndrome (MS) definition

The International Diabetes Federation (IDF) definition of metabolic syndrome (MS; Alberti et al. 2006) was used, in which individuals were classified as having MS if the waist perimeter was >94 cm in men or >80 cm in women plus two or more of the following: (1) arterial blood pressure >130/85 mmHg or antihypertensive treatment, (2) triglycerides >150 mg/dl or use of treatment for hypertriglyceridemia, (3) high density lipoprotein (HDL) ≤40 mg/dl in men or ≤50 mg/dl in women or (4) fasting glucose ≥100 mg/dl or diabetes.

Data analysis

Statistical descriptive analyses were performed expressing categorical data as percentages and continuous data as means and standard deviations. Analyses were performed for the whole cohort or separately for men and women when required. To compare proportions by gender or other categorical variables a chi square test or a Fisher's exact test was used. The correlations between hormone concentrations, mental and depressive status, and functional capacity measurements were done using the Spearman correlation coefficient (r_s). For further study of the relationship between these variables, an initial univariate analysis was carried out by using a linear regression. A multivariate regression analysis was additionally performed to adjust the effects of the variables showing an association in the univariate analysis with mental status, depressive state, MNA-SF or functional capacity with a p value <0.20. Statistical significance was considered when the p value was <0.05.

Results

Phenotype description and hormones concentrations at basal time point

Descriptive data of the physical characteristics, as well as age, BMI and abdominal perimeter, MMSE, MNA-SF, GDS, Barthel and Lawton scores are shown in Table 1. Globally, men performed better than women in most of the parameters evaluated indicating a much better ageing condition in men. Metabolic syndrome according to IDF criteria was present in 57.9% of the cohort (54.9% in men and 61% in women). Both males and females showed a high prevalence of obesity, as mean BMI was above normal in either sex, and a prevalence of 30% of obesity was found for the total sample. This tendency to obesity was paralleled by elevated abdominal perimeter, with no differences in absolute values in relation to gender. Only 3/110 obese individuals showed obesity with a healthy metabolic profile (no hypertension, no hypertriglyceridemia, no low HDL, no abnormal glucose values). Twenty-four subjects died during the follow-up period of 2 years, 14 (9.2%) men and 10 (6.2%) women.

In women, DHEA and DHEAs showed a decrease with age ($r_s = -0.17$, $p = 0.04$ and $r_s = -0.16$, $p < 0.05$, respectively), while testosterone, estradiol and estrone, IGF-I, IGFBP-3 and IGF-I/IGFBP3 ratio did not show changes in the age range studied (71–102 year old). In men, a decrease with age was only observed in testosterone ($r_s = -0.19$, $p = 0.02$) with no changes in the rest of hormones.

Estrogens were higher in men than in women, either estradiol (48.7 ± 14.7 pg/mL vs 32.1 ± 12.7 pg/mL, $p < 0.05$) and estrone (42.9 ± 21.4 pg/mL vs $27.9 \pm$

16.1 pg/mL, $p = 0.049$). Testosterone showed a positive correlation with estradiol in men but not in women ($\beta = 0.33$, $p < 0.001$) and IGF-I did correlate with IGFBP-3 in both genders.

Relationships of hormones with cognition, depressive status, functional capacity and nutritional assessment at basal time point

No relationship was observed in men with any of the steroids and other hormones studied with cognition and mood at basal time point, while cognitive function in women showed a negative correlation with DHEA ($\beta = -0.79$, $p = 0.03$), cortisol ($\beta = -0.27$; $p < 0.01$) and a positive correlation with IGF-I ($\beta = 0.03$, $p = 0.04$), as well as higher education ($\beta = 3.40$, $p < 0.001$) after adjustment for depressive status (GDS score); higher education also correlated with cognitive function in men. These results in women were also observed when the memory items of the MMSE were separately analyzed, with persistence of the negative correlation of DHEA, although the positive correlation of IGF-I and the negative correlation of cortisol lost statistical significance. When a multivariate logistic regression analysis was performed the higher education showed the maximal protective effect ($OR = 6.25$, $p < 0.001$) for preserved cognitive status in both genders; in women, OR for deteriorated cognition for age, DHEA and cortisol were 1.14 ($p < 0.001$), 1.57 ($p = 0.02$) and 1.09 ($p = 0.02$), respectively. No correlations were observed between DHEAs and any of the variables evaluated. Furthermore, no association with depression and any of the other hormones studied were found in either men or women.

The results of the multivariate analysis showed that Barthel was related to age ($\beta = -0.18$, $p < 0.01$), gender ($\beta = -0.26$, $p = 0.04$) and IGF-I ($\beta = 0.12$,

Table 1 Descriptive data of the study sample

	Men ($n = 153$)	Women ($n = 160$)	p value
Age	76.7 ± 5.4	77.3 ± 6.4	n.s.
BMI	27.2 ± 3.7	29.2 ± 4.8	< 0.001
Abdomen perimeter	101.8 ± 11.4	101.5 ± 11.1	n.s.
MMSE	30.0 ± 4.9	27.8 ± 6.5	0.002
Lawton	5.5 ± 2.0	6.9 ± 1.8	< 0.001
Barthel	97.4 ± 6.0	94.8 ± 9.2	0.005
Depression score	29/138 (20%)	71/146 (48.6%)	< 0.001
MNA-SF	14.1 ± 1.4	13.4 ± 2.0	0.001

ns $p \geq 0.05$, BMI body mass index, MMSE mini-mental state examination, MNA-SF, Mini Nutritional Assessment

$p=0.04$) with no significant influence of steroid hormones; MNA-SF was associated to GDS ($\beta=-0.22$, $p<0.001$), cortisol ($\beta=-0.18$, $p<0.01$) and DHEA ($\beta=0.15$, $p=0.03$); MMSE was related to age ($\beta=-0.24$, $p<0.001$), academic status ($\beta=0.42$, $p<0.001$) and DHEA ($\beta=-0.16$, $p<0.01$); GDS was associated to gender ($\beta=0.26$, $p=0.03$), estradiol ($\beta=0.15$, $p=0.04$) and DHEAs ($\beta=-0.14$ and $p=0.06$).

Evolution of MNA, MMSE, functional capacity and GDS

According to the MNA, only 1.4% of men and 6.2% of women were at risk of malnutrition ($p=0.06$) at basal time point. During the follow-up period, 13% of men and 20% of women showed a $>5\%$ weight loss, and nutritional status according to MNA-SF categories deteriorated in 18% of men and 39% of women. In both men and women, the reduction of the MNA-SF score during the follow-up period was statistically significant with a decrease of 1.53 points in men and 1.72 points in women. In men, $>5\%$ weight loss was related with age (9.6% in 70- to 80-year-old group vs 22.9% in the >80 -year-old group; $p=0.06$) but not with any of the co-morbidities considered except possible depression ($\text{GDS}\geq 2$; 10.0% with no depression vs 26.9% with possible depression; $p=0.03$). Women showed a more pronounced decline in nutritional status over the 2-year follow-up period in comparison to men. In women, $>5\%$ weight loss was not related with any of the variables considered and $>10\%$ weight loss was related with GDS (13.0% if possible depression vs 3.3% if no depression; $p=0.05$) and MMSE (22.2% if cognitive deterioration vs 4.8% if no cognitive deterioration; $p=0.049$).

According to MMSE, at basal time point, 22 women (15.3%) and 10 men (7.3%) had moderate impairment in cognitive status, 14 women (9.7%) and 9 men (6.6%) had mild impairment and the rest had a normal cognitive function (108 women, 75%; 118 men, 86%). MMSE results showed a negative correlation with age ($r_s=-0.27$, $p<0.001$). At 2-years follow-up in 235 out of 285 who were still alive, 9 women (7.3%) and 4 men (3.6%) showed severe impairment in cognitive status, 12 women (9.7%) and 4 men (3.6%) moderate impairment, 16 women (12.9%) and 8 men (7.2%) mild impairment and the rest had normal cognitive function (87 women, 70.2%; 95 men, 85.6%).

A notable difference in depressive status was found according to gender after GDS evaluation, as 71 out of 146 women showed depressive scores (48.6%) in comparison to 29 out of 138 men (21%); there were no changes in GDS scores with older ages. At 2-years follow-up, GDS scores showed 47 out of 124 women (37.4%) in comparison 13 out of 111 men (11.7%).

Barthel score was optimal in 172 out of 300 individuals (57.3%), 66 out of 152 women (43.4%) and 106 out of 148 men (71.6%). Physical activity, measured as outdoor self-reported walking hours per day, was 0.87 h/day in women and 1.56 h/day in men (median, $p<0.001$). During the follow-up period, the Barthel score improved in 8.8%, did not change in 63.4% and worsened in 27.8% of participants (25.2% worsened from 5 to 19 points and 2.6% worsened 20 points or more). The decline of Barthel score was correlated with MNA-SF ($r=-0.18$; $p<0.01$) in the whole cohort and was also related to age, as individuals with a Barthel loss $>10\%$ had a median age of 80.3 years and used to walk outdoors 0.64 h a day in comparison to 76.3 years old and 1.21 h a day in $<10\%$ Barthel loss ($p<0.01$ and $p=0.02$, respectively).

Relationships between hormone levels and changes in nutritional status, functional capacity, MMSE and GDS at 2-years follow-up

When we evaluated the association between the hormonal profile at basal time point and the changes observed at follow-up in MNA-SF, Barthel, GDS and MMSE scales, a significant association was found with estradiol and the difference in MNA-SF value for the whole cohort ($p<0.01$, after adjusting for age and gender, $\beta=-0.17$, $p=0.03$). Men with $>5\%$ weight loss had lower median basal testosterone (8.57 pg/ml vs 12.14 pg/ml in no losers; $p=0.03$). In women, weight loss was not related to basal hormonal levels. The change in Barthel scale was significantly associated with testosterone ($p=0.02$, after adjusting for age and gender, $\beta=-0.52$, $p<0.001$), and SHBG ($p<0.01$, after adjusting for age and gender, $\beta=0.18$, $p<0.01$). DHEA was associated with the change in MMSE ($p=0.01$, after adjusting for age, gender, GDS scale and academic status, $\beta=-0.26$, $p=0.01$). Results are shown in Table 2.

Table 2 Relationships between hormone levels and changes in nutritional status, functional capacity, MMSE and GDS at 2-years follow-up

Whole cohort			Men		Women	
			Crude analysis	After adjusting	Crude analysis	After adjusting
Difference in MNA	17Beta-estradiol	$p=0.009$	$\beta=-0.173$; $p=0.031^a$	–	$p=0.013$	$\beta=-0.229$; $p=0.018^b$
Difference in Barthel	Testosterone	$p=0.021$	$\beta=-0.520$; $p=0.000^a$	$\beta=-0.308$; $p=0.002^b$	ns	–
	SHBG	$p=0.002$	$\beta=0.185$; $p=0.007^a$	$\beta=0.208$; $p=0.037^b$	$p=0.022$	$\beta=0.190$; $p=0.042^b$
Difference in MMSE	Testosterone	ns	–	$\beta=-0.238$; $p=0.028^c$	ns	–
	DHEA	$p=0.014$	$\beta=-0.257$; $p=0.011^d$	–	$p=0.009$	$\beta=-0.258$; $p=0.011^c$

MNA Mini Nutritional Assessment, MMSE mini-mental state examination.

^a After adjusting for age and gender.

^b After adjusting for age.

^c After adjusting for age, GDS scale and academic status.

^d After adjusting for age, gender, GDS scale and academic status.

Frailty at basal time and follow-up and its relationship to hormone levels

According to Fried criteria, 4.6% of individuals (14 out of 304; 4/151 men and 10/153 women) were frail at basal time point. Frailty was associated to IGF-I levels (96.3 ± 14.9 mcg/dl in frail individuals vs 103.3 ± 36.9 mcg/dl in non-frail individuals, $p=0.02$; after adjusting for age and gender $p=0.45$) and a trend between frailty and DHEAs was also found (28.8 ± 26.3 mcg/dl in frail individuals vs 49.4 ± 41.8 mcg/dl in non-frail individuals, $p=0.09$, after adjusting for age and gender $p=0.29$).

At 2-years follow-up, 31.4% of individuals were frail (83 out of 264 in which follow-up was possible and frailty criteria were available; 26/131 men and 57/133 women) and 77 out of 258 (29.8%) became frail at 2-years follow-up. The change from non-frail to frail was associated to lower levels of testosterone at the beginning of the study ($p<0.001$), estradiol ($p<0.01$), IGF-I ($p=0.09$) and SHBG ($p=0.06$). When categorizing by gender, a significant association with frailty development was observed only in men with testosterone levels at the beginning of the study (10.7 ± 4.3 vs 13.0 ± 4.2 pg/ml, $p=0.02$; which remained significant after adjusting for age, $p=0.02$). A trend was also observed in men for DHEA and estradiol ($p=0.08$ for both). In women, no associations were found with hormone levels and frailty development.

ER22/23EK polymorphism of the GC-R gene and cardiometabolic status

The ER22/23EK polymorphism of the GC-R gene was found in 8/309 (2.5%) of the total population (four women and four men). No differences in anthropometric variables, including waist circumference and lipid profile were found in relation to this polymorphism; abnormal glycaemia was found in 51.9% of non-carriers vs 42.9% of carriers ($p=0.71$); a blood pressure $>130/85$ was found in 88% of non-carriers vs 62.5% of carriers ($p=0.05$); metabolic syndrome (IDF criteria) was found in 58% of non-carriers and in 28.6% of carriers ($p=0.14$). Barthel and MNA scores were not different among carriers and non-carriers; in the heterozygous carriers of ER22/23EK, cortisol was lower (15.4 ± 4.9 vs 18.4 ± 6.8 g/dl) as well as DHEAs (28.1 ± 15.0 vs 48.4 ± 23.0 g/dl), although without reaching statistical

significance ($p=0.20$ and 0.17 , respectively). Carriers of this polymorphism showed similar levels of cognitive status and mood as non-carriers in our cohort. No changes at 2 year of follow-up were found in relation to this polymorphism with the changes in functional capacity, MNA, MMSE and GDS.

Discussion

In this population of independently living old men and women, we found an expected decline in global mental function and certain hormones with age. IGF-I did not show changes in the range of age of our cohort, probably indicating that no further decline in the circulating levels of IGF-I may be expected to happen over 75 years of age when a sample of population is studied, although on an individual basis some decline may be possible. A considerable percentage of women, about half of the sample in our cohort—the double of men—showed a depressive mood score with no remarkable changes in relation to age. Also, a similar situation was seen in relation to cognition with more women showing cognitive dysfunction in either category explored (global and memory items of the MMSE) in comparison to men. The endocrine system may influence the dynamics of ageing, and reciprocally, changes in the synthesis and metabolism of different families of hormones are determined and timed by age (Lamberts et al. 1997). In our study, it was also shown that estrogens exposure is higher in old men than in old women, because of the continuous conversion of testosterone to estrogens takes place in peripheral tissues, explaining why testosterone and estradiol depicted a high positive correlation in men. Therefore, overall, men have a more prolonged gonadal hormonal exposure during life than women. This point may have implications for physical, mental and mood performance when comparisons between genders are considered, and mostly in terms of frailty development or robust condition during ageing. Taken overall, our results seem to indicate a protective effect of gonadal steroids in both genders in relation to nutritional, functional, cognitive and mood which decline over time. Individuals with higher IGF-I also performed better than those in the lower range. DHEA and its sulphate remains the main gonadal prohormone in women, with the potential of being converted subse-

quently to either androgens or estrogens, but always at a considerably lower absolute value than those observed in men. DHEA has been related to mood status in women of the Rancho Bernardo Study (Barrett-Connor et al. 1999b; Barrett-Connor et al. 1999c) and an interesting debate has been running over the last decade in relation to the possible beneficial effects of DHEA supplementation in old women with the aim of achieving circulating levels in the range of younger women. Up to now, no consistent results have been obtained that justify long-term supplementation with this steroid in the general population of old women (Gurnell and Chatterjee 2001; Nair et al. 2006) and it is currently just recommended as substitution treatment for young women with primary adrenal insufficiency, where an improvement in self esteem, mood and general well-being have been described using this treatment (Hunt et al. 2000). Despite this lack of a formal recommendation, DHEA is available and used in some countries without medical prescription. Our results seem to indicate that DHEA is rather protective for the whole cohort, and particularly in women, as at a 2-year follow-up, women with a lower DHEA had a more global cognitive impairment and also a lower memory performance. Other studies have also tried to evaluate the relationship between adrenal steroids and cognition in women, and in some of them overexposure to cortisol has been associated to cognitive impairment (Lupien et al. 1997; Seeman et al. 1994), and in others, i.e. Kalmijn et al. (Kalmijn et al. 1998), a relationship with mental impairment was found with the cortisol/DHEAs ratio rather than with cortisol or DHEAs individually, by virtue of which a higher ratio was associated to an impaired cognitive performance. DHEA itself has been proposed as a neuroprotective steroid, but most of the information has been obtained in experimental models in which DHEA seems to act as a natural counteractive factor of cortisol, the latter being believed to have increasing neurotoxic actions when DHEA is decreasing as a consequence of ageing at the hippocampus and other important sites of the central nervous system (Bologa et al. 1987; Sapolsky et al. 1990). We did not find any relationship between cortisol/DHEA ratio and cognitive or memory performance in our cohort of women. More than cortisol, or the cortisol/DHEAs ratio, a relevant factor may be the individual sensitivity or resistance to circulating cortisol according to some polymor-

phisms of the glucocorticoid receptor gene, as previously described in the cohort of the Rotterdam study (Van Rossum and Lamberts 2004; Van Rossum et al. 2004; Van Rossum et al. 2005). In our study, due to the an unexpected low prevalence of ER22/23EK polymorphism of the GC-R gene in comparison to the Rotterdam study (2% vs 8%), we were underpowered to show any relationship with certain variables, although our carriers had less frequency of hypertension, agreeing with previous publications of the Rotterdam study.

Also remarkable in our study is that testosterone showed a cognitive protective effect in men, thus indicating that hormonal status was important in our cohort for development of frailty, as MNA and weight loss were also associated to testosterone. The most prominent factor associated to a good cognitive status were age itself and higher education, the latter having a greater influence than any other biological factors considered. Therefore, although the hormonal changes seen in ageing may be of some importance, non-hormonal factors are important driving factors determining the cognitive status of old persons.

Somatotropic axis activity was also weakly associated to better cognitive scores in women, a fact that has also been reported in other studies in men (Aleman et al. 1999); this may be due to some neuroprotective actions of GH and IGF-I. In old men, substitutive treatment with GH and/or testosterone has shown contradictory results in mood changes (Barrett-Connor et al. 1999a; Tenoer 1992; Wang et al. 1996; Brill et al. 2002). In our cohort, men seemed to be overall exposed to more factors associated to better psychophysical condition, with a better academic background, more hours per day of exercise, and higher levels of somatotrophic axis activity and sexual hormones but significant statistical associations were not found.

We did not find any relationship between depressive status and adrenal or sexual steroids in men and women when analyzed separately; although double the number of women in relation to men had depressive scores and all gonadal hormones were much higher in men, it is difficult to elucidate whether these gender differences of elderly mood may be influenced by these different hormonal profiles. A lack of statistical power of our study may explain our results, as other studies with more individuals have shown a positive relationship of

testosterone and less depression in men (Barrett-Connor et al. 1999c).

In relation to frailty status in men, frailty development has been associated to lower levels of testosterone. However, we found no association between frailty development in women and hormonal status. These results are probably explained by a greater and longer hormonal exposure in men than in women, who present globally lower adrenal and gonadal steroids in comparison to men. So, differences in men in frailty development may be explained partially by hormonal status while in women other non-hormonal factors may contribute to frailty.

In conclusion, our study indicates that in a population of old people living independently, gonadotropic, somatotrophic and adrenal axis may participate, to some extent, to the development of impairment of health status that characterizes the ageing condition, and particularly in women.

Acknowledgements We thank Cristina Mas for technical assistance.

Special thanks to Nicola Van Berckel for reviewing the English version of the manuscript.

This work was supported by grants from the Catalan Agency for health and Technology Assessment #095/16/02; iMSERSO #E094 and E88/03 and Fis #PIO 070601.

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