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Luteinizing Hormone and Different Genetic Variants, as Indicators of Frailty in Healthy Elderly Men

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ABSTRACT

We investigated the possible clinical correlates between the serum LH concentration and characteristics of frailty and determined the presence and concentration of a genetic LH variant in an independently living population of elderly men. After exclusion of subjects with severe mobility problems and signs of dementia, 403 healthy men (aged 73–94 yr) were randomly selected from a population-based sample. Total testosterone (T), sex hormone-binding globulin (SHBG), and leptin were determined by RIA. Non-SHBG-bound T was calculated. LH and the presence of the genetic LH variant were measured using immunofluorometric assays. The characteristics of frailty were leg extensor strength using dynamometry, bone mineral density of total body and proximal femur, and body composition, including lean mass and fat mass, measured by dual energy x-ray absorptiometry. Disability was further assessed by the Modified Health Assessment Questionnaire and by a measure of physical performance.

LH significantly increased with age and inversely correlated with T and non-SHBG-bound T. LH was inversely related to muscle strength and lean mass, and both relations were independent of T. LH was positively related to self-reported disability (Modified Health Assessment Questionnaire); 12.5% of the study population was heterozygous for the LH variant allele. T levels and the degree of frailty were not different in the wild-type LH group compared with the heterozygote LH variant group. A significant positive relation between LH and fat mass as well as leptin was only present in the heterozygote LH variant group.

In conclusion, serum LH levels increases with age in independently living elderly men and correlates inversely with a variety of indicators of frailty. The observed relation between LH and frailty, independent of T, suggests that LH reflects serum androgen activity in a different way than T, possibly reflecting more closely the combined feedback effect of estrogen and androgen. A difference in biological response between the two LH forms is suggested, as a difference exists in the relation between LH and fat mass, respectively, and leptin in the heterozygote LH variant subjects *vs.* the wild-type LH subjects. (*J Clin Endocrinol Metab* **84:** 1334–1339, 1999)

PHYSICAL frailty in the elderly is defined as "a state of reduced physiological reserves associated with increased susceptibility to disability" (1). Age-related disability is characterized by generalized weakness, impaired mobility and balance, and poor endurance. Clinical correlates of physical frailty include falls, fractures, impairment in activities of daily living, and loss of independence; falls contribute to 40% of admissions to nursing homes (2). Falls are significantly associated with slow gait, poor physical performance, and lack of muscle strength (3–5).

In a previous study we demonstrated that low testosterone (T) levels in a population of healthy elderly men were associated with reduced muscle strength and bone mineral density and increased fat mass (unpublished data). As a negative feedback relationship exists between T and LH, the circulating LH level may also serve as an indicator of frailty.

Recently, a common variant form of LH was detected in apparently healthy individuals, caused by point mutation-based substitutions of two amino acids ($Trp^{8}Arg$ and $Ile^{15}Thr$) in the LH β-subunit (6–8). Suggestions have been made that the *in vivo* bioactivity of the LH variant is lower than that of the wild-type hormone due to its shorter circulatory half-time (9, 10). Raivio *et al.* (10) suggested that the occurrence of the variant LH may be a factor contributing to delayed pubertal tempo in otherwise healthy boys. No data are available yet describing the presence and bioactivity of this LH variant in elderly populations.

We investigated serum LH levels in relation to characteristics of frailty and determined the presence and concentration of the genetic LH variant in an independently living population of elderly men. The purpose was to determine whether any of the variable alterations in pituitary-testicular function with aging could be related to the occurrence of a biologically dissimilar variant form of LH.

Subjects and Methods

Subjects

A group of 403 independently living men, aged 73 yr or above, participated in this study. Participants were recruited by letters of invitation, which were sent to the oldest male inhabitants of Zoetermeer, a medium-sized town in the midwestern part of The Netherlands. All participants provided informed consent, and the study was approved by the medical ethics committee of Erasmus University Hospital Rotterdam. Participants were judged sufficiently healthy to participate in the study when they were physically and mentally able to visit the study

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center independently. No additional health-related eligibility criteria were used.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum concentrations of total T (TT; nanomoles per L) and sex hormone-binding globulin (SHBG; nanomoles per L) were all measured by RIA using commercial kits (Diagnostic System Laboratories, Webster, TX). The intraassay coefficients of variation (CVs) were, respectively, 8.1% and 3.0%. The interassay CVs were, respectively, 10.5% and 4.4%. Non-SHBG-bound T (non-SHBG-T; nanomoles per L) was calculated according to a method described by Södergård *et al.* (11). Leptin (micrograms per L) was also measured by RIA (Lilly Research Laboratories, Giessen, Germany). Albumin (grams per L) was measured by photometry using a commercial kit (ALB, Boehringer Mannheim, Mannheim, Germany).

LH (international units per L) was measured by an immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). The LH variant was recognized after calculation of the ratio of the results of two LH assays. The Delfia method for LH (LHspec), which uses two LH β-subunit-specific monoclonal antibodies (mAb) (12, 13), served as a reference method (assay 2). In the other assay (assay 1), the capture mAb recognizes a conformational epitope present in the wild-type α/β LH dimer but not in the variant form of LH or the free subunits, and the detection mAb recognizes an epitope in the α-subunit (6). The ratio of LH values measured by the two assays (assay 1/assay 2) was used to assess the variant or wild-type LH status. Three separate categories of this ratio were obtained: 1) normal ratio (>0.9), 2) low ratio (0.2–0.9), and 3) zero ratio (<0.15). A normal ratio individual has two wild-type LH alleles, a low ratio individual is heterozygous for the LH variant allele, and a zero ratio individual is homozygous for the variant LHβ gene, as confirmed by DNA analysis (9, 14, 15). The sensitivity of the two immunofluorometric assays was 0.05 IU/L, and the intra- and interassay CVs were less than 4% and 5%, respectively, at LH concentrations at and above the lowest standard concentration (0.6 IU/L of WHO International Reference Preparation 80/552).

Measures of muscle strength

Isometric grip strength was measured using an adjustable hand-held dynamometer (JAMAR dynamometer) at the nondominant hand (16). Each test was repeated three times, and the average was used in the analyses. Leg or knee extensor strength (LES) was measured as described previously (17, 18) using the Hoggan MicroFET hand-held dynamometer. To obtain one main outcome measurement for leg extensor strength, maximum LES (maxLES) was defined as the maximum strength for the right or left leg, whichever is largest, in a position of 120° extension. Statistical analyses were based on the physical unit momentum [Newton meters (Nm)], obtained by multiplying the maximum strength (in Nm) and the distance of the dynamometer to the knee joint (in meters).

Bone mineral density and body composition measurements

Total body bone mineral density was measured using dual energy x-ray absorptiometry (Lunar Corp., Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter, and Ward's triangle. In addition, total and trunk lean body mass and fat mass were measured (19). Quality assurance for dual energy x-ray absorptiometry, including calibration, was performed routinely every morning, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Physical performance

Lower extremity function, or physical performance score, was assessed as described by Guralnik *et al.* (20), including measurements of standing balance, walking speed, and ability to rise from a chair. Three tests of standing balance were considered in hierarchical difficulty in assigning a single score of 0–4 for standing balance. For the 8-ft walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1–4, corresponding to the quartiles of time needed to complete the task, with the fastest times scoring as 4. A summary performance scale was created by summing the category scores for the walking, chair stand, and balance test.

Satisfaction in performing activities of daily living was assessed by using a self-administered questionnaire from the Stanford Modified Health Assessment Questionnaire (MHAQ), as described by Pincus *et al.* (21), in which high score means low ability. The final score of the MHAQ was not normally distributed. Analyses were performed after logarithmic transformation.

Data analyses

Results were expressed, unless otherwise stated, as the mean and SD with the interquartile range. Relations between variables were assessed using linear regression for continuous variables and logistic regression for binary variables and were described as the linear regression coefficient (β) and its SE. Multiple regression analysis was used to adjust for age and BMI as well as to assess the contributions of different independent variables to the dependent variable. Correlations between variables were assessed using Pearson's correlation coefficient r. Pearson χ^2 test was used to assess differences in variables between groups. If not mentioned otherwise, all analyses were performed after adjustment for age.

Results

Descriptive data of all parameters measured are shown in Table 1. Their relations with age are shown in Table 2. LH was significantly positively related with age and SHBG (Table 3). An inverse relation existed between LH and the different T measurements (Fig. 1 and Table 3). Albumin and leptin levels were not related with LH.

T and frailty

T was independently related to muscle strength, bone mineral density, and fat mass. Linear regression coefficients for relations between non-SHBG-T and maxLES, respectively, and total body bone mineral density were $\beta = 1.93 \pm 0.53$ and $\beta = 0.01 \pm 0.002$ ($P < 0.001$). Total body

TABLE 1. Descriptive data of the study population (n = 403)

	Mean ± SD	Interquartile range
Age (yr)	77.8 ± 3.58	75–80
BMI	25.45 ± 3.04	23.32–27.25
Total T (nmol/L)	8.83 ± 2.98	7.19–10.66
SHBG (nmol/L)	31.5 ± 14.4	23.2–37.5
Non-SHBG-bound T (nmol/L)	5.68 ± 1.89	4.62–6.76
LH (IU/L)	9.38 ± 8.41	4.57–11.15
Leptin (μg/L)	5.35 ± 4.21	2.45–6.90
Albumin (g/L)	45.6 ± 2.76	43.8–47.5
IGS (kg)	34.3 ± 6.9	30.0–38.7
MaxLES (Nm)	103.2 ± 20.9	89.4–117.1
Total fat mass (kg)	21.2 ± 6.4	17.4–24.5
Total lean mass (kg)	51.7 ± 5.6	47.8–55.5
Total body BMD (g/cm^2)	1.17 ± 0.10	1.11–1.23
Femoral neck BMD (g/cm^2)	0.88 ± 0.14	0.78–0.97
Femoral ward BMD (g/cm^2)	0.72 ± 0.16	0.60–0.82
Femoral trochanter BMD (g/cm^2)	0.85 ± 0.15	0.76–0.94
Physical performance (points)	8.45 ± 2.43	7–10
MHAQ (points)	10.70 ± 4.30	8–12

IGS, Isometric grip strength; MaxLES, maximum leg extensor strength; MHAQ, Modified Health Assessment Questionnaire. Normative values for healthy young men are 9.72–30.54 nmol/L for total T, 10–55 nmol/L for SHBG, and 3.6–17.1 IU/L for LH.

fat mass decreased 0.53 ± 0.15 kg/nmol·L non-SHBG-T. T was not related to lean mass or the physical performance score.

TABLE 2. Relationship between age and the hormones and the characteristics of frailty

	Age (yr; $\beta \pm$ SE)	*P* value
LH (IU/L)	0.06 ± 0.01	<0.001
Total T (nmol/L)	−0.04 ± 0.04	0.37
SHBG (nmol/L)	0.92 ± 0.20	<0.001
Non-SHBG-bound T (nmol/L)	−0.07 ± 0.03	0.01
Leptin (μg/L)	−0.02 ± 0.01	0.04
Albumin (g/L)	−0.07 ± 0.04	0.06

The β coefficient denotes changes in unit per year. For example, LH increases 0.06 IU/L·yr.

TABLE 3. Associations of LH with hormones, SHBG, albumin, and characteristics of frailty

	LH (IU/L; n = 403; $\beta \pm$ SE)	*P* value
Hormones (dependent variables)		
Total T (nmol/L)	−0.12 ± 0.02	<0.001
SHBG (nmol/L)	0.31 ± 0.09	<0.001
Non-SHBG-bound T (nmol/L)	−0.09 ± 0.01	<0.001
Leptin(log) (μg/L)	0.002 ± 0.005	0.63
Albumin (g/L)	−0.002 ± 0.02	0.91
Characteristics of frailty (dependent variables)		
MaxLES (Nm)	−0.41 ± 0.12	<0.001
IGS (kg)	−0.09 ± 0.04	0.03
Lean mass (kg)	−0.11 ± 0.09	<0.001
Fat mass (kg)	−0.03 ± 0.04	0.43
TBBMD (g/cm^2)	−0.0005 ± 0.005	0.38
PPS (points)	−0.001 ± 0.001	0.17
MHAQ(log) (points)	0.01 ± 0.002	<0.001

β denotes linear regression coefficient. For example, total T decreases 0.12 nmol/L per IU/L LH. Maximum leg extensor strength (MaxLES), isometric grip strength (IGS), total body bone mineral density (TBBMD), and Modified Health Assessment Questionnaire (MHAQ) are also adjusted for body mass index.

LH and frailty

The relations between LH and the measures of frailty are shown in Table 3. LH was inversely related to maxLES (Fig. 2a). LH was also inversely related to lean mass, independent of maxLES (Fig. 2b). LH was not associated with bone mineral density or fat mass in the group as a whole. A positive relation existed between LH and MHAQ. MHAQ and maxLES were strongly related, but the relation between LH and MHAQ was independent of maxLES. Subjects with LH levels in the highest quartile had 10.1% lower maxLES values and 18.7% higher MHAQ values compared to subjects with LH levels in the lowest quartile (after adjustment for age and BMI).

Because we demonstrated that low T levels were accompanied by reduced muscle strength, a multiple regression analysis was performed including LH and T. Both T (TT and non-SHBG-T) and LH were significantly, but independently, related to maxLES and isometric grip strength. Multiple regression coefficients of the relations between maxLES and, respectively, LH and non-SHBG-T were $\beta = -0.29 \pm 0.13$ ($P = 0.03$) and $\beta = 1.42 \pm 0.56$ ($P = 0.01$). Non-SHBG-T and LH were also both related to MHAQ. However, in a multiple regression analysis including LH and non-SHBG-T, only LH remained significantly inversely related to MHAQ ($\beta = 0.007 \pm 0.002$; $P < 0.001$ and $\beta = -0.008 \pm 0.009$; $P = 0.40$, respectively).

LH variants

The heterozygote form of the LH variant was present in 12.5% (50 men) in this study population of elderly men. Mean concentrations of TT, non-SHBG-T, LH, SHBG, leptin, and albumin were not different in the wild-type LH group compared with those in the heterozygote LH variant group. The homozygote form of the LH variant was present in only 2 subjects (0.5%). Their TT values were 6.32 and 9.97 nmol/L.

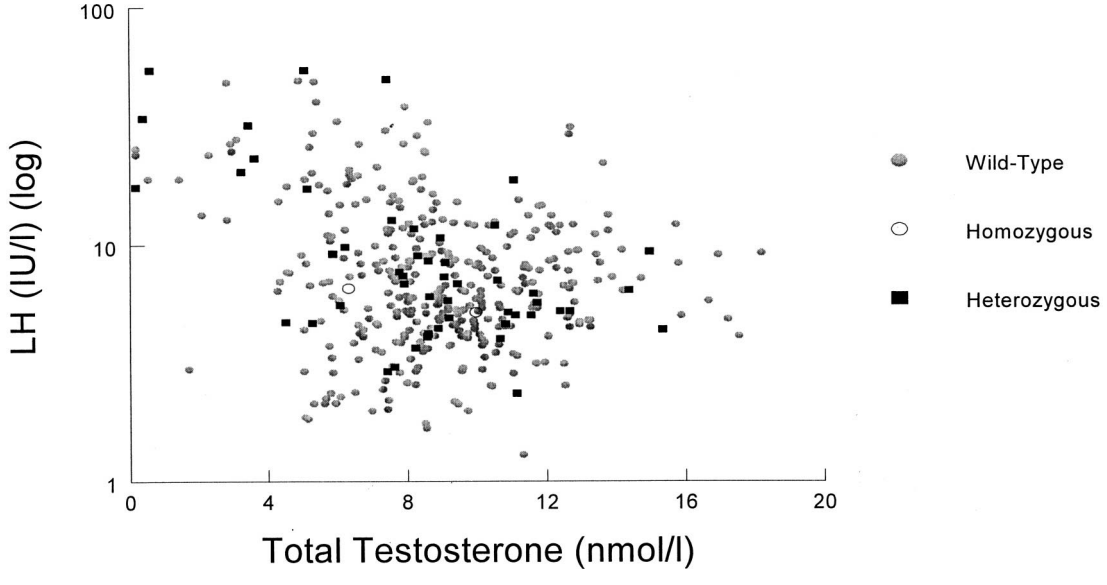

FIG. 1. Relation between TT and LH (logarithmically transformed) of wild-type LH variant subjects, heterozygote LH variant subjects, and homozygote LH variant subjects.

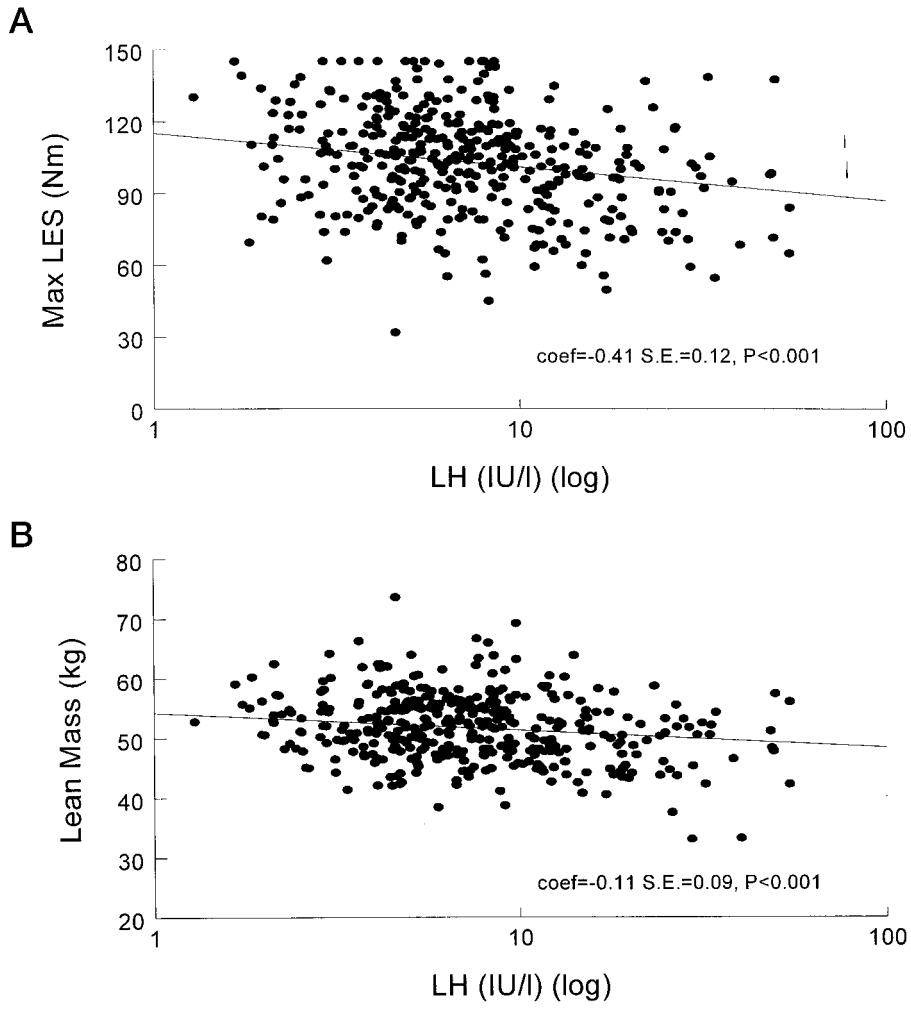

FIG. 2. Relation between LH in internationsl units per L and maxLES in Nm (a) and lean body mass in kilograms (b). Coef, Coefficient of linear regression.

TABLE 4. Age-adjusted linear regression analysis between LH and hormones, respectively, and characteristics of frailty in the wild-type LH group and in the heterozygote LH variant group

Dependent	Wild-type LH (n = 347; $\beta \pm$ SE)	P	Heterozygote LH variant (n = 50; $\beta \pm$ SE)	P
Hormones and SHBG				
Age (yr)	0.44 ± 0.11	<0.001	1.73 ± 0.39	<0.001
BMI	−0.05 ± 0.02	0.02	0.06 ± 0.04	0.14
Total T (nmol/L)	−0.11 ± 0.02	<0.001	−0.16 ± 0.04	<0.001
Non-SHBG-bound T (nmol/L)	−0.0004 ± 0.0001	<0.001	−0.0005 ± 0.0001	<0.001
SHBG (nmol/L)	0.45 ± 0.10	<0.001	0.05 ± 0.13	0.69
Leptin(log) (μg/L)	−0.004 ± 0.006	0.46	0.03 ± 0.01	0.02
Characteristics of frailty				
MaxLES (Nm)	−0.49 ± 0.14	<0.001	−0.46 ± 0.28	0.11
IGS (kg)	−0.09 ± 0.05	0.05	−0.15 ± 0.11	0.17
Lean mass (kg)	−0.16 ± 0.04	<0.001	0.022 ± 0.070	0.75
Fat mass (kg)	−0.03 ± 0.04	0.43	0.23 ± 0.07	0.001
TBBMD (g/cm^2)	−0.0001 ± 0.001	0.92	−0.002 ± 0.001	0.19
PPS (points)	−0.001 ± 0.001	0.26	−0.001 ± 0.001	0.26
MHAQ(log) (points)	0.006 ± 0.002	0.007	0.012 ± 0.004	0.004

β, Linear regression coefficient. Maximum leg extensor strength (MaxLES), isometric grip strength (IGS), total body bone mineral density (TBBMD), physical performance (PPS), and Modified Health Assessment Questionnaire are also adjusted for BMI.

The relations between LH and T and age, respectively, were not different in the two LH groups (Table 4).

None of the characteristics of frailty was different in the heterozygote LH variant group compared to the wild-type LH group. Mean values of the measures of frailty in the two variant LH homozygote individuals were also within the ranges of the other groups. The relations between LH, on the one hand, and the different measures of frailty, on the other,

in the two LH variant groups are shown in Table 4. Interestingly, LH was positively associated with fat mass and leptin only in the heterozygote LH variant group. This remained after adjustment for T (TT or non-SHBG-T).

Discussion

In an independently living population of healthy elderly men, LH significantly increased with age. This gonadotropin was inversely associated with T (TT and non-SHBG-T). LH was independent of T, negatively related to several characteristics of frailty, *i.e.* maxLES and lean mass, and positively related to self-reported disability. In this population, 12.5% were heterozygous for the LH variant allele. In the heterozygote LH variant subjects, but not in those with wild-type hormone, LH was positively related to fat mass and leptin.

Conflicting results have been reported concerning the question of whether LH increases with age or remains relatively stable (22, 23). One reason may be that the aging-induced decrease in T is primarily testicular in some men, mainly due to hypothalamo-pituitary insufficiency in others and of mixed origin in a third group. In our study population, LH increased significantly with age. The inverse relation we found between LH and TT could be due to the rather low T levels of the subjects in our study population. It is likely that in subjects with low T levels, LH cannot increase T, because of a primary disturbance of Leydig cell function.

The strong inverse relation of LH with muscle strength, lean mass, and self-reported disability, which is independent of T, suggests that LH also reflects the process of frailty. This association has not been described previously. Our observation might imply that LH monitors androgen effects in a different manner than T, probably because LH levels reflect the sum of systemic T levels as well as locally produced 5α-dihydrotestosterone and estrogens, which are produced via the local conversion of T. We did measure estradiol in this study; however, this hormone could not explain the independent relation of LH to the characteristics of frailty. The mechanism of this relationship remains to be elucidated.

Previously, a common variant form of LH has been detected in apparently healthy individuals due to the point mutation-based substitutions described above. There is a large variation in the common frequency of this polymorphism in different populations (0–50%) (15), and presently research is underway to investigate its possible clinical correlates. The prevalence of heterozygozity for the LH variant allele in the current cohort of subjects is in agreement with frequencies measured previously in The Netherlands, which found a mean frequency of 14.3% (95% confidence interval, 5.7–22.9) in 63 men and women aged 15 yr and older (15). The stability of the prevalence across age groups suggests that there is no selection regarding survival of the LH variant. It has been proposed that the *in vivo* bioactivity of the LH variant is lower than that of the wild-type hormone due to its shorter circulatory half-time (9, 10). On the other hand, this may be compensated for by the higher bio/immuno ratio of the variant hormone at the LH target cell level (8, 24). Neither the mean T, SHBG, and leptin levels nor the mean characteristics of frailty values differed between subjects with the wild-type LH and subjects with the heterozygote form of the LH variant. However, from subjective assessment of the findings presented in Fig. 1, it appears that the proportion heterozygous for the LH variant was relatively large in a group of subjects with low T and high LH concentrations. This is compatible with a reduced *in vivo* bioactivity of LH in the heterozygous individuals. Further, we observed differences in the relations between LH and leptin as well as fat mass between subjects with the different LH forms. The nature of these differences remains to be explained.

In conclusion, in independently living elderly men, LH increases with age and is inversely related to T. The observed relation between LH and frailty, independent of T, suggests that LH levels reflect the overall serum androgen activity in a different manner from T, possibly reflecting the combined effect of estrogen and androgen feedback action at the hypothalamic-pituitary level. The hormone levels and the degree of frailty are not different in the wild-type and heterozygote LH groups. However, a difference in biological response between the two LH forms is suggested, as a difference exists in the relation between LH and fat mass, respectively, and leptin in the heterozygote LH variant subjects *vs.* the wild-type LH subjects.

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