Relationship Between Low Levels of Anabolic Hormones and 6-Year Mortality in Older Men

The Aging in the Chianti Area (InCHIANTI) Study

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Background: Aging in men is characterized by a progressive decline in levels of anabolic hormones, such as testosterone, insulinlike growth factor 1 (IGF-1), and dehydroepiandrosterone sulfate (DHEA-S). We hypothesized that in older men a parallel age-associated decline in bioavailable testosterone, IGF-1, and DHEA-S secretion is associated with higher mortality independent of potential confounders.

Methods: Testosterone, IGF-1, DHEA-S, and demographic features were evaluated in a representative sample of 410 men 65 years and older enrolled in the Aging in the Chianti Area (InCHIANTI) study. A total of 126 men died during the 6-year follow-up. Thresholds for lowest-quartile definitions were 70 ng/dL (to convert to nanomoles per liter, multiply by 0.0347) for bioavailable testosterone, 63.9 ng/mL (to convert to nanomoles per liter, multiply by 0.131) for total IGF-1, and 50 µg/dL (to convert to micromoles per liter, multiply by 0.027) for DHEA-S. Men were divided into 4 groups: no hormone in the lowest quartile (reference) and 1, 2, and 3 hormones in the lowest quartiles. Kaplan-Meier survival and Cox proportional hazards models adjusted for confounders were used in the analysis.

Results: Compared with men with levels of all 3 hormones above the lowest quartiles, having 1, 2, and 3 dysregulated hormones was associated with hazard ratios for mortality of 1.47 (95% confidence interval [CI], 0.88-2.44), 1.85 (95% CI, 1.04-3.30), and 2.29 (95% CI, 1.12-4.68), respectively (test for trend, $P<.001$). In the fully adjusted analysis, only men with 3 anabolic hormone deficiencies had a significant increase in mortality (hazard ratio, 2.44; 95% CI, 1.09-5.46 (test for trend, $P<.001$).

Conclusions: Age-associated decline in anabolic hormone levels is a strong independent predictor of mortality in older men. Having multiple hormonal deficiencies rather than a deficiency in a single anabolic hormone is a robust biomarker of health status in older persons.

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I T IS CURRENTLY BELIEVED THAT anabolic-catabolic imbalance that favors catabolism is a key factor of accelerated aging in men.[1] The imbalance is mostly related to abnormalities in 3 anabolic endocrine axes, gonadal, adrenal, and somatotropic, with a decline in testosterone, dehydroepiandrosterone sulfate (DHEA-S), and insulinlike growth factor 1 (IGF-1), respectively.[1] Anabolic impairment may facilitate the decline in muscle mass, increase in fat mass, development of insulin resistance, and several medical conditions that, in turn, affect mortality.[1,2]

The contribution of single hormonal deficiency to age-related morbidity and mortality has been described in previous studies. Shores et al[3] showed that testosterone, the main anabolic hormone in men, is a predictor of mortality in male veterans. The extent to which this finding is valid for the general population is still in question. According to Roth et al,[4] DHEA-S has been considered one of the mediators of the relationship between caloric restriction and longevity in both animals and humans. Barrett-Connor et al[5] found that low DHEA-S levels predict cardiovascular mortality in older men, and Cappola et al[6] found that in older disabled women the relationship between DHEA-S and mortality is not linear. However, further studies[7] performed in the older population were unable to validate these findings. Signaling of IGF-1 has been considered a determinant of longevity, probably because of beneficial effects on muscle, vasculature, and metabolism.[8] However, although IGF-1 was shown to be a predictor of cardiovascular mortality in older men,[9,10] the role of IGF-1 as a single determinant of longevity is still debated.[11]

On the basis of these contradictory data, it is unlikely that a single anabolic hormone deficiency could be considered a re-

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liable index of the aging process. Interestingly, plurihormonal dysregulation rather than single hormonal derangement has been associated with the frailty syndrome[12,13] or the metabolic syndrome in older men.[14] In men with chronic heart failure, deficiency of more than 1 anabolic hormone identifies patients with higher mortality rates.[15] However, data concerning the relationship between parallel deficiency of anabolic hormones and mortality in a general population of older men, regardless of specific disease, are still lacking. Using data from the Aging in the Chianti Area (InCHIANTI) study, we hypothesized that a decline in the level of multiple anabolic hormones rather than in a single hormone predicts mortality during a 6-year follow-up in older men.

METHODS

STUDY SAMPLE

The InCHIANTI study is an epidemiologic study of a representative sample of the population living in Tuscany, Italy. We selected from this population 410 men 65 years and older (age range, 65-92 years) who had complete data on levels of testosterone, DHEA-S, IGF-1, sex hormone–binding globulin (SHBG), and albumin. Six-year vital status for the original InCHIANTI cohort was ascertained with data from the Tuscany Region Mortality General Registry maintained by the Registry Office of the Tuscany Region, including death certificates. One hundred twenty-six men died during the 6-year follow-up period. None of the men in the study were taking DHEA, testosterone, or growth hormone, as determined from examination of all prescription and over-the-counter medication containers. The Italian National Institute of Research and Care on Aging institutional review board ratified the study protocol. Participants consented to participate and to have their blood samples analyzed for scientific purposes.[16]

BIOLOGICAL SAMPLES

At an initial home interview, participants were given a plastic container and received detailed instructions for a 24-hour urine collection. Blood samples were obtained from participants after a 12-hour fast and after a 15-minute rest. Aliquots of serum and 24-hour urine were stored at –80°C and were not thawed until analyzed.

HORMONE MEASUREMENT

Total testosterone and DHEA-S levels were assayed using commercial radioimmunologic kits (Diagnostic Systems Laboratories, Webster, Texas). For total testosterone, the minimum detection limit was 0.86 ng/dL (to convert to nanomoles per liter, multiply by 0.0347); intra-assay and interassay coefficients of variation for 3 different concentrations were 9.6%, 8.1%, and 7.8% and 8.6%, 9.1%, and 8.4%, respectively. For DHEA-S, the minimum detection limit was 1.7 µg/dL (to convert to nanomoles per liter, multiply by 27.2); intra-assay and interassay coefficients of variation for the 3 different concentrations were 4.1%, 5.3%, and 4.7% and 4.8%, 7.0%, and 4.6%, respectively. The SHBG was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California), which has a sensitivity of 0.000046 µg/dL (to convert to nanomoles per liter, multiply by 34.7). In our laboratory, interassay and intra-assay coefficients of variation for 3 concentrations were less than 4%.[17] Serum albumin was measured with a commercial enzymatic test (Roche Diagnostics GmbH, Mannheim, Germany).

Bioavailable testosterone (serum-free and albumin-bound testosterone but not SHBG-bound testosterone) and free testosterone were calculated with the Vermeulen formula.[18] Serum concentrations of total IGF-1 were measured in duplicate from frozen specimens by immunoradiometric assay, using commercial reagents (Diagnostic Systems Laboratories). Interassay and intra-assay coefficients of variation for 3 concentrations (low, medium, and high) were all less than 10%. Serum interleukin 6 (IL-6) was measured in duplicate by high-sensitivity enzyme-linked immunosorbent assays (BioSource, Camarillo, California). The lowest detectable concentration was 0.1 pg/mL, and the interassay coefficient of variation was 4.5%.

COMORBIDITY AND OTHER VARIABLES

Diseases were ascertained by an experienced physician according to preestablished criteria that combine information from self-reported physician diagnoses, current pharmacologic treatment, medical records, clinical examinations, and blood tests. Diseases included in the current analysis were coronary heart disease (including angina and myocardial infarction), congestive heart failure, stroke, diabetes mellitus, hypertension, Parkinson disease, peripheral artery disease, asthma, cancer, and chronic obstructive pulmonary disease (COPD). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Smoking history was determined from self-report and dichotomized in the analysis as current smoking vs ever smoked or never smoked. Educational level was recorded as years of school. Cognitive function was evaluated using the Mini-Mental State Examination, and the total score was adjusted for educational level and age.[19]

Depressive symptoms were measured with the 20-item Center for Epidemiologic Studies–Depression scale. Participants with a Center for Epidemiologic Studies–Depression scale score of 16 or more were considered to be depressed.[20] Physical activity in the year before the interview was coded as (1) sedentary: completely inactive or light-intensity activity less than 1 hour per week; (2) light physical activity: light-intensity activity 2 to 4 hours per week; and (3) moderate to high physical activity: light activity at least 5 hours per week or more or moderate activity at least 1 to 2 hours per week. Daily total energy intake (in kilocalories) and alcohol intake (in grams) were estimated by the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire.[21]

STATISTICAL ANALYSIS

Variables are reported as mean ± SD, median and interquartile range, or number and percentage. Because of skewed distributions, log-transformed values for DHEA-S, total IGF-1, bioavailable testosterone, and IL-6 were used in regression analyses. Participants' main characteristics according to the number of dysregulated hormones were determined by age-adjusted linear regression models (analysis of covariance) and the Mantel-Haenszel χ^2 test. Survival analysis (Kaplan-Meier) and Cox proportional hazards models adjusted for age, BMI, log(IL-6), educational level, cognitive function, depression, physical activity, caloric and alcohol intake, smoking, coronary heart disease (including angina and myocardial infarction), congestive heart failure, stroke, diabetes, hypertension, Parkinson disease, peripheral artery disease, asthma, cancer, and COPD were used to assess the effects of single or multiple hormones in the lowest quartile on mortality. Covariates for adjustment were included in the model if in univariate analysis they were statistically associated with mortality. All analyses were performed with the SAS statistical package, version 9.1 (SAS Institute Inc, Cary, North Carolina).

Table 1. Characteristics of the General Population at Baseline According to Anabolic Status Calculated as the Number of Anabolic Hormones in the Lowest Quartiles[a]

| | No. of Hormones in the Lowest Quartiles | | | | P Value[b] |
Characteristic	0	1	2	3	
No. of patients	207	126	57	20	
Age, y	72.27 (5.20)	75.13 (6.81)	79.52 (7.56)	82.97 (7.69)	<.001
BMI	27.44 (3.21)	27.13 (3.01)	26.56 (2.99)	26.69 (3.40)	.90
Bioavailable testosterone, ng/dL	111.88 (32.43)	89.90 (36.34)	52.77 (30.57)	39.02 (18.72)	<.001
Total insulinlike growth factor 1, ng/mL	141.05 (51.45)	122.75 (50.06)	100.24 (54.90)	45.83 (14.43)	<.001
Dehydroepiandrosterone sulfate, µg/dL	109.01 (55.52)	69.91 (62.65)	41.26 (31.60)	21.75 (14.90)	<.001
Interleukin 6, median (IQR), pg/mL	0.36 (−0.16 to 0.77)	0.48 (−0.07 to 0.90)	0.50 (0.09 to 1.13)	1.02 (0.17 to 2.01)	.49
Cardiovascular disease, No. (%)	8 (3.86)	10 (7.94)	1 (1.75)	1 (5.00)	.92
Stroke, No. (%)	11 (5.31)	11 (8.73)	5 (8.77)	2 (10.00)	.41
Parkinson disease, No. (%)	3 (1.45)	1 (0.79)	1 (1.75)		.26
Hypertension, No. (%)	54 (26.09)	41 (32.54)	20 (35.09)	4 (20.00)	.07
Cancer, No. (%)	9 (4.35)	4 (3.17)	4 (7.02)	1 (5.0)	.62
COPD, No. (%)	28 (13.53)	22 (17.46)	11 (19.30)	4 (20.00)	.17
Mini-Mental State Examination score	25.88 (3.55)	25.33 (4.34)	23.26 (6.14)	22.45 (6.16)	.64
CES-D total score	9.05 (6.52)	10.76 (8.18)	9.62 (6.01)	11.06 (6.78)	.19
Peripheral artery disease, No. (%)	16 (7.73)	12 (9.52)	6 (10.53)	1 (20.00)	.32
Caloric intake, g/d	2278.83 (581.89)	2123.32 (527.69)	1981.49 (572.97)	1929.25 (566.48)	.33
Alcohol intake, g/d	24.71 (18.16)	24.47 (21.79)	17.14 (17.20)	20.71 (11.88)	.32
Smoking (current vs ever or never smoked)	47 (22.71)	27 (21.43)	8 (14.04)	3 (15.00)	.16
Physical activity					
Sedentary level	17 (8.21)	19 (15.08)	18 (31.58)	3 (15.00)	
Light level	170 (82.13)	99 (78.57)	35 (61.40)	15 (75.00)	.002
Moderate-high level	20 (9.66)	8 (6.35)	4 (7.02)	2 (10.00)	
Educational level, y	6.16 (3.15)	6.43 (3.81)	5.83 (3.90)	4.35 (1.53)	.20

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CES-D, Center for Epidemiologic Studies–Depression; COPD, chronic obstructive pulmonary disease; IQR, interquartile range.

SI conversion factors: To convert testosterone to nanomoles per liter, multiply by 0.0347; insulinlike growth factor 1 to nanomoles per liter, multiply by 0.131; and dehydroepiandrosterone sulfate to micromoles per liter, multiply by 0.027.

[a] Values are expressed as mean±SD unless otherwise indicated.

[b] Differences in variables among patients with different anabolic status were tested using age-adjusted analysis of variance.

RESULTS

Serum levels used to define the lowest quartiles of the hormones considered in this study were as follows: 70 ng/dL for bioavailable testosterone, 50 µg/dL for DHEA-S, and 63.86 ng/mL for total IGF-1 (to convert to nanomoles per liter, multiply by 0.131). Four groups were created according to baseline serum levels of these hormones: no hormone in the lowest quartiles (reference group) (n=207) and 1 hormone (n=126), 2 hormones (n=57), and 3 hormones in the lowest quartiles (n=20) (the group with plurihormonal deficiency). Compared with living participants, men who died during the 6 years of follow-up were older and more likely to have a history of sedentary lifestyle, stroke, peripheral vascular disease, cognitive impairment, and higher serum log(IL-6) levels (data not shown).

Compared with men with levels of all hormones above the lowest quartile, men with 1, 2, or 3 hormones in the lowest quartiles were older and more likely to have a history of sedentary lifestyle (**Table 1**).

After adjusting for age, we did not find a statistically significant relationship between lowest quartiles of bioavailable testosterone and IGF-1 and all-cause mortality (**Table 2**), whereas a low DHEA-S level was associated with significantly higher mortality. Using the hormone levels as continuous variables, none of the 3 hormones were associated with significantly higher mortality. The hazard ratios (HRs) for mortality associated with having a hormone level in the lowest quartile vs no hormone level in the lowest quartile were 1.48 (95% confidence interval [CI], 0.89-2.45) for bioavailable testosterone, 1.36 (95% CI, 0.78-2.38) for IGF-1, and 1.37 (95% CI, 0.84-2.37) for DHEA-S. However, the association of DHEA-S with mortality was no longer significant after adjusting for potential confounders, including age, BMI, educational level, smoking, alcohol and caloric intake, physical activity, log(IL-6) levels, and chronic disease including diabetes, hypertension, peripheral artery diseases, coronary heart disease, congestive heart failure, stroke, Parkinson disease, COPD, asthma, and cancer (**Table 3**).

Remarkably, the HR for mortality progressively increased with the number of anabolic hormones in the lowest serum quartile level. Compared with men with levels of all hormones above the lowest quartile, men with 1, 2, or 3 hormones in the lowest quartile had an HR for mortality of 1.47 (95% CI, 0.88-2.44), 1.85 (95% CI, 1.04-3.30), and 2.29 (95% CI, 1.12-4.68) (test for trend, P=.006), respectively (Table 2). After adjusting for confounders, including age, BMI, educational level, smoking, alcohol and caloric intake, physical activity, log

Table 2. Relationship Between Hormonal Dysregulation and All-Cause Mortality in Older Men: Unadjusted Analysis[a]

Characteristic	Hazard Ratio (95% Confidence Interval)	Age-Adjusted P Value	P Value for Trend[b]
Bioavailable testosterone <70 ng/dL[c]	1.31 (0.84-2.02)	.23	.65
Insulinlike growth factor 1 <63.86 ng/dL[c]	1.30 (0.82-2.07)	.27	.32
Dehydroepiandrosterone sulfate <50 ng/dL[c]	1.70 (1.13-2.55)	.01	.28
Multiple hormonal dysregulation[d]			<.001
0 (n=207)			
1 (n=106)	1.47 (0.88-2.44)	.14	
2 (n=57)	1.85 (1.04-3.30)	.04	
3 (n=20)	2.29 (1.12-4.68)	.02	

SI conversion factors: To convert testosterone to nanomoles per liter, multiply by 0.0347; insulinlike growth factor 1 to nanomoles per liter, multiply by 0.131; and dehydroepiandrosterone sulfate to micromoles per liter, multiply by 0.027.
[a] Results reported in the table are from separate models.
[b] Obtained by fitting the same model with hormone levels as continuous variables.
[c] Top level of the lowest quartile.
[d] Hormones in the lowest quartile.

Table 3. Relationship Between Hormonal Dysregulation and All-Cause Mortality in Older Men: Adjusted Analysis[a]

Characteristic	Hazard Ratio (95% Confidence Interval)	P Value[b]	P Value for Trend[c]
Bioavailable testosterone <70 ng/dL[d]	1.48 (0.89-2.46)	.13	.48
Insulinlike growth factor 1 <63.86 ng/mL[d]	1.36 (0.78-2.38)	.28	.91
Dehydroepiandrosterone sulfate <50 µg/dL[d]	1.38 (0.84-2.26)	.21	.97
Multiple hormonal dysregulation[e]			<.001
0 (n=207)			
1 (n=106)	1.04 (0.56-1.95)	.89	
2 (n=57)	1.34 (0.67-2.65)	.41	
3 (n=20)	2.44 (1.09-5.46)	.03	

[a] Results reported in the table are from separate, fully adjusted models.
[b] P values adjusted for age, body mass index, cancer, log(interleukin 6), education, cognitive function, depression, physical activity, caloric and alcohol intake, smoking, coronary heart disease (including angina and myocardial infarction), congestive heart failure, stroke, diabetes, hypertension, Parkinson disease, peripheral artery disease, asthma, cancer, and chronic obstructive pulmonary disease.
[c] Obtained by fitting the same model with hormone levels as continuous variables.
[d] Top level of the lowest quartile.
[e] Hormones in the lowest quartile.

(IL-6) levels, and chronic disease such as diabetes, hypertension, peripheral artery diseases, coronary heart disease, congestive heart failure, stroke, Parkinson disease, COPD, asthma, and cancer, the HRs for mortality were 1.04 (95% CI, 0.56-1.95), 1.34 (95% CI, 0.67-2.65), and 2.44 (95% CI, 1.09-5.46), respectively (test for trend, $P<.001$) (Table 3). Survival analysis (Kaplan-
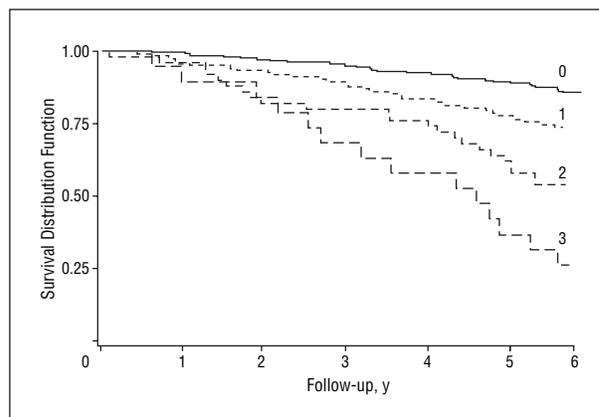
Figure. Relationship between the number of anabolic hormones in the lowest serum level quartile and 6-year survival in older men. The survival distribution function according to the number of dysregulated hormones is shown for the 6 years of follow-up. The solid black line indicates the survival rates for patients with 0 dysregulated hormones; short-dashed line, survival rates for patients with 1 dysregulated hormone; medium-dashed line, survival rates for patients with 2 dysregulated hormones; and long-dashed line, survival rates for patients with 3 dysregulated hormones. The thresholds for anabolic hormonal deficiency were 70 ng/dL (to convert to nanomoles per liter, multiply by 0.037) for bioavailable testosterone, 63.9 ng/mL (to convert to nanomoles per liter, multiply by 0.13) for total insulinlike growth factor 1, and 60 µg/dL (to convert to micromoles per liter, multiply by 0.027) for dehydroepiandrosterone sulfate (log rank=62.92; $P<.001$).

Meier analysis) between the number of anabolic hormones in the lowest serum level quartile and mortality after 6 years of follow-up in older men is reported in the **Figure**. The mean±SD survival time expressed in years was 5.58±0.07 in group 0, 5.18±0.13 in group 1, 4.70±0.24 in group 2, and 3.98±0.39 in group 3 ($P=.01$, fully adjusted). The mortality rates (events per 100 person-years) were 2.9 in group 0, 6.2 in group 1, 16.7 in group 2, and 46.0 in group 3 ($P=.007$, test for trend).

COMMENT

We found that independent of age and multiple potential confounders, low circulating levels of multiple anabolic hormones, including testosterone, IGF-1, and DHEA-S (in the lowest quartiles of the population), were an independent predictor of mortality during 6 years of follow-up in older men. On the contrary, serum levels of each of these hormones considered separately were not associated with significantly differential mortality.

These findings are consistent with the report of Jankowska et al[15] that indicated that in patients affected by chronic heart failure, deficiency of more than 1 anabolic hormone identifies groups with a higher mortality rate. However, in contrast to the population in the study by Jankowska and colleagues, our cohort consisted of older people who were not selected according to a specific disease. In addition, we were able to show that association between multiple hormone dysregulation and mortality is not accounted for by potential confounders, such as cardiovascular risk factors and cardiovascular morbidity.

The concept that relatively low levels of anabolic hormones may be an independent cause of mortality, especially in older persons, is not surprising. Several lines of

evidence suggest that the aging process is associated with a decline in anabolic hormones and increase in catabolic hormones.[22] In this study, we evaluated the 3 key anabolic hormones: bioavailable testosterone, DHEA-S, and IGF-1. Each of these anabolic hormones has a direct impact on lipid and glucose metabolism.[23-25] Evidence has indicated that low testosterone, IGF-1, and DHEA-S levels are good predictors of cardiovascular disease and diabetes in men.[5,10,26] Furthermore, we have previously shown that a low testosterone level is inversely related with inflammatory markers and predicts the development of anemia in the older population.[27,28] Both cardiovascular disease and anemia are 2 independent predictors of mortality in older men.[29]

Interestingly, in this study, we did not find any significant association between decline in a single hormone level and overall mortality. Two previous reports[30,31] performed in older men found no association between testosterone and all-cause mortality. As already mentioned, Shores et al[3] recently showed an association between testosterone and mortality in male veterans, but their study design was retrospective; therefore, the existence of a systematic bias cannot be excluded. The Veterans Affairs clinic–based population generally has greater morbidity and lower socioeconomic status than the general population, so the external validity of these findings is questionable.

Observational and intervention studies[5-7,9,10] that tested the relationship between DHEA-S and IGF-1 and mortality have reported inconsistent findings. Whether these 2 hormones represent biomarkers of longevity is still an enigma.[4,11] Nair et al[32] recently showed that neither DHEA nor low-dose testosterone replacement in elderly people has beneficial effects on body composition, physical performance, insulin sensitivity, or quality of life.

In accordance with our working hypothesis, the HR for mortality increased progressively with the number of anabolic hormones in the lowest quartiles. Compared with no dysregulated hormones, having 3 dysregulated anabolic hormones was a significant and independent risk factor for mortality. It has been suggested that anabolic hormonal deficiency is a surrogate of disease severity, especially in men.[1,33] However, even after adjusting for extensive information on chronic morbidity, our findings were substantially unchanged.

Multisystem disorders that are widely prevalent in aging, such as the metabolic syndrome, frailty syndrome, and chronic heart failure, are more significantly associated with multiple hormonal dysregulation rather than a single hormonal derangement.[12-15] Studies[15] have found that the risk of death increases progressively with the number of dysregulated hormones and becomes 2.5 times higher when 3 hormones are dysregulated compared with no dysregulation.

It is conceivable that DHEA-S, testosterone, and IGF-1 have synergistic effects. For example, DHEA-S can be converted to testosterone in peripheral tissues, and some of the peripheral actions of both DHEA-S and testosterone may be mediated via tissue-generated IGF-1.[25,34,35] In preliminary analyses performed in the Baltimore Longitudinal Study on Aging population, both DHEA-S and testosterone had a joint protective effect on 30-year mortality rates.[36]

Our study has limitations. First, testosterone, IGF-1, and DHEA-S were only measured once. Second, information on cause-specific mortality (cardiovascular and cancer) was also not available; hence, the specific mechanism underlying the relationship between anabolic hormones and mortality remains unknown. Moreover, the number of participants with 3 dysregulated hormones was small. Third, although IGF-1 is secreted from the liver under growth hormone stimulus, it is only an indirect marker of growth hormone activity. Fourth, one measure of bioavailable testosterone was estimated from total testosterone and SHBG with the Vermeulen formula, and we did not measure estradiol levels. Finally, because of the nature of the analysis, our findings cannot be directly compared with previous data from the literature.

Nevertheless, our study has several strengths. First, this is the only large, population-based prospective study, to our knowledge, to evaluate the relationship between multiple deficiencies in testosterone, DHEA-S, and IGF-1 level and mortality in older men. Second, information on hormonal levels was complemented by measures such as caloric intake, inflammatory markers, physical activity, cognition, and mood, all factors that potentially affect the relationship between anabolic hormones and mortality. Third, we used bioavailable testosterone for analysis, which is a measure of androgen bioactivity at the tissue level, is more reliable and valid than total testosterone, and was strongly correlated with free testosterone ($r = 0.98$ for Pearson correlation coefficient; $P < .001$) in our sample. Finally, the existence of a relationship between plurihormonal decline and mortality, even after adjusting for multiple confounders, provides evidence of the biological plausibility of our working hypothesis. These findings strongly suggest a causal pathway between age-related deficiency in multiple anabolic hormones and high prevalence of overall mortality in older men.

The results of this study have important clinical and conceptual implications. Since previous epidemiologic studies in elderly patients focused on only a single hormonal decline rather than multiple anabolic deficiency, we suggest that assessment of these 3 anabolic hormones should always be considered in older men. Future studies should also clarify the link between impairment of these 3 different hormonal axes and cause-specific mortality in older men. The findings of this study raise the possibility that a multiple dysregulation rather than a single dysregulation of the anabolic hormones is a powerful marker of poor health status and perhaps accelerates aging in older men. Once our findings have been confirmed in other populations, the door will open to clinical trials of multiple hormonal replacement in elderly men.

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