Improved prediction of all-cause mortality by a combination of serum total testosterone and insulin-like growth factor I in adult men

Nele Friedrich a,⁎, Harald J. Schneider b, Robin Haring a, Matthias Nauck a, Henry Völzke c, Heyo K. Kroemer d, Marcus Dör r e, Jens Klotsche f, Caroline Jung-Sievers g, David Pittrow h, Hendrik Lehner t i, Winfried März j, Lars Pieper k, Hans-Ulrich Wittchen l, Günter K. Stalla n

⁎ Corresponding author. Tel.: +49 3834 19655; fax: +49 3834 865502.
E-mail addresses: nele.friedrich@uni-greifswald.de (N. Friedrich), Harald.Schneider@med.uni-muenchen.de (H.J. Schneider), robin.haring@uni-greifswald.de (R. Haring), matthias.nau@uni-greifswald.de (M. Nauck), voelzke@uni-greifswald.de (H. Völzke), kroemer@uni-greifswald.de (H.K. Kroemer), mdoerr@uni-greifswald.de (M. Dör r), klotsche@psychologie.tu-dresden.de (J. Klotsche), carolinesievers@gmx.de (C. Jung-Sievers), david.pittrow@mailbox.tu-dresden.de (D. Pittrow), Hendrik.Lehnert@uk-sh.de (H. Lehner t), maerz@synlab.de (W. März), pieper@psychologie.tu-dresden.de (L. Pieper), wittchen@psychologie.tu-dresden.de (H-U. Wittchen), henri.wallaschofski@uni-greifswald.de (H. Wallaschofski), stalla@mpip-sykli.mpg.de (G.K. Stalla).

These authors contributed equally to this work.

a Institute of Clinical Chemistry and Laboratory Medicine, Ernst Moritz Arndt University, Sauerbruchstraße N 27, D-17475 Greifswald, Germany
b Medizinische Klinik – Innenstadt, Ludwig-Maximilians-University of Munich, Ziemensstr. 1, D-80336 Munich, Germany
c Institute for Community Medicine, Ernst Moritz Arndt University, Sauerbruchstraße N K, D-17475 Greifswald, Germany
d Institute of Pharmacology, Ernst Moritz Arndt University, Loeffler Str. 23 d, D-17475 Greifswald, Germany
e Department Internal Medicine B, Ernst Moritz Arndt University, Loeffler Str. 23 a, D-17475 Greifswald, Germany
f Institute of Clinical Psychology and Psychotherapy, Technische Universität Dresden, Chemnitzer Str. 46, D-01187 Dresden, Germany
g Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, D-80804 Munich, Germany
h Institute of Clinical Pharmacology, Fiedlerstrasse 27, Technische Universitaet Dresden, D-01307 Dresden, Germany
i 1st Dept. of Medicine, University of Lübeck, Ratzeburger Aller 160, D-23538 Lübeck, Germany
j Institute for Community Medicine, Ernst Moritz Arndt University, Sauerbruchstraße NK, D-17475 Greifswald, Germany
k Synlab Medizinisches Versorgungszentrum Heidelberg GmbH, Wasserturmstraße 71, 69214 Eppelheim, Germany
l Medizinische Klinik – Innenstadt, Ludwig-Maximilians-University of Munich, Ziemensstr. 1, D-80336 Munich, Germany
m Institute of Clinical Psychology and Psychotherapy, Technische Universität Dresden, Chemnitzer Str. 46, D-01187 Dresden, Germany
n Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, D-80804 Munich, Germany

Contents lists available at SciVerse ScienceDirect
Steroids journal homepage: www.elsevier.com/locate/steroids

A R T I C L E   I N F O
Article history:
Received 11 July 2011
Received in revised form 11 October 2011
Accepted 11 October 2011
Available online 20 October 2011

Keywords:
IGF-I
Testosterone
Mortality
Study of Health in Pomerania (SHIP)
DETECT

ABSTRACT
Objective: Lower levels of anabolic hormones in older age are well documented. Several studies suggested that low insulin-like growth factor I (IGF-I) or testosterone levels were related to increased mortality. The aim of the present study was to investigate the combined influence of low IGF-I and low testosterone on all-cause mortality.

Methods and results: From two German prospective cohort studies, the DETECT study and SHIP, 3942 men were available for analyses. During 21,838 person-years of follow-up, 8.4% (n = 330) of men died. Cox model analyses with age as timescale and adjusted for potential confounders revealed that men with levels below the 10th percentile of at least one hormone [hazard ratio (HR) 1.38 (95% confidence-interval (CI) 1.06–1.78), p = 0.02] and two hormones [HR 2.88 (95% CI 1.32–6.29), p < 0.01] showed a higher risk of all-cause mortality compared to men with non-low hormones. The associations became non-significant by using the 20th percentile as cut-off showing that the specificity increased with lower cut-offs for decreased hormone levels. The inclusion of both IGF-I and total testosterone in a mortality prediction model with common risk factors resulted in a significant integrated discrimination improvement of 0.5% (95% CI 0.3–0.7%, p = 0.03).

Conclusions: Our results prove that multiple anabolic deficiencies have a higher impact on mortality than a single anabolic deficiency and suggest that assessment of more than one anabolic hormone as a biomarker improve the prediction of all-cause mortality.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction
Insulin-like growth factor I (IGF-I) and total testosterone are anabolic hormones involved in the aging process with a strong age-dependent decline in their concentration [1,2]. IGF-I, as the main mediator of the growth hormone actions, is involved in cell replication and proliferation, protein synthesis, as well as glucose and lipid metabolism [3]. Low IGF-I levels have recently been related to a broad range of morbidities including chronic obstructive pulmonary disease [3], osteoporosis [3], metabolic syndrome (MetS), or diabetes mellitus [4,5], as well as overall mortality [6]. Testosterone represents the principal male sex hormone and is
linked to the regulation of e.g. fertility, libido, muscle mass and glucose metabolism [7]. Low total testosterone levels have been associated with hypertension [7], insulin resistance [8], the MetS, and type 2 diabetes [9].

In addition to the associations with major co-morbidities, we recently have demonstrated that low levels of IGF-I [6] and total testosterone [10] were directly associated with increased risk of mortality in a population-based samples of men. In particular, men with either low IGF-I or total testosterone levels had an almost two-fold higher risk of all-cause mortality.

The impact of multiple low serum hormone concentrations, including IGF-I, total testosterone and dehydroepiandrosterone sulphate on mortality was investigated in two previous studies [11,12]. The InCHIANTI study [12] as well as a Polish study in men with chronic heart failure [11] reported that the risk of mortality constantly increases with the number of deficient hormones. However, both studies were limited by relatively small study populations of 410 [12] or 208 [11] men, respectively, and by a narrow age-range. Furthermore, these studies suggested that a combination of more than one hormone might be superior in the prediction of mortality. However, the quantification of the improvement in risk prediction by the combined biomarkers as one of the key questions has not been answered satisfactorily.

Therefore the objective of the present study was to verify the previously described association between the combination of low hormone levels and all-cause mortality based on IGF-I and total testosterone levels in a population of 4092 men aged 18–91 years from two studies. Furthermore, we examined whether the prediction of mortality on the basis of a model containing hormone levels is superior to a model with known risk factors.

2. Materials and methods

2.1. Design and subjects

DETECT (Diabetes Cardiovascular Risk-Evaluation: Targets and Essential Data for Commitment of Treatment) is a large, multistage, and nationally representative study in Germany [13]. On 16th and 18th September 2003, 3188 GPs completed a standardized assessment of the diagnostic and therapeutic profile of 55,518 eligible patients at the target days, with 7519 in 851 primary care settings added. A net sample (after exclusion of migrants or deceased persons) comprised 6267 eligible subjects. All subjects received a maximum of three written invitations. In cases of non-response, letters were followed by several phone calls or by home visits if contact by phone was not possible. Finally, 2116 men and 2192 women (response proportion 69%) participated. All participants gave written informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the University of Greifswald. Of the 2116 male participants 125 subjects with missing data for IGF-I or total testosterone and 117 patients with missing data for used confounders were excluded. Valid data including mortality follow-up were available in 1911 men. Excluded men (n = 242) were on average 4 years younger than men included in the analyses. However, no differences regarding the age-adjusted proportions/mean of smoking, physical activity, diabetes, increased blood pressure or WHtR were found.

2.2. Instruments and measurements

Information on age, sex, socio-demographic characteristics, and medical histories were assessed by standardised interviews and medical records by the primary care physicians in DETECT and by standardised computer-assisted personal interviews in SHIP. In both studies, physicians and study nurses were advised to measure weight, height, blood pressure, and waist circumference according to written, standardized instructions given in a manual. Smoking was categorised into smokers and non-smokers. Subjects who participated in physical training during summer or winter for at least two hours a week were classified as being physically active. A systolic and diastolic blood pressures of >140 and >90 mmHg, respectively, were considered increased. Diabetes mellitus was defined as history of diabetes as reported by patients or physicians in DETECT and SHIP, respectively, intake of oral antidiabetic drugs or insulin, or hemoglobin A1c (HbA1c) levels >6.5%. For both studies, we defined liver disease as physician’s or self-reported diagnosis of liver disease or aspartate-amino-transferase or alanin-amino-transferase > population mean +2 * standard deviation. Kidney failure was assumed if the estimated glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease (MDRD) formula was <30 ml/min [17]. Missing values in categorical variables were treated as separate group.

In DETECT, blood samples were collected and shipped by courier at room temperature within 24 h to the central laboratory. Blood samples were taken during fasting or non-fasting and fasting state was recorded. Upon arrival in the central laboratory, the samples were centrifuged immediately and serum and plasma was stored at −20 °C until further processing. In SHIP non-fasting blood samples were taken between 07:00 a.m. and 04:00 p.m., analysed immediately for routine clinical chemistry parameters and stored at −80 °C for IGF-I and testosterone analyses. In both studies IGF-I was determined with an automated chemiluminescence system (Nichols Institute Diagnostics, San Clemente, CA, USA). The analytical sensitivity was 6 ng/ml. In SHIP, total testosterone levels were measured using competitive chemiluminescent enzyme immunoassays on an Immulite 2500 analyzer (Siemens Immulite 2500 DHEA-S04, ref. LSKDS, lot 106; and Siemens Immulite 2500 Total
3. Results

3.1. General characteristics

Comparisons regarding general baseline characteristics are presented in Table 1. Men with one or two low hormone levels had a higher WHR, were less physically active and more often affected by diabetes mellitus and liver diseases compared to men with non-low IGF-I and total testosterone levels, respectively. No differences in smoking habits were apparent between the different groups.

3.2. Association of IGF-I and total testosterone levels to all-cause mortality

During the 21,838 person-years (SHIP 15,299 person-years, DETECT 6538 person-years) of follow-up 8.4% (n = 330) of men died. The crude mortality rate ratios are given in Table 2. Men with two hormones involved had, independently of the used categorization, higher crude rate ratios. Results of univariable Kaplan–Meier survival analysis (Fig. 1) also revealed that the decrease in cumulative survival was more evident in men with one (p < 0.01) or two (p < 0.01–0.05) hormones with low levels compared to men with two non-low hormones.

These findings were confirmed by multivariable Cox regression analyses adjusted for WHR, smoking, and physical activity. Men with one or both hormones below the 10th or 15th percentile had higher risks of all-cause mortality compared to men with two non-low hormones (Table 3). Furthermore, the mortality risk is higher for 2 low hormones compared to 1 low hormone which implies a dose–response relation. The additional inclusion of diabetes, liver disease, increased blood pressure, and high-sensitive C-reactive protein as confounders did not affect these results for the 10th percentile. However, in analyse using the 15th percentile as cut-off, only men with 2 low hormones had still a significant higher mortality risk. Therefore, the risk estimates and thus the specificity increased with lower cut-offs to define low levels of IGF-I and total testosterone.

Further factors which were significantly related to all-cause mortality (model with 10th percentile as cut-off) were smoking and IGF-I were smoking [HR 2.41 (95% CI 1.83–3.17), p < 0.01], diabetes [HR 1.45 (95% CI 1.14–1.84), p < 0.01], liver disease [HR 2.26 (95% CI 1.52–3.38), p < 0.01], increased blood pressure [HR 0.79 (95% CI 0.63–0.99), p = 0.04], and high-sensitive C-reactive protein [per 10 mg/l HR 1.16 (95% CI 1.08–1.24), p < 0.01].

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of low hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (N = 2885)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.7 (15.1)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>74.4</td>
</tr>
<tr>
<td>Smokers</td>
<td>25.5</td>
</tr>
<tr>
<td>Physically active (%)</td>
<td>0.1</td>
</tr>
<tr>
<td>&lt;2 h/week</td>
<td>46.3</td>
</tr>
<tr>
<td>&gt;2 h/week</td>
<td>50.7</td>
</tr>
<tr>
<td>WHTR</td>
<td>0.56 (0.07)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>16.6</td>
</tr>
<tr>
<td>Liver disease (%)</td>
<td>5.3</td>
</tr>
<tr>
<td>Increased blood pressure (%)</td>
<td>51.7</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>146.3 (48.0)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>17.3 (5.3)</td>
</tr>
</tbody>
</table>

WHR = waist-to-height ratio; IGF-I = insulin-like growth factor I. Low hormone levels were defined according to the age-specific 15th percentile.
*χ2-test (nominal data) or Mann–Whitney test (interval data) were performed, category “missing values” were not included in tests.
The exclusion of men with a GFR <30 ml/min [n = 23; one hormone <10th percentile: HR 1.38 (95% CI 1.06–1.78), p = 0.02; two hormones <10th percentile: HR 2.88 (95% CI 1.32–6.29), p = 0.01] did not change the results substantially. Moreover, we performed an additional analysis in the subgroup of men older than 60 years (n = 1805). Also these analyses showed that men with one [HR 1.41 (95% CI 1.06–1.86), p = 0.02] or two <10th percentile: HR 2.90 (95% CI 1.24–6.79), p = 0.01] low hormone levels had a higher mortality risk.

After the inclusion of IGF-I and total testosterone to the standard model (with age, smoking, systolic blood pressure, diabetes, WHtR, total cholesterol, and HDL cholesterol as predictors), the discrimination slope increased from 9.42% to 9.92%; resulting in an absolute IDI of 0.5% (95% CI 0.3–0.7%, p = 0.03) and a relative IDI of 5.3%. AUC for the standard model was 0.809 and slightly increased to 0.811 after the inclusion of IGF-I and total testosterone as additional predictors (p = 0.52).

4. Discussion

In the present study we revealed that the mortality risk in adult men successively increases with the combination of low IGF-I and testosterone levels, independent of age and major confounders. This is in good agreement with previous results of the InCHIANTI study, a cohort study that investigated the relation of low levels of IGF-I, bioavailable testosterone, and dehydroepiandrosterone sulfate with mortality in 410 men 65 years or older [12]. In contrast to previous studies including the SHIP cohort [6,10], which revealed a higher all-cause mortality in men with low IGF-I [6,19,20] or low total testosterone levels [10,21,22], in the InCHIANTI study low IGF-I or low bioavailable testosterone as single hormones were not related to mortality [12]. Small patient and event numbers (InCHIANTI 126 deaths vs. SHIP 359 deaths) might be explain the different findings.

Furthermore, the cut-off to define low free or total testosterone levels plays an important role as shown in Fig. 1. The InCHIANTI study used a higher value of 2.4 nmol/l (lowest quartile) to define low testosterone levels, independent of age and major confounders [23]. This cut-off might be too unspecific to detect an association between low testosterone levels and mortality as we show in our study population for the 20th percentile. A further study in 208 men with chronic heart failure, also investigating IGF-I and testosterone, similarly revealed decreasing survival rates with an increase in the number of low hormones [11]. Furthermore, each single hormone level was inversely related to mortality in this study. In contrast to former studies our present study...
population consisted of men who were not selected by age or according to a specific disease with per se increased mortality risk. Our cohort covered a broader age-range and included over 4000 men with 359 cases of death [11,12].

Changes in IGF-I and testosterone levels due to the aging process are well documented. Both IGF-I and total testosterone levels significantly decline with age and thus it seems consequential that lower hormone levels are related to increased morbidity [3,24]. Whether IGF-I or total testosterone levels directly influence mortality, or represent biomarkers of underlying morbidity is still subject of debate. However, the concept that low levels of hormones may play an independent causal role in longevity, is not surprising. Multisystemic disorders that are widely prevalent in aging subjects, such as the metabolic syndrome, frailty syndrome, and chronic heart failure, are more likely associated with multiple hormonal dysregulation rather than a single hormonal derangement [11,25]. There is increasing evidence that aging or subclinical diseases are associated with a decline in hormones and increase in catabolic hormones [26]. Therefore, hormonal deficiency might be a surrogate parameter of disease severity, especially in men. In face of the present findings, it was beyond the scope of our study to analyze if the association of hormonal dysregulation with increased mortality is also present for other non-anabolic hormones such as, for example, thyroid hormone levels. Even though, an analysis of the SHIP cohort revealed no independent association of thyrotropin levels with mortality [27], further studies are needed for a conclusive answer. In face of the fact that we used age-specific low hormone levels it is arguable to break the problem only down to the aging process. One possible, but clearly theoretical, explanation might be a complex interaction between genetic background, individual risk behaviour and hormone regulation. Several genome-wide association studies showed that genetic variants of the fat mass- and obesity-associated gene (FTO) are related to obesity [28,29], which in turn represents a major cause for low testosterone levels in men. Furthermore, another investigation on the SHIP collective revealed that the FTO risk alleles were associated with lower IGF-I levels independently of obesity [30]. Moreover, obesity and the FTO gene are associated with an abnormal glucose metabolism as well as cardiovascular disease in men and therefore, are risk factors for mortality [31]. All these facts contribute to the complex interplay between hormone regulation and mortality. However the present study has, due to its epidemiological design, not the ability to clarify specific pathways which explain the presence of both low IGF-I and testosterone levels and their relation to a higher mortality.

The ongoing discovery of new biomarkers presents opportunities and challenges for biostatisticians and clinicians to evaluate and judge these new biomarkers and to develop new risk models for a more individualized medical approach. Therefore we estimated the IDI to examine whether the prediction of all-cause mortality by a combination of both hormones is superior. In the present study, the estimates of IDI suggest that the addition of IGF-I and total testosterone to the model with established mortality risk factors did significantly improve the discriminatory property of the prediction model. However, the improvement was low.

The aim of our study was to verify the previously described association between the combination of low hormone levels and all-cause mortality based on low IGF-I and total testosterone levels in large setting. The analysed studies were conducted in the same country with the same health care system and covered a wide age-range. However, both studies based on different backgrounds (primary care and population-based) which results in differences with respect to e.g. age, WHTR, and proportion of diabetes or increased blood pressure (Table A.2). One further strength was the use of the same assay in both studies to measure IGF-I levels. Limitations might have arisen from the single determination of IGF-4 or total testosterone which was measured only at baseline. Additionally, hormone levels may vary because of individual fasting or the time of day in particular for total testosterone. Blood samples were taken between 07:00 a.m. and 04:00 p.m., however, a previous investigation in SHIP showed only minor differences in total testosterone levels between serum samples drawn before and after noon [32], therefore, this variation is expected to be minimal. Furthermore, different assay systems to determine total testosterone were used (SHIP: Siemens Immulite 2500, DETECT: Roche Diagnostics) and the assays were not cross-checked between both studies. Therefore, low total testosterone levels were separately defined by percentiles in both studies. However, analyses using the overall 10th percentile to define low hormone levels confirmed our main results (Table A.3). A further limitation arise from the missing measurement of sex hormone-binding globulin, therefore no calculation of the free and bioavailable testosterone was possible. Moreover, the follow-up in our study, particularly in the DETECT study was rather short and less than 10% of all participants died during follow-up. Therefore we can only draw conclusions on early death and we do not know if our findings extend to longer-term follow-up. Furthermore, analyses with cause-specific mortality would further clarify the relation between multiple anabolic deficiencies and mortality. Unfortunately due to the small number of subjects with both low IGF-I and testosterone levels and inferential


