Received Date : 17-May-2012
Returned for Revision: 06-Sep-2012
Finally Revised Date : 12-Sep-2012
Accepted Date : 12-Sep-2012
Article category : R

Title

Associations of Insulin-like Growth Factor-I and its binding proteins, and testosterone, with frailty in older men

Short title

IGF-I, binding proteins, testosterone and frailty

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Key words

Insulin-like growth factor-I, IGF-binding proteins, testosterone, frailty, male ageing

Acknowledgements

The authors thank Sylvia Johnson of DSL Australia for advice regarding the assays of IGF-I and its binding proteins, Zoe Hyde for advice regarding the FRAIL scale, staff of Fremantle Hospital for assistance with the assays, management and staff of Shenton Park Hospital, and all the participants in the Western Australian Abdominal Aortic Aneurysm Program and the Health In Men Study. We acknowledge assistance received from the Data Linkage Unit of the Health Department of Western Australia.

Disclosures

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Abstract

Objective

Ageing is associated with frailty, and decreased anabolic hormones, insulin-like growth factor-I (IGF-I) and testosterone. We hypothesised that components of the IGF-I system, in conjunction with testosterone, modulate frailty risk in the elderly. We examined associations between IGF-I, its binding proteins IGFBP1 and IGFBP3, and testosterone with frailty in men.

Design

Observational study of 3,447 community-dwelling men aged 70-89 years assessed in 2001-4, with 1,654 re-assessed in 2008-9.

Methods

Baseline total IGF-I, IGFBP1, IGFBP3 and testosterone were assayed. Frailty was assessed using the FRAIL scale, comprising 5 domains: fatigue; difficulty climbing stairs; difficulty walking >100 m; >5 illnesses; weight loss >5%. Men with ≥3 domains were considered frail.

Results

At baseline 527 men (15.3%) were frail. Frail men had lower IGFBP3 (3,630 ng/ml vs not frail: 3,800 ng/ml, $p<0.001$) and comparable IGFBP1 (23.5 vs 21.5 ng/ml, $p=0.09$). In multivariate analyses, higher IGFBP1 was associated with increased prevalence of frailty (highest vs lowest quartile Q4:Q1, adjusted odds ratio [OR]=1.39, 95%CI=1.03-1.88). New onset frailty arose in 260 (17.5%) of 1,484 men. Lower baseline IGF-I predicted new onset frailty (Q1:Q4 OR=1.48, 95%CI=1.00-2.20) as did higher IGFBP1 (Q4:Q1 OR=1.59, 95%CI=1.01-2.50). Men with both IGF-I and free testosterone in Q1 had greater odds of prevalent frailty (OR=2.13, 95%CI=1.54-2.95).

Conclusions

Older men with higher IGFBP1 level, or both lower IGF-I and testosterone, are more likely to be frail, while those with lower IGF-I and higher IGFBP1 are more likely to become frail. Components of the IGF-I system may be biomarkers or independent predictors of frailty.

Introduction

Ageing is characterised by an increase in the prevalence of frailty, conceptualised by deterioration of multiple organ systems leading to loss of physiological reserve, diminished capacity to cope with stressors, and increased risk of disability and death [1,2]. One definition of frailty utilises the presence of three or more of five components: unintentional weight loss, exhaustion, poor grip strength, slow walking speed, or low physical activity [1]. The FRAIL scale has been proposed as an alternative tool for screening for frailty [3]. This utilises five elements of Fatigue, Resistance, Ambulation limitation, Illnesses, and Loss of weight, with frailty represented by the presence of three or more of these elements. Both scales reflect the presence of sarcopenia, or loss of lean muscle mass, which in turn leads to decreased muscle function which is regarded as a key factor in the development of frailty.

The underlying mechanisms that contribute to development of frailty as people age remain poorly understood. Insulin-like growth factor-I (IGF-I) is an anabolic hormone that regulates body composition and maintains muscle mass [4]. Ageing is associated with a decline in pituitary growth hormone secretion, resulting in reduced liver production of insulin-like growth factor-I (IGF-I) [5]. The bioavailability of IGF-I is modulated by binding to circulating IGF-binding proteins (IGFBPs), with the majority of circulating IGF-I carried within a ternary complex bound to IGFBP3 and the acid labile subunit (ALS) [6]. IGFBP1 binds a smaller fraction of plasma IGF-I, but in a manner responsive to metabolic status [7]. Insulin suppresses hepatic IGFBP1 synthesis, decreasing the amount available for binding

IGF-I and accounting for the association of reduced IGFBP1 with insulin resistance or the metabolic syndrome [8]. IGF-I has been investigated as a potential contributor to risk of frailty in women [9,10]. However, despite its role as an anabolic hormone and its decline with age, there are limited data exploring the role of circulating IGF-I and its binding proteins in the genesis of frailty in ageing men.

We have previously shown that examining different components of the IGF-I system can be more informative than analysing circulating levels of IGF-I alone [11]. Therefore we examined associations of IGF-I and its binding proteins IGFBP1 and IGFBP3 with frailty in a population-based cohort of community-dwelling older men. Testosterone is another anabolic hormone [12]. As lower testosterone levels are associated with frailty [13], we assessed whether stratifying by both IGF-I and testosterone levels would identify men most at risk of being or becoming frail. Additionally, indices of insulin resistance are predictors of frailty [14], prompting us to exclude men with metabolic syndrome in a supplementary analysis.

Methods

Study population

The Health In Men Study (HIMS) is a longitudinal study of men who originally participated in a trial of screening for abdominal aortic aneurysm [15]. Briefly, between 1996-99 (wave 1: W1), 12,203 community-dwelling men aged 65 years and older resident in Perth, Western Australia attended a clinic and completed a questionnaire, providing a range of demographic and risk factor data. In 2001-04 (wave 2: W2), 4,249 of surviving men attended a clinic, completed a second questionnaire and provided a blood sample. In 2008-09 (wave 3: W3), surviving men were mailed a third questionnaire. A participant flow chart is shown in Figure

1. These men were predominantly of Caucasian ethnicity. Demographic, medical and medications data were collected. The Human Research Ethics Committee of the University of Western Australia approved the study protocol and all men gave written informed consent before entering the study.

Assessment of frailty

Frailty was assessed at W2 and at W3 using the FRAIL scale [3]. This screening tool assesses five domains: **F**atigue, **R**esistance, **A**mbulation, **I**llnesses, and **L**oss of weight. Fatigue, resistance and ambulation were assessed from responses to the SF-36 Health Survey [16]. Participants scored positive for **F**atigue if they responded "all of the time", "most of the time", or "a good bit of the time" to the questions "did you feel worn out?" or "did you feel tired?", or answered "some of the time", "a little of the time", or "none of the time" to the question "did you have a lot of energy?". Deficits were recorded for **R**esistance or **A**mbulation if participants reported that they were "limited a lot" or "limited a little" in their ability to climb one flight of stairs, or walk 100 metres respectively. A deficit was recorded for **I**llness if the participant reported more than 5 of the following: arthritis, diabetes, angina or myocardial infarction, hypertension, stroke, asthma, chronic bronchitis, emphysema, osteoporosis, colorectal cancer, skin cancer, depression or an anxiety disorder, Alzheimer's disease or other dementia, or leg ulcers. Participants scored positive for **L**oss of weight if their weight decreased by more than 5% between W1 and W2, or between W2 and W3. Men were classified as being frail if they presented problems in three or more of these five domains.

Assessment of medical comorbidities

Questionnaire responses were utilised to collect data on medical comorbidities. Smoking history was categorised as current, ex-smoker or life-long non-smoker. Hypertension was defined as a recorded blood pressure ≥140/90 or having a diagnosis of hypertension or receiving treatment for high blood pressure. Dyslipidemia was defined as having HDL <0.9 mmol/L, LDL ≥3.4 mmol/L, triglycerides ≥1.8 mmol/L or total cholesterol ≥5.5 mmol/L, or receiving lipid-lowering therapy. Diabetes was defined as having been diagnosed with or receiving treatment for diabetes, fasting glucose level >7 mmol/L or non-fasting glucose >11.1 mmol/L. Cardiovascular disease (CVD) was defined as history of angina, acute myocardial infarction or stroke by questionnaire responses, or hospital diagnoses of these conditions prior to Wave 2. Additional information on medical co-morbidities was obtained from the Western Australian Data Linkage System which links together records from the Mental Health Information System, cancer register, death register and hospital morbidity data [17].

Laboratory assays

Blood samples were collected between 0800 and 1030 hours. Plasma was prepared immediately following phlebotomy and stored at -80^{o}C until assayed. Hormone assays were performed as previously described [11]. Briefly, total IGF-I, IGFBP1 and IGFBP3 were assayed using reagent kits of single lot numbers from Diagnostics Systems Laboratories Inc (DSL, supplied by Beckman Coulter, Gladesville, NSW, Australia). The assays were automated using a Grifols Triturus ELISA processor (Vital Diagnostics, Castle Hill, NSW, Australia). For measurement of total IGF-I, samples were pretreated with acid to displace IGF-I from binding proteins, followed by neutralisation and addition of binding inhibitors prior to assay. Between-run imprecision (coefficient of variation) was 12.2% and 8.6% at

117 and 216 ng/ml IGF-I; 8.6% and 5.2% at 3.1 and 49 ng/ml IGFBP1; and 16.8% and 4.4% at 540 and 4300 ng/ml IGFBP3. All assays were carried out on freshly thawed aliquots of EDTA plasma in a series of runs performed between January 2008 and February 2009. Testosterone results measured by immunoassay in this cohort have been previously reported, as have calculated free testosterone values [18]. Coefficient of variation for testosterone was 11.2% at 7.2 nmol/l and 8.9% at 18 nmol/l [18].

Statistical analysis

Data were analysed with the statistical package Stata, version 11.2 (StataCorp, 2011). Continuous variables were presented as mean ± standard deviation (SD) and comparisons performed using Student's t-test. Nominal variables were presented as percentages (%) and compared with the Chi-squared test. Logistic regression analysis was used to assess odds ratio for frailty according to quartiles of IGF-I, IGFBP1 or IGFBP3. To determine if there were trends across quartiles of IGF-I, IGFBP1 and IGFBP3, the data were tested for linearity and entered as a continuous variable in the model. A trend was considered to exist if the odds ratio for these quartiles when entered as a continuous variable was significantly different from 1 ($p<0.05$). Regression analyses were adjusted for age, BMI, smoking, fasting status at time of blood collection and diabetes. Cross-sectional analyses were conducted to test associations of IGF-I and its binding proteins with presence of frailty at W2. Longitudinal analyses were performed after excluding men with frailty at W2, to test associations of IGF-I and its binding proteins with new onset of frailty at W3. As men with lower total IGF-I and higher IGFBP1 levels might be expected to have less bioavailable IGF-I, associations with frailty were examined after stratifying men accordingly. Finally, logistic regression models were used to assess risk of frailty for men with both IGF-I and free testosterone levels in the

lowest quartile of their respective values. In a supplementary analysis, we excluded men with metabolic syndrome. P values of <0.05 were considered significant.

Results

IGF-I, IGFBP3 and IGFBP1 levels in frail men

Of 4,249 men who were assessed at W2, after excluding men for whom suitable plasma aliquots could not be retrieved and men with incomplete data, IGF-I, IGFBP1 and IGFBP3 data were available for 3,970 men. Descriptive data from this cohort have been published previously showing that IGF-I and IGFBP3 levels were negatively correlated with increasing age, while IGFBP1 levels were positively correlated [11]. We then excluded 333 men who reported use of testosterone, hormonal therapies interfering with androgen production or action, and prostate cancer. Another 190 men had incomplete data, leaving 3,447 men for the analysis. Demographic, physical and biochemical data for these men are shown in Table 1. 527 men were classified as frail at baseline (15.3%), and these men had comparable IGFBP1 and lower IGFBP3 levels compared with men who were not.

Associations of IGF-I, IGFBP1 and IGFBP3 with prevalent frailty

In univariate analyses, higher IGFBP3 was associated with reduced odds ratio (OR) of frailty (highest vs lowest quartile, Q4:Q1 OR=0.59, 95% confidence interval [CI]=0.46-0.77, p<0.001 for trend) (Table 2). However, this association was attenuated after adjustment for covariates. In the multivariate analysis, higher IGFBP1 was associated with increased OR for frailty (Q4:Q1: adjusted OR=1.39, 95%CI=1.03-1.88, p=0.035 for trend).

Associations of IGF-I, IGFBP1, IGFBP3 and IGF-I/IGFBP3 with new onset frailty

Of the 3,447 men in the cross-sectional analysis, 2,959 were alive at W3. Of these, 1,654 completed and returned the questionnaire at W3. Men who did not respond were older (77.6 vs 75.6 years, p<0.001) and more likely to be frail (14.9 vs 10.9%, p=0.001) at W2. Excluding 170 men who were frail at W2 there were 1,484 men for the longitudinal analysis, aged 76-93 years at W3. 260 of these men who were not frail at W2 had become frail at W3 (17.5%). In univariate analysis, lower IGF-I level was associated with increased risk of new onset frailty (Q1:Q4 OR=1.66, 95%CI=1.13-2.43, p=0.015 for trend). Higher IGFBP3 was associated with reduced risk (p=0.038 for trend) (Table 3). However, following adjustment for covariates both these associations were attenuated. There were trends toward an association between lower IGF-I and increased odds of new onset frailty (Q1:Q4, adjusted OR=1.48, 95%CI=1.00-2.20, p=0.081 for trend) and higher IGFBP1 and increased odds of new onset frailty (Q4:Q1, adjusted OR=1.59, 95%CI=1.01-2.50, p=0.085 for trend).

Stratification by low IGF-I and high IGFBP1 level

To investigate these trends further, we stratified men according to IGF-I level in the highest quartile and IGFBP1 in the lowest (>173 and <11.8 ng/ml respectively), IGF-I in the lowest quartile and IGFBP1 in the highest (<103 and >32.9 ng/ml respectively) with the remaining men as the reference group (Table 4). The interaction term for IGF-I and IGFBP1 was not significant (z=0.52, p=0.604). In the multivariate analysis, men with IGF-I <103 ng/ml and IGFBP1 >32.9 ng/ml possessed a two-fold increased risk for new onset frailty (adjusted OR=2.08, 95%CI=1.22-3.53).

Stratification by low IGF-I and low free testosterone level

To assess whether men with both low IGF-I and low free testosterone levels would have the greatest risk of being or becoming frail, we stratified men using thresholds for the lowest quartile of IGF-I (<103 ng/ml) and the lowest quartile for free testosterone (<222 pmol/L) (Table 5). In univariate analyses, men with both IGF-I and testosterone values in the lowest quartiles had increased odds of being frail at baseline (OR=2.90, 95%CI=2.14-3.94). In multivariate analyses the association of low IGF-I and low testosterone with prevalent frailty remained (OR=2.13, 95%CI=1.54-2.95). There was a significant interaction between IGF-I and free testosterone in the analysis of frailty at baseline (z=-3.45, p=0.001). Men with both low IGF-I and free testosterone were more likely to be frail compared to men with low free testosterone alone (OR=1.45, 95%CI=1.01-2.07, p=0.04).

There was no significant association of low IGF-I and low testosterone with new onset frailty in univariate (OR=1.53, 95%CI 0.85-2.75) or multivariate analyses (OR=1.12, 95%CI=0.59-2.10). In the adjusted analysis, men with low IGF-I and normal free testosterone were more likely to become frail (OR=1.50, 95%CI=1.05-2.15).

Supplementary analysis

Insulin resistance is associated with lower IGFBP1, and men with low IGFBP1 are more likely to have metabolic syndrome [8,11]. To examine whether presence of metabolic syndrome might confound the association of IGFBP1 with frailty, we performed a sensitivity analysis excluding all men with metabolic syndrome. Metabolic syndrome was defined according to the NCEP-ATPIII classification as 3 or more of: waist circumference >102 cm, fasting plasma glucose ≥5.6 mmol/L or a known diagnosis of diabetes, fasting serum

triglycerides ≥1.7 mmol/L, fasting HDL cholesterol <1.03 mmol/L or blood pressure ≥130/85 mmHg [11]. In multivariate analysis, higher IGFBP1 remained associated with increased odds of frailty at baseline (Q4:Q1 adjusted OR=1.63, 95%CI=1.07-2.47, p=0.017 for trend).

Discussion

We found that there was no independent association of either IGF-I or IGFBP3 with prevalent frailty or new onset of frailty in community-dwelling older men. However, men with higher IGFBP1 levels were more likely to be frail, while those with either IGF-I in the lowest quartile or IGFBP1 in the highest quartile were more likely to become frail. Men with both lower IGF-I and lower free testosterone levels were more likely to be frail.

These results are relevant as previous studies have suggested a role for IGF-I in the maintenance of muscle mass and prevention of sarcopenia [4]. IGF-I mediates growth and repair in skeletal muscle, hence the expectation that lower IGF-I levels might predispose to frailty (for review, see [19]). However, GH supplementation at a dose sufficient to increase circulating IGF-I did not enhance the improvement in muscle strength following exercise training in older men [20]. A systematic review of GH therapy in healthy older adults concluded that the available data were limited, with GH treatment resulting in modest decreases in fat mass and increases in lean body mass [21]. A previous study of 494 women aged 70-79 years reported an increased odds ratio for frailty in those with IGF-I in the lowest quartile, which did not reach statistical significance [9]. A cross-sectional analysis reported a bivariate U-shaped association: compared to women with low white blood cell (WBC) counts and high IGF-I levels defined by tertiles, women with low WBC counts and low IGF-I levels, high WBC counts and low IGF-I, and high WBC and high IGF-I levels had

increased odds of frailty [10]. Our findings in a large cohort of older men demonstrate no independent associations of IGF-I or IGFBP3 with frailty, and thus do not support the concept that interventions which raise IGF-I levels might prevent frailty.

Lower circulating IGF-I has been associated with an increased risk of heart failure and occlusive atherosclerotic disease such as stroke and coronary heart disease [22-24]. Lower IGF-I levels have also been associated with all-cause or CVD-related mortality [25,26]. A recent meta-analysis reported a U-shaped association of IGF-I with mortality, with increased mortality associated with low and high IGF-I levels (10^{th} vs 50^{th} and 50^{th} vs 90^{th} percentiles, [27]). However, the hazard ratios were modest (1.56 and 1.29 respectively). Published studies are not wholly consistent, for instance in this cohort we found no independent association of IGF-I levels with either all-cause or cardiovascular mortality [28]. We did find that lower and higher IGF-I and IGFBP3 levels were associated with metabolic syndrome [11], which is consistent with the findings of the meta-analysis with respect to mortality [27]. Therefore the IGF-I system may contribute to, or be a biomarker for, cardiovascular disease in ageing. However, circulating IGF-I in isolation is not closely associated with the outcome of frailty.

In this study lower IGFBP1 levels were associated with lower odds of frailty at baseline. Lower IGFBP1 levels are associated with metabolic syndrome, but this association remained unchanged after exclusion of all men with metabolic syndrome who would be expected to have a greater degree of insulin resistance. Therefore, the finding is unlikely to be due to confounding from this source. We postulate that lower IGFBP1 levels might reflect reduced binding capacity for IGF-I and therefore the presence of increased proportions of unbound or bioavailable IGF-I, and that this fraction of IGF-I could be the most relevant [7,26]. Of note,

men with both lower IGF-I and higher IGFBP1 levels were significantly more likely to become frail, showing a two-fold increased risk in the multivariate analysis. Thus higher IGFBP1, or the combination of lower IGF-I and higher IGFBP1, could reflect reduced levels of bioavailable or bioactive IGF-I and loss of its anabolic action predisposing to sarcopenia. It is also possible that IGFBP1 may act independently of IGF-I on a cellular level to influence specific tissues [29]. Higher IGFBP1 levels have been associated with increased all-cause and cardiovascular mortality [28,30,31]. Therefore, further studies are needed to clarify whether IGFBP1 is a biomarker or a causal factor for divergent health outcomes including frailty. Additional studies are also required to explore the interaction of IGFBP1 and IGF-I levels on other health outcomes.

Lower testosterone levels are associated with frailty in both cross-sectional and longitudinal analyses from this cohort and elsewhere [13,32]. The combination of both testosterone and GH therapy appears to additively increase lean body mass and reduce fat mass when compared to either agent alone [33,34]. Therefore men with both low IGF-I and low testosterone levels could be most at risk for frailty. We demonstrated that men with low IGF-I and low free testosterone levels were more likely to be frail, but not more likely to become frail over time. We offer two explanations for this observation. The combination of low testosterone and low IGF-I may be a biomarker for existing frailty, rather than an independent predictor of its onset *de novo*. If so, underlying ill-health may be a common factor underlying both low anabolic hormone levels and frailty. Alternatively, it is possible that the reduced number of men re-assessed in 2008-09 might have resulted in inadequate statistical power to demonstrate a longitudinal association of low testosterone and low IGF-I levels with frailty. Of note, in this analysis men with low IGF-I and normal free testosterone

had a significantly increased risk of becoming frail, supporting the relevance of the IGF-I system to the development of frailty.

The strengths of this study include the large sample size, the availability of results for both IGF-I and its binding proteins and for testosterone, adjustment for several potential confounders in the multivariate analyses, and the combination of cross-sectional and longitudinal analyses. Study limitations include the fact that the cohort comprised men who returned for assessment and blood sampling from an earlier population-based sample and only a proportion of surviving men responded to the questionnaire at W3. Therefore a "healthy survivor" effect is possible which would make our results more conservative and applicable to generally healthier community-dwelling older men. The limited response at W3 may have reduced our power to detect underlying associations of IGF-I and its binding proteins with new onset frailty. We used a single blood sample, and did not have the capacity to measure free or unbound IGF-I levels either by immunoassay or ultracentrifugation, nor were we able to measure circulating IGF-I bioactivity [26]. Therefore our assessment was limited to circulating total IGF-I, IGFBP1 and IGFBP3. We measured testosterone by immunoassay, and calculated free testosterone values [18]. We used the FRAIL scale to assess frailty, rather than a measure based on the Fried criteria [1]. However, we have shown that the FRAIL scale is a robust predictor of mortality in this cohort of older men [13]. A component of the FRAIL scale is loss of weight, which may be associated gain of insulin sensitivity and therefore with higher IGFBP1 levels [8], or with higher testosterone levels [35]. If this were the case, it would result in frail men having higher IGFBP1 and testosterone levels, hence would tend to attenuate rather than accentuate the association between low IGFBP1 and low testosterone with frailty.

In conclusion, this study shows that investigation of IGF-I in concert with its binding proteins including IGFBP1, or with other anabolic hormones such testosterone, is more predictive of frailty than assessment of circulating IGF-I alone. Men with low IGF-I and low testosterone levels are more likely to be frail, while those with low IGF-I and high IGFBP1 levels are more likely to become frail. Further research is required to clarify whether components of the IGF-I system are biomarkers for underlying ill-health reflected by the presence of frailty, or causative factors contributing to its development.

Funding

This work was supported by the National Health and Medical Research Council of Australia (NHMRC) Project Grants 279408, 379600, 403963, 513823 and 634492, and by a Clinical Investigator Award to BBY from the Sylvia and Charles Viertel Charitable Foundation, New South Wales, Australia.

References

1. Fried, L.P., Tangen, C.M., Walston, J., Newman, A.B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W.J., Burke, G. & McBurnie, M.A. (2001) Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, **56**, M146-156.
2. Ahmed, N., Mandel, R. & Fain, M.J. (2007) Frailty: an emerging geriatric syndrome. *American Journal of Medicine*, **120**, 748-753.
3. Abellan van Kan, G., Rolland, Y., Bergman, H., Morley, J.E., Kritchevsky, S.B. & Vellas, B. (2008) The I.A.N.A. Task Force on frailty assessment of older people in clinical practice. *Journal of Nutrition, Health and Aging*, **12**, 29-37.

4. Perrini, S., Laviola, L., Carreira, M.C., Cignarelli, A., Natalicchio, A. & Giorgino, F. (2010) The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. *Journal of Endocrinology*, **205**, 201-210.
5. Sherlock, M. & Toogood, A.A. (2007) Aging and the growth hormone/insulin like growth factor-I axis. *Pituitary*, **10**, 189-203.
6. Ohlsson, C., Mohan, S., Sjogren, K., Tivesten, A., Isgaard, J., Isaksson, O., Jansson, J-O. & Svensson, J. (2009) The role of liver-derived insulin-like growth factor-I. *Endocrine Reviews*, **30**, 494-535.
7. Juul, A. (2003) Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Hormone & IGF Research*, **13**, 113-170.
8. Kotronen, A., Lewitt, M., Hall, K., Brismar, K. & Yki-Jarvinen, H. (2008) Insulin-like growth factor binding protein 1 as a novel specific marker of hepatic insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism*, **93**, 4867-4872.
9. Cappola, A.R., Xue, Q-L. & Fried, L.P. (2009) Multiple hormone deficiencies in anabolic hormones are found in frail older women: The Women's Health and Aging Studies. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, **64**, A243-248.
10. Leng, S.X., Hung, W., Cappola, A.R., Yu, Q., Xue, Q-L. & Fried, L.P. (2009) White blood cell counts, insulinlike growth factor-1 levels, and frailty in community-dwelling older women. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, **64**, A499-502.
11. Yeap, B.B., Chubb, S.A.P., Ho, K.K.Y., Setoh, J.W.S., McCaul, K.A., Norman, P.E., Jamrozik, K. & Flicker, L. (2010) Insulin-like growth factor-I and its binding proteins 3

and 1 are differentially associated with metabolic syndrome in older men. *European Journal of Endocrinology*, **162**, 249-257.

12. Tracz, M.J., Sideras, K., Boloña, E.R., Haddad, R.M., Kennedy, C.C., Uraga, M.V., Caples, S.M., Erwin, P.J. & Montori, V.M. (2006) Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. *Journal of Clinical Endocrinology and Metabolism*, **91**, 2011-2016.
13. Hyde, Z., Flicker, L., Almeida, O.P., Hankey, G.J., McCaul, K.A., Chubb, S.A.P. & Yeap, B.B. (2010) Low free testosterone predicts frailty in older men. The Health in Men Study. *Journal of Clinical Endocrinology and Metabolism*, **95**, 3165-3172.
14. Barzilay, J.I., Blaum, C., Moore, T., Xue, Q.L., Hirsch, C.H., Walston, J.D. & Fried, L.P. (2007) Insulin resistance and inflammation as precursors of frailty. The Cardiovascular Health Study. *Archives of Internal Medicine*, **167**, 635-641.
15. Norman, P.E., Flicker, L., Almeida, O.P., Hankey, G.J., Hyde, Z. & Jamrozik, K. (2009) Cohort Profile: The Health In Men Study (HIMS). *International Journal of Epidemiology*, **38**, 48-52.
16. Ware, J.E., Snow, K.K., Kosinski, M. & Gandek, B. (1993) SF-36 Health Survey Manual and Interpretation Guide. Boston: The Health Institute.
17. Holman, C.D., Bass, A.J., Rouse, I.L. & Hobbs, M.S. (1999) Population-based linkage of health records in Western Australia: development of a health services research linked database. *Australian and New Zealand Journal of Public Health*, **23**, 453-459.
18. Yeap, B.B., Almeida, O.P., Hyde, Z., Norman, P.E., Chubb, S.A.P., Jamrozik, K. & Flicker, L. (2007) In men older than 70 years, total testosterone remains stable while free testosterone declines with age. The Health In Men Study. *European Journal of Endocrinology*, **156**, 585-594.

19. Giovannini, S., Marzetti, E., Borst, S.E. & Leeuwenburgh, C. (2008) Modulation of GH/IGF-1 axis: potential strategies to counteract sarcopenia in older adults. *Mechanisms of Ageing and Development*, **129**, 593-601.

20. Taaffe, D.R., Pruitt, L., Reim, J., Hintz, R.L., Butterfield, G., Hoffman, A.R. & Marcus, R. (1994) Effect of recombinant growth hormone on the muscle strength response to resistance exercise in elderly men. *Journal of Clinical Endocrinology and Metabolism*, **79**, 1361-1366.

21. Liu, H., Bravata, D.M., Olkin, I., Nayak, S., Roberts, B., Garber, A.M. & Hoffman, A.R. (2007) Systematic review: the safety and efficacy of growth hormone in the healthy elderly. *Annals of Internal Medicine*, **146**, 104-115.

22. Juul, A., Scheike, T., Davidsen, M., Gyllenborg, J. & Jorgensen, T. (2002) Low serum insulin-like growth factor 1 is associated with increased risk of ischemic heart disease. A population-based case-control study. *Circulation*, **106**, 939-944.

23. Johnsen, S.P., Hundborg, H.H., Sorensen, H.T., Orskov, H., Tjonneland, A., Overvad, K. & Jorgensen, J.O. (2005) Insulin-like growth factor (IGF) I, -II and IGF binding protein-3 and risk of ischemic stroke. *Journal of Clinical Endocrinology and Metabolism*, **90**, 5937-5941.

24. Vasan, R.S., Sullivan, L.M., D'Agostino, R.B., Roubenoff, R., Harris, T., Sawyer, D.B., Levy, D. & Wilson, P.W. (2003) Serum insulin-like growth factor 1 and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. *Annals of Internal Medicine*, **139**, 642-648.

25. Laughlin, G.A., Barrett-Connor, E., Criqui, M.H. & Kritz-Silverstein, D. (2004) The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular mortality in older adults: the Rancho Bernardo study. *Journal of Clinical Endocrinology and Metabolism*, **89**, 114-120.

26. Brugts, M.P., van den Beld, A.W., Hofland, L.J., van der Wansem, K., van Koetsveld, P.M., Frystyk, J., Lamberts, S.W.J. & Janssen, J.A.M.J.L. (2008) Low circulating insulin-like growth factor I bioactivity in elderly men is associated with increased mortality. *Journal of Clinical Endocrinology and Metabolism*, **93**, 2515-2522.

27. Burgers, A.M.G., Biermasz, N.R., Schoones, J.W., Pereira, A.M., Renehan, A.G., Zwahlen, M., Egger, M. & Dekkers, O.M. (2011) Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *Journal of Clinical Endocrinology and Metabolism*, **96**, 2912-2920.

28. Yeap, B.B., Chubb, S.A.P., McCaul, K.A., Ho, K.K.Y., Hankey, G.J., Norman, P.E. & Flicker, L. (2011) Associations of Insulin-like Growth Factor-I and its binding proteins 1 and 3 with all-cause and cardiovascular mortality in older men. The Health In Men Study. *European Journal of Endocrinology*, **164**, 715-723.

29. Wheatcroft, S.B. & Kearney, M.T. (2009) IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis. *Trends in Endocrinology and Metabolism*, **20**, 153-162.

30. Harrela, M., Qiao, Q., Koistinen, R., Tuomilehto, J., Nissinen, A., Seppala, M. & Leinonen, P. (2002) High serum insulin-like growth factor binding protein-1 is associated with increased cardiovascular mortality in elderly men. *Hormone and Metabolic Research*, **34**, 144-149.

31. Hu, D., Pawlikowska, L., Kanaya, A., Hsueh, W-C., Colbert, L., Newman, A.B., Satterfield, S., Rosen, C., Cummings, S.R., Harris, T.B. & Ziv, E. (2009) Serum insulin-like growth factor-1 binding proteins 1 and 2 and mortality in older adults: the Health, Aging, and Body Composition Study. *Journal of the American Geriatric Society*, **57**, 1213-1218.

32. Travison, T.G., Nguyen, A.H., Naganathan, V., Stanaway, F.F., Blyth, F.M., Cumming, R.G., Le Couteur, D.G., Sambrook, P.N. & Handelsman, D.J. (2011) Changes in reproductive hormone concentrations predict the prevalence and progression of the frailty syndrome in older men: the Concord Health and Ageing in Men Project. *Journal of Clinical Endocrinology and Metabolism*, **96**, 2464-2474.

33. Giannoulis, M.G., Sonksen, P.H., Umpleby, M., Breen, L., Pentecost, C., Whyte, M., McMillan, C.V., Bradley, C. & Martin, F.C. (2006) The effects of growth hormone and/or testosterone in healthy elderly men: a randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism*, **91**, 477-484.

34. Sattler, F.R., Castaneda-Sceppa, C., Binder, E.F., Schroeder, E.T., Wang, Y., Bhasin, S., Kawakubo, M., Stewart,Y., Yarasheski, K.E., Ulloor, J., Colletti, P., Roubenoff, R. & Azen, S.P. (2009) Testosterone and growth hormone improve body composition and muscle performance in older men. *Journal of Clinical Endocrinology and Metabolism*, **94**, 1991-2001.

35. Grossmann, M. (2011) Low testosterone in men with Type 2 diabetes: significance and treatment. *Journal of Clinical Endocrinology and Metabolism*, **96**, 2341-2353.

Figure legend

Participant flow chart showing the recruitment of men into the Health In Men Study cohort, and the numbers assessed at each of the successive stages.

Table 1

Baseline sociodemographic, clinical and biochemical characteristics of 3,447 community-dwelling men aged 70-89 years stratified according to the absence or presence of frailty. BMI=Body Mass Index; CVD=cardiovascular disease.

Variable	Not frail (≤2 domains)	Frail (≥3 domains)	P value
	n (%)	n (%)	
	2920 (84.7)	527 (15.3)	
FRAIL scale components			
Fatigue	955 (32.7)	500 (94.9)	<0.001
Resistance	485 (16.6)	517 (98.1)	<0.001
Ambulation	147 (5.0)	453 (86.0)	<0.001
Illness	46 (1.6)	97 (18.4)	<0.001
Loss of weight	488 (16.7)	186 (35.3)	<0.001
Smoking status			
Never smoked	1038 (35.6)	121 (23.0)	
Ex-smoker	1728 (59.2)	364 (69.1)	<0.001
Current smoker	154 (5.3)	42 (8.0)	
BMI			
<18.5kg/m^2 (underweight)	17 (0.6)	4 (0.8)	
18.5-24.9 kg/m^2 (normal)	994 (34.0)	171 (32.5)	
25.0-29.9 kg/m^2 (overweight)	1506 (51.6)	238 (45.2)	<0.001
>29.9 kg/m^2 (obese)	403 (13.8)	114 (21.6)	
Hypertension	2211 (75.7)	415 (78.8)	0.13
Dyslipidemia	2201 (75.4)	405 (76.9)	0.47
Diabetes mellitus	420 (14.4)	122 (23.2)	<0.001
CVD	571 (19.6)	186 (35.3)	<0.001
Depression	124 (4.2)	73 (13.9)	<0.001
Dementia	98 (3.4)	72 (13.7)	<0.001
	Median (25th, 75th percentiles)	**Median (25th, 75th percentiles)**	
Age (years)	75.8 (74.0, 78.7)	78.3 (75.0, 81.2)	<0.001

IGF-I (ng/ml)	133.0 (101.0, 171.0)	129.0 (94.9, 171.0)	0.18
IGFBP1 (ng/ml)	21.5 (12.1, 35.2)	23.5 (12.1, 39.8)	0.09
IGFBP3 (ng/ml)	3800 (3190, 4390)	3630 (2940, 4250)	<0.001
Total testosterone (nmol/l)	14.9 (11.9, 18.5)	13.6 (10.6, 16.9)	<0.001
Free testosterone (pmol/l)	274 (226, 330)	248 (196, 304)	<0.001

Table 2

Associations of IGF-I, IGFBP1 and IGFBP3 with prevalent frailty

Logistic regression models showing association of plasma total IGF-I, IGFBP1 and IGFBP3 by quartiles to prevalent frailty in 3,447 community-dwelling men aged 70-89 years. OR=odds ratio, CI=confidence interval. P-values are shown for overall trends. Quartiles of IGF-I were 100, 133, 171 ng/ml. Quartiles of IGFBP1 were 12.1, 21.7, 35.8 ng/ml. Quartiles of IGFBP3 were 3160, 3770, 4370 ng/ml.

		IGF-I		IGFBP1		IGFBP3	
		OR (95%CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
	Quartile						
Univariate	1	1.22 (0.95-1.58)		1.00		1.00	
	2	0.87 (0.66-1.14)	0.184	0.89 (0.68-1.17)	0.071	0.68 (0.53-0.88)	<0.001
	3	0.93 (0.72-1.21)		0.90 (0.69-1.18)		0.64 (0.50-0.83)	
	4	1.00		1.26 (0.98-1.63)		0.59 (0.46-0.77)	
Adjusted*	1	1.06 (0.81-1.39)		1.00		1.00	
	2	0.78 (0.59-1.04)	0.866	1.03 (0.77-1.38)	0.035	0.78 (0.60-1.02)	0.065
	3	0.91 (0.69-1.19)		1.08 (0.80-1.45)		0.81 (0.62-1.06)	
	4	1.00		1.39 (1.03-1.88)		0.76 (0.58-1.00)	

* adjusted for age, BMI, smoking, fasting status at time of blood sampling and diabetes.

Numbers of men in each quartile and the percentage of these (%) who were frail at baseline were: IGF-I Q1: 841 (18.1), Q2: 866 (13.5), Q3: 876 (14.4), Q4: 864 (15.3); IGFBP1 Q1: 859 (15.1), 865 (13.8), 864 (13.9), 859 (18.4); IGFBP3 Q1: 852 (20.0), 858 (14.6), 867 (13.8), 870 (12.9).

Table 3

Associations of baseline IGF-I, IGFBP1 and IGFBP3 with new onset frailty

Logistic regression models showing association of baseline plasma total IGF-I, IGFBP1 and IGFBP3 by quartiles to new onset of frailty in 1,484 community-dwelling men aged 76-93 years at follow-up. OR=odds ratio, CI=confidence interval. P-values are shown for overall trends. Quartiles of IGF-I were 103, 135, 173 ng/ml. Quartiles of IGFBP1 were 11.8, 20.6, 32.9 ng/ml. Quartiles of IGFBP3 were 3260, 3890, 4450 ng/ml.

		IGF-I		IGFBP1		IGFBP3	
		OR (95%CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
	Quartile						
Univariate	1	1.66 (1.13-2.43)		1.00		1.00	
	2	1.16 (0.79-1.70)	0.015	1.22 (0.84-1.78)	0.217	0.99 (0.68-1.45)	0.028
	3	1.17 (0.79-1.72)		1.07 (0.73-1.58)		0.76 (0.52-1.11)	
	4	1.00		1.37 (0.93-2.02)		0.70 (0.48-1.03)	
Adjusted*	1	1.48 (1.00-2.20)		1.00		1.00	
	2	1.08 (0.72-1.60)	0.081	1.32 (0.88-1.96)	0.085	1.12 (0.75-1.67)	0.289
	3	1.13 (0.76-1.69)		1.19 (0.77-1.82)		0.94 (0.63-1.41)	
	4	1.00		1.59 (1.01-2.50)		0.85 (0.57-1.27)	

*Adjusted for age, BMI, smoking, fasting status at blood sampling and diabetes.

Numbers of men in each quartile and the percentage of these (%) who had become frail were: IGF-I Q1: 326 (22.4), Q2: 388 (16.8), Q3: 379 (16.9), Q4: 391 (14.8); IGFBP1 Q1: 386 (15.5), 397 (18.4), 388 (16.5), 313 (20.1); IGFBP3 Q1: 313 (20.1), 345 (20.0), 406 (16.0), 420 (15.0).

Table 4

Logistic regression models showing odds ratio of frailty (A) at baseline in 3,447 men aged 70-89 years and (B) at follow-up in 1,484 men aged 76-93 years, stratified according to baseline plasma total IGF-I and IGFBP1 levels. OR=odds ratio, CI=confidence interval. For IGF-I the threshold for lowest quartile was 103 ng/ml, highest quartile 173 ng/ml, and IGFBP1 lowest quartile 11.8 ng/ml, highest quartile 32.9 ng/ml. Numbers of men in each subgroup who were frail (A) or had become frail (B) are shown in brackets (n).

		A		B	
		n	OR (95%CI)	n	OR (95% CI)
Univariate	IGF-I>173 and IGFBP1<11.8 ng/ml	272 (36)	0.86 (0.60-1.24)	123 (14)	0.60 (0.34-1.08)
	IGF-I<103 and IGFBP1>32.9 ng/ml	279 (56)	1.42 (1.04-1.94)	89 (24)	1.75 (1.07-2.85)
	Reference	2896 (435)	1.00	1272 (222)	1.00
Adjusted*	IGF-I>173 and IGFBP1<11.8 ng/ml	272 (36)	0.85 (0.58-1.25)	123 (14)	0.61 (0.34-1.11)
	IGF-I<103 and IGFBP1>32.9 ng/ml	279 (56)	1.36 (0.97-1.89)	89 (24)	2.08 (1.22-3.53)
	Reference	2896 (435)	1.00	1272 (222)	1.00

ljusted for age, BMI, smoking, fasting status at blood sampling, diabetes.

Note: p value for trend could not be determines due to deviation from linearity

Table 5
Logistic regression models showing odds ratio of frailty (A) at baseline in 3,444 men aged 70-89 years and (B) at follow-up 1,483 men aged 76-93 years, stratified according to baseline plasma total IGF-I and free testosterone in the lowest quartile of values. OR=odds ratio, CI=confidence interval. P-values are shown for overall trends. Threshold for the lowest quartile of IGF-I was 103 ng/ml and for lowest quartile of free testosterone (FT) 222 pmol/L. Numbers of men in each subgroup who were frail (A) or had become frail (B) are shown in brackets (n).

		A		B	
		n	OR (95%CI)	n	OR (95% CI)
Univariate	IGF-I<103 ng/ml and FT<222 pmol/L	245 (72)	2.90 (2.14-3.94)	72 (16)	1.53 (0.85-2.75)
	IGF-I<103 ng/ml and FT>222 pmol/L	596 (80)	1.08 (0.83-1.42)	254 (57)	1.55 (1.10-2.19)
	IGF-I>103 ng/ml and FT<222 pmol/L	609 (124)	1.78 (1.41-2.26)	241 (43)	1.16 (0.80-1.69)
	IGF-I>103 ng/ml and FT>222 pmol/L	1994 (250)	1.00	916 (144)	1.00

Adjusted*	IGF-I<103 ng/ml and FT<222 pmol/L	245 (72)	2.13 (1.54-2.95)	72 (16)	1.12 (0.59-2.10)
	IGF-I<103 ng/ml and FT>222 pmol/L	596 (80)	1.02 (0.78-1.35)	254 (57)	1.50 (1.05-2.15)
	IGF-I>103 ng/ml and FT<222 pmol/L	609 (124)	1.47 (1.15-1.89)	241 (43)	1.08 (0.73-1.60)
	IGF-I>103 ng/ml and FT>222 pmol/L	1994 (250)	1.00	916 (144)	1.00

* adjusted for age, BMI, smoking, fasting status at blood sampling and diabetes.
Note: p value for trend could not be determined due to deviation from linearity.

Figure 1