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Proinsulin and IGFBP-1 predicts mortality in an elderly population

**Simona I. Chisalita MD, PhD ¹, Ulf Dahlström MD, PhD ²,
Hans J. Arnqvist MD, PhD ³ and Urban Alehagen MD, PhD ²**

¹ Department of Acute Healthcare, County Council of Östergötland, Linköping, Cell Biology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Sweden,

² Division of Cardiovascular Medicine, Department of Medicine and Health Sciences, Faculty of Health Sciences, Linköping University, Department of Cardiology UHL, County Council of Östergötland, Linköping, Sweden

³ Cell Biology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Department of Endocrinology and Gastroenterology UHL, County Council of Östergötland, Linköping, Sweden

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Declaration of interest

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To whom correspondence should be addressed

Simona I. Chisalita

Department of Acute Healthcare

County Council of Östergötland, Linköping

S-581 85 Sweden

Telephone: + 46 010 1030000

Fax: +46 010 1037077

E-mail: ioana.simona.chisalita@liu.se

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Abstract

Background. High IGFBP-1 in elderly subjects is related to all-cause and cardiovascular (CV) mortality. We studied the relation of IGFBP-1 to cardiometabolic risk factors and cardiovascular and all-cause mortality, and also the impact of proinsulin and insulin on this association in an unselected elderly primary health care population.

Hypothesis. Our hypothesis was that proinsulin and insulin may have an impact on the association of high IGFBP-1 levels with all-cause and CV-mortality in elderly.

Design, Setting and Participants. Cross-sectional and prospective observational in a rural Swedish population. 851 persons aged 66-81 years, were evaluated by medical history, clinical examination, electrocardiography, echocardiography, and fasting plasma samples, and were followed prospectively for up to 12 years.

Results. At baseline, in a multivariate analysis, IGFBP-1 was associated with gender, N-terminal proBNP (NT pro-BNP), blood glucose, body mass index (BMI), insulin and proinsulin, estimated glomerular filtration rate (eGFR) and hemoglobin (Hb).

During the follow-up period there were 230 deaths (27%), of which 134 (16%) were due to CV mortality. When divided into tertiles there was a significant difference for CV mortality and all-cause mortality between tertiles of IGFBP-1 and proinsulin. For insulin there was a significant difference only for all-cause mortality. After adjustment for well-known risks factors, proinsulin and IGFBP-1 had significant impact on all-cause mortality but only proinsulin on CV mortality.

Conclusion. Only proinsulin is an independent predictor for both all-cause mortality and CV mortality when comparing IGFBP-1, insulin, and proinsulin as prognostic biomarkers for CV and all-cause mortality in an elderly population.

Keywords. Insulin-like growth factor binding protein-1, proinsulin, insulin, elderly population.

Introduction

The insulin-like growth factors and insulin belong to a common ancient family having a structure highly preserved among vertebrate and invertebrate. IGF-I and insulin share an evolutionary precursor hormone (1). By binding 99% of circulating IGF-I, IGF-BPs act as a natural store, which prolongs IGF-I half-life and modulates its biological activity (2). Both low and high levels of circulating IGF-I have been reported to be associated with increased mortality (3-6), whereas high IGF-1 levels have been related with cancer mortality (7).

Insulin-like growth factor binding protein-1 (IGFBP-1) is produced in the liver and is negatively regulated by insulin (8). IGFBP-1 modulates the bioavailability of IGF-I by binding free IGF-I (9, 10). Low levels of IGFBP-1 are associated with insulin resistance and with the metabolic syndrome (11). Several cardiometabolic risk factors such as obesity, hyperinsulinemia, diabetes, hypertension and dyslipidemia are associated with IGFBP-1 (12-15). In elderly subjects, elevated IGFBP-1 levels have been reported to be related to chronic heart failure (16, 17), and all-cause and cardiovascular (CV) mortality (18-22). It has been shown that IGFBP-1 inhibits cancer cell growth and migration, and therefore low IGFBP-1 levels result in higher risk of cancer mortality (23). However data on IGFBP-1 levels and mortality are conflicting (18, 19, 21, 24-27).

Insulin is synthesized as pre-proinsulin, which is processed to proinsulin by removal of the signal peptide, and then further processed to mature insulin by prohormone convertases and carboxypeptidase (28). Secretory signals trigger the release of insulin and C-peptide in equimolar amounts together with small amounts of uncleaved proinsulin. Hyperinsulinemia has been reported to predict all-cause (29) and CV mortality (30, 31). Proinsulin is related to CV risk factors, such as dyslipidemia and hypertension, in diabetic and non-diabetic subjects (32, 33). Proinsulin concentration is more strongly correlated with CV risk factors than fasting or non-

fasting insulin levels in both diabetic and non-diabetic subjects (34). Whether proinsulin has an impact on the association of IGFBP-1 in elderly subjects with overall and CV-related mortality is not known. High proinsulin levels have been associated with increased risk for cancer mortality (35). It has been speculated that this could be due to the different binding activity of proinsulin to the insulin receptors isoform A which could induce cell proliferation and migration (36).

Our hypothesis was that proinsulin and insulin may have an impact on the association of high IGFBP-1 levels with all-cause and CV-mortality in elderly. To test this hypothesis we studied the relation of IGFBP-1 as well as proinsulin and insulin to cardiometabolic risk factors and CV and total mortality in an unselected elderly population.

Research design and methods

The study population has previously been described in detail (37). Briefly, all individuals aged 66 to 81 years residing in a south-eastern Swedish rural municipality with a population of 10,300 were chosen. Of 1162 eligible subjects, 851 (73.3%) agreed to participate and give blood samples that are analysed in this evaluation.

All participants (851 persons) were examined by one of three experienced cardiologists. A medical history was taken, and a clinical examination, including weight and height, was performed. An electrocardiogram and Doppler echocardiography were obtained. The New York Heart Association functional class (NYHA Class) was assessed. Blood pressure was measured to the nearest 5 mm Hg, with the patient resting in a supine position. The study protocol was approved by the Ethics Review Board in Linköping.

Blood samples and biochemical analysis

Blood samples were obtained at baseline from fasting subjects after a resting period of 30 minutes. The samples were collected in pre-chilled plastic tubes containing EDTA (Terumo EDTA K-3), placed on ice, and centrifuged at 3000g for 10 minutes at 4°C, and then immediately stored at -70°C pending analysis. Total plasma IGF1 was measured by a one-step enzyme-linked immunosorbent assay (ELISA) with a commercial kit (R&D Systems, Minneapolis, MN, USA). At our lab, interassay coefficients of variation (CV) for high (1688 µg/L) and low (4 µg/L) controls were 7.8% and 20.0% respectively. Total IGF1 was measured by a one-step ELISA after acid-ethanol extraction from its binding protein using a commercial kit (Human IGF-I Quantikine ELISA Kit R&D Systems, Minneapolis, MN, USA). Interassay coefficients of variation were 10.9, 5.9, and 18.2% for high (278 µg/L), medium (116 µg/L), and low (45 µg/L) controls respectively. Insulin and proinsulin concentrations were measured by a one-step

enzyme-linked immunosorbent assay (ELISA) with a commercial kit (DAKO, Glostrup, Denmark). N-terminal proBNP (NT-proBNP) was measured using an electrochemiluminescence immunoassay (Elecsys 2010, Roche Diagnostics, Mannheim, Germany), first described by Karl (38). The analytical range was 5 to 35,000 ng/L (0.6 to 4130 pmol/L). The total coefficient of variation was 4.8% at the level of 217 ng/L (26 pmol/L) (n = 70) and 2.1% at the level of 4261 ng/L (503 pmol/L) at our laboratory.

Estimated glomerular filtration rate (eGFR) was calculated by use of the Cockcroft-Gault formula (39).

Doppler echocardiography

Doppler echocardiography (Accuson XP-128c) was performed on all patients in the left supine position. Both M-mode and two-dimensional methodologies were used. The left ventricular systolic function was determined semi-quantitatively, with the global systolic function classified as follows: normal (ejection fraction [EF] $\geq 50\%$); mild impairment (EF 40% to 49%); moderately impaired function (EF 30% to 40%); and severely impaired function (EF $< 30\%$). The method has been validated against the modified Simpson algorithm (40-41).

Concomitant diseases

Diabetes mellitus (DM) was defined as a fasting plasma blood glucose concentration ≥ 7.0 mmol/L, or current treatment for diabetes (diet, oral therapy or insulin). Ischemic heart disease (IHD) was defined as a history of coronary artery disease (CAD), angina pectoris, treatment for angina pectoris, a previously verified myocardial infarction, coronary artery bypass surgery, percutaneous coronary intervention, and/or ECG changes characteristic of IHD (evaluated by three experienced cardiologists).

Follow-up of included patients

All patients were followed-up for 12 years (7.9 ± 2.6) (mean \pm SD) and no patient was lost during this period. During the follow-up period all patients received standard treatment according to clinical routines.

When patients were reported to have died, death certificates were obtained from the Swedish Central Population Registry and information about cause of death and/or autopsy reports, if available, were analysed by the authors. All autopsies were performed at the University Hospital in Linköping. All-cause mortality and CV mortality were chosen as end points. CV mortality was defined as death caused by HF (heart failure), fatal arrhythmia, sudden death, IHD, or cerebrovascular death as deduced from the autopsy report or death certificate issued by the physician in charge of the patient.

Statistics

For normally distributed variables, data are presented as mean \pm SD. Patients were stratified into tertiles of IGFBP-1, insulin, proinsulin, and NT-proBNP. The differences between tertiles 1-3 of IGFBP-1 with regard to the characteristics of the studied population were analysed by ANOVA using Bonferroni as post hoc analysis. As IGFBP-1, insulin, proinsulin, and NT-proBNP concentrations were skewly distributed, they were all ¹⁰log transformed to normal distribution. Differences in distribution of patients with diabetes mellitus between tertiles 1-3 of IGFBP-1 were analysed by Chi-square test. The association between IGFBP-1 and cardiometabolic risk factors was analysed by Spearman's correlation test and also by multivariate regression. HOMA-IR (Homeostasis Model of Assessment - Insulin Resistance) as a marker of insulin resistance was calculated (fasting Glucose(mg/dl) x fasting Insulin(μ U/mL) / 405).

Cox proportional hazard regression analyses were used to analyse the risk of both all-cause and CV mortality during the total follow-up period. A multivariate Cox proportional hazard

regression analysis was used to assess the impact of gender, age >75 years, Hb<120 g/L, DM, IHD, EF<40%, eGFR <60 mL/min and tertiles of NT pro-BNP, IGFBP-1, insulin, and proinsulin levels. Survival analysis was made using Kaplan–Meier survival curve analysis. A p-value less than 0.05 was considered statistically significant. Data analysis was performed using the commercially available statistical analysis software package IBM SPSS statistic version 20 (SPSS Inc. Headquarters, Chicago, Illinois, USA).

Results

IGFBP-1 levels and characteristics of the study population

To analyse the characteristics of the study population in relation to IGFBP-1, tertiles of IGFBP-1 were used (Table 1a). When tested by ANOVA there were highly significant differences ($p < 0.001$) between tertiles of IGFBP-1 for age, eGFR < 60 , Hb, BMI and cardiometabolic risk factors: $^{10}\log$ proinsulin, $^{10}\log$ insulin, triglycerides and also $^{10}\log$ NT pro-BNP. Significant differences were also found for blood glucose ($p < 0.05$) and DM ($p < 0.02$). Insulin ($r = -0.24$, $p < 0.001$) and proinsulin ($r = -0.17$, $p < 0.001$) were negatively and highly significantly correlated to IGFBP-1 levels. We found a positive correlation between levels of CRP and HOMA ($r = 0.16$, $p < 0.001$), and a tendency to correlation between levels of IGFBP-1 and HOMA ($r = 0.07$, $p = 0.053$).

When tested by ANOVA using Bonferroni as post hoc analysis there were differences ($p < 0.001$) between tertile 1 compared to tertile 3 of IGFBP-1 for age, BMI, triglycerides, $^{10}\log$ insulin, $^{10}\log$ proinsulin, $^{10}\log$ NT-proBNP, eGFR and Hb (Table 1b). Significant differences between tertile 1 to 2 and/or 2 to 3 were found for age, BMI, triglycerides, $^{10}\log$ proinsulin, $^{10}\log$ insulin, $^{10}\log$ NT-proBNP, eGFR, and Hb, respectively (Table 1b). Using a Chi-square test, no significant differences in distribution of patients with DM between tertiles 1-3 of IGFBP-1 were found. In multivariate regression analysis with $^{10}\log$ IGFBP-1 as a dependent variable and age, gender, BMI, DM, blood glucose, Hb, eGFR, $^{10}\log$ proinsulin, $^{10}\log$ insulin, $^{10}\log$ NT-proBNP, BT systolic and triglyceride as independent variables. Gender ($p < 0.001$), BMI, ($p = 0.02$), blood glucose ($p < 0.001$), Hb ($p < 0.001$), GFR ($p < 0.001$), $^{10}\log$ proinsulin ($p = 0.002$), $^{10}\log$ insulin ($p = 0.003$) and $^{10}\log$ NT-proBNP ($p < 0.001$) had a significant impact. In order to eliminate the impact of age on changes in hormonal and others variables with age, we analysed each of them as dependent

variables in a univariate analysis with tertiles of IGFBP-1 as fixed variables and age as a co-variable. The result show that the significant differences between tertile 1 to 3 IGFBP-1 are still available when adjusted for age for BMI ($p<0.001$), Hb ($p<0.001$), GFR ($p<0.001$), $^{10}\log$ proinsulin ($p<0.001$), $^{10}\log$ insulin ($p<0.001$) and $^{10}\log$ NT-proBNP ($p<0.001$), but not for blood glucose (NS). With regard to DM by using a logistic regression with DM as dependent variable and tertiles of IGFBP-1 and age as co-variables a significant difference was shown between tertile 1 to 3 ($p=0.04$) and 1 to 2 ($p=0.006$).

CV mortality and all-cause mortality in relation to tertiles of IGFBP-1, insulin, and proinsulin

The study population was followed for up to 12 years (7.9 ± 2.6). During the follow-up period there were 230 deaths (27%) of which 134 (16%) were due to CV mortality. For all-cause and CV mortality incidence rate/10,000 person/ years were 323 and 134, respectively. For IGFBP-1, CV mortality and all-cause mortality increased with a linear trend with increased tertiles 1-3. For proinsulin, and insulin, a U-shaped trend was found, with lower values in tertile 2 (Figure 1, Table 2).

IGFBP-1, insulin, and proinsulin as prognostic biomarkers for all-cause mortality

In order to assess the prognostic potential of IGFBP-1, insulin, and proinsulin for all-cause mortality, univariate Cox proportional hazard regression analyses were used (Table 3a) during the whole follow-up period. The risk for all-cause mortality was higher for participants with plasma IGFBP-1 and proinsulin concentrations in the 3rd tertiles compared to those in the 1st tertile

(Table 3a). A multivariate Cox proportional hazard regression analysis was then performed using a model including well-known variables for CV mortality (Table 3b) and tertiles of IGFBP-1, insulin and proinsulin. Also in the multivariate analysis the risk for all-cause mortality was higher for participants with plasma IGFBP-1 and proinsulin concentrations in the 3rd tertiles compared to those in the 1st tertile. When tertiles of IGF-1/IGFBP-1 ratio was introduced instead of IGFBP-1 tertiles in Cox proportional hazard regression analysis, similar results were obtained (data not shown).

IGFBP-1, insulin and, proinsulin as prognostic biomarkers for CV mortality

In order to assess the prognostic potential of IGFBP-1, insulin, and proinsulin for CV mortality, univariate Cox proportional analysis was used (Table 3a) during the follow-up period. For the whole period, the risk for CV mortality was lower for participants with plasma IGFBP-1 and proinsulin concentrations in the 1st tertile compared to those in the 3rd tertile (Table 3a). For proinsulin the opposite result was obtained when the 1st tertile was compared to the 2nd tertile indicating a U-shaped relation. In the univariate analysis we found no association between insulin and CV mortality (Table 3a).

A multivariate Cox proportional hazard regression analysis was then performed using a model including other well-known variables for CV mortality (Table 3b) and tertiles of IGFBP-1, insulin and proinsulin. When the 1st tertile was compared to the 2nd and 3rd tertile there were no significant effects. However, there was a significant difference between the proinsulin tertiles ($p = 0.005$) but not the other tertiles and the hazard ratio when comparing the 2nd to the 3rd tertile of proinsulin was 0.5 with a CI of 0.3 – 0.7 ($p < 0.001$). The results show that only proinsulin had a significant impact on CV mortality for the whole period.

When tertiles of IGF-1/IGFBP-1 ratio was introduced instead of IGFBP-1 tertiles in Cox proportional hazard regression analysis, similar results were obtained.

IGFBP-1, insulin and, proinsulin as prognostic biomarkers for cancer mortality

In our study individuals with fasting proinsulin levels >11.7 pmol/L (tertile3) had a 3-fold risk of cancer mortality compared to tertile 1 up to 12 years of follow-up ($p=0.016$), whereas there were no significant differences between tertiles of insulin or IGFBP-1. When tertiles of IGF-1/IGFBP-1 ratio was introduced instead of IGFBP-1 tertiles in Cox proportional hazard regression analysis, similar results were obtained.

Discussion

In this study, which compared IGFBP-1, insulin, and proinsulin as prognostic biomarkers for CV and all-cause mortality followed up to 12 years, we found that after adjustment for well-known CV risk factors, only proinsulin was an independent predictor for both all-cause mortality and CV mortality. To our knowledge, this is the only study which has investigated the prognostic impact of IGFBP-1, insulin, and proinsulin concomitantly on CV and all-cause mortality in an unselected elderly population with concomitant diseases representative for their age. A new observation shown here is also the negative association between IGFBP-1 and proinsulin.

Cardiometabolic risk factor

Metabolic syndrome includes disturbances in glucose metabolism, insulin resistance, overweight, dyslipidemia and hypertension (42, 43). In this study we found that IGFBP-1 inversely correlated with BMI, triglycerides and diastolic BP, which was consistently found in previous studies (13, 15, 19, 21, 44). Our results showed that blood glucose was positively correlated to IGFBP-1 levels, as was also reported by Kajante and Hu (13, 21), whereas in other studies an inverse correlation was found (15, 19). It is well known that IGFBP-1 is down-regulated by insulin, and as expected, we found a negative correlation between IGFBP-1 and insulin levels (19, 44, 45). It is possible that the different results for blood glucose reflect different states of insulin resistance in the studied populations (44). There was a negative and highly significant correlation between IGFBP-1 and proinsulin. To our knowledge, the association between IGFBP-1 and proinsulin has not been studied before.

Impact of insulin and proinsulin on mortality

In multivariate analysis, only proinsulin was an independent risk factor for all-cause and CV mortality. Our findings are similar to the results obtained in the Hoorn study (46), in which proinsulin but not insulin was found to be an independent risk factor for all-cause and CV mortality after adjustment for age and sex. Studying CV mortality, Zethelius et al. (34) found in a 27-year follow-up study of a male population that insulin and proinsulin at baseline in univariate analysis were risk factors, whereas in multivariate analysis, only proinsulin was an independent risk factor. An association of elevated proinsulin with atherosclerosis or CV diseases has also been reported by other groups (32, 33).

IGFBP-1 and mortality

In an univariate Cox regression we found that IGFBP-1 was a risk factor for all-cause mortality and CV mortality during the follow-up period. In studies restricted to older men with an average follow-up of five years, similar results were reported by Yeap et al. (15) and Harrela et al. (18). In a multivariate Cox regression we found a significant prognostic outcome of IGFBP-1 on all-cause mortality but not on CV-mortality in contrast to Yeap et al. (15) and Harrela et al. (18) who also found an association with CV mortality. When only analysing the association between high IGFBP-1 levels and all-cause mortality, Kaplan et al. (20) and Hu et al. (21) found that high IGFBP-1 predicted mortality. A probable explanation for the discrepant results in the multivariate analysis is the fact that different risk factors were used for adjustment. In our multivariate model, NT-proBNP was a very strong risk indicator, which was not taken into consideration in previous studies addressing the association between IGFBP-1 and mortality.

Proinsulin as an independent predictor factor for both all-cause mortality and CV mortality and cancer mortality

In our study results from the multivariate analysis show that only proinsulin was an independent risk factor for all-cause, CV and cancer mortality. As we mention above our findings are in concordance with previous results reported in the Hoorn study (46) and by Zethelius et al. (34), where in the multivariate analyses proinsulin was found to be an independent risk factor for all-cause and CV mortality after adjustment. In our study we take into consideration besides proinsulin and insulin, IGFBP-1 which has not been done before, and found that proinsulin of all 3 hormones has a great importance as an independent prognostic risk factor for mortality. The association between high proinsulin and increased mortality may be explained by high proinsulin reflecting a process that has an impact on mortality or by proinsulin having a direct biologic effect that is negative. Beta-cell stress is associated with an increased proinsulin secretion (47). Insulin resistance is a cause of beta-cell stress and is also associated with increased mortality (48). It is therefore conceivable that high proinsulin is a marker of insulin resistance. Proinsulin binds with very low affinity to the insulin receptor and there is no specific receptor for proinsulin (49). Whether proinsulin or its split products have direct effects of their own is controversial.

IGFBP-1, insulin and, proinsulin as prognostic biomarkers for cancer mortality

In our study individuals with fasting proinsulin levels >11.7 (tertile3) have a 3-fold risk of cancer mortality compared to tertile 1 up to 12 years of follow-up. Data are in accordance with a recent publication by Walraven et al. (35). The mechanism behind this association could possibly be explained by the different binding activity of proinsulin to the two insulin receptors (IR) isoform A and B (36). Malaguarnera et al. have shown that proinsulin is a selective IR-A ligand by

having an almost equipotent to insulin effect on cell proliferation and migration in cells expressing IR-A (36).

It has been suggested that the ratio of IGF-1 to IGFBP-1, the IGF/IGFBP-1 ratio probably represents a more reliable marker of IGF-1 bioactivity and a more powerful indicator of adverse outcomes than IGF-1 and IGFBP (14). When tertiles of IGF-1/IGFBP-1 ratio was introduced instead of IGFBP-1 tertiles in the Cox proportional hazard regression analysis in our study, similar results were obtained for CV, all-cause and cancer mortality.

The main strength of the present population is that the participants were recruited directly from the community without sampling, and every participant was followed over a long period of time, up to 12 years (7.9 ± 2.6). No patient was lost during follow-up. Nevertheless, this study has some limitations. The analysis of hormones and other biochemistry was only done at baseline. This is an observational study, which is why conclusions about the causal role of proinsulin on mortality can only be speculative. Another limitation is the limited age span of study participants (66 to 81 years), which makes extrapolation of the results to other age groups unwise.

Conclusion

When comparing IGFBP-1, insulin, and proinsulin as prognostic biomarkers for CV and all-cause mortality in an elderly population we found only proinsulin to be an independent predictor for both all-cause mortality and CV mortality. Our results suggest that proinsulin is an important prognostic biomarker even in an elderly population, and highlight the importance of proinsulin not only as a cardiometabolic risk factor, but also as a biomarker for CV mortality. We consider that this unique biomarker, proinsulin, is a candidate for development of a prognostic panel of biomarkers for all-cause and CV mortality.

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Tables

Table 1a. Population baseline characteristics and cardio-metabolic variables presented as tertiles 1 to 3 of IGFBP-1.

| | IGFBP-1 | | | p value |
|--|--------------------------|----------------------------|----------------------------|---------|
| | tertile 1 | tertile 2 | tertile 3 | |
| | 1.1-11.7 µg/L (n=283) | 11.72-22.6 µg/L (n=284) | 22.7-490.6 µg/L (n=284) | |
| Variable | | | | |
| Male, n (%) | 137 (33.0) | 136 (32.8) | 142 (34.2) | NS |
| Female, n (%) | 146 (33.5) | 148 (33.9) | 142 (32.6) | NS |
| Age, (mean ± SD), years | 72.0 (3.2) | 73.0 (3.5) | 73.9 (3.4) | <0.001 |
| Current smokers, n (%) | 27 (34.6) | 17 (21.8) | 34 (43.6) | 0.045 |
| Clinical History | | | | |
| Diabetes mellitus, n (%) | 41 (31.1) | 34 (25.8) | 57 (43.2) | 0.02 |
| CAD, n (%) | 36 (28.3) | 45 (35.4) | 46 (36.2) | NS |
| EKG, n (%) | 13 (27.7) | 14 (29.8) | 20 (42.6) | NS |
| IHD, n (%) | 44 (28.0) | 56 (35.7) | 57 (36.3) | NS |
| NYHA I, n (%) | 175 (33.7) | 182 (35.1) | 162 (31.2) | NS |
| NYHA II, n (%) | 83 (31.2) | 87 (32.7) | 96 (36.1) | NS |
| NYHA III, n (%) | 25 (39.7) | 15 (22.7) | 26 (39.4) | NS |
| Clinical variables | | | | |
| BMI (mean ± SD), kg/m ² | 28.5 (4.1) | 26.9 (3.8) | 24.6 (3.9) | <0.001 |
| EF<40%, n (%) | 8 (19.0) | 15 (35.7) | 19 (45.2) | NS |
| Clinical examination | | | | |
| blood pressure systolic (mean±SD), mmHg | 162.2 (17.4) | 158.9 (19.2) | 159.1 (20.6) | NS |
| blood pressure diastolic (mean±SD), mmHg | 86.5 (8.4) | 85.3 (9.2) | 84.1 (9.0) | 0.06 |
| Laboratory analyses | | | | |
| IGF-1 (mean ± SD), µg/L | 85.2 (27.7) | 78.7 (26.6) | 73.5 (26.2) | <0.001 |
| ¹⁰ log proinsulin (mean ± SD), pmol/L | 0.95 (0.40) | 0.93 (0.33) | 0.83 (0.35) | <0.001 |
| ¹⁰ log insulin (mean ± SD), pmol/L | 1.76 (0.29) | 1.65 (0.29) | 1.57 (0.36) | <0.001 |
| blood glucose (mean ± SD), mmol/l | 4.62 (1.23) | 4.59 (1.35) | 4.87 (1.84) | 0.05 |
| HOMA | 1.6 (59.6) | 1.3 (63.1) | 1.0 (94.3) | NS |
| cholesterol, mean (SD), mmol/L | 6.07 (1.1) | 6.1 (1.1) | 6.0 (1.2) | NS |
| triglycerides, mean (SD), mmol/L | 1.6 (0.7) | 1.5 (0.7) | 1.37 (0.7) | <0.001 |
| log NT-proBNP (mean ± SD), pmol/L | 2.02 (0.39) | 2.11 (0.44) | 2.29 (0.48) | <0.001 |
| eGFR<60 mL/min, n (%) | 60 (16.6) | 124 (34.3) | 178 (49.2) | <0.001 |
| Hb, mean (SD), g/L | 143.7 (12.6) | 140.6 (11.1) | 136.3 (12.8) | <0.001 |
| Treatment | | | | |
| ACE-inhibitor, n (%) | 58 (33.5) | 56 (32.4) | 59 (34.1) | NS |
| β-blocker, n (%) | 61 (30.5) | 71 (35.5) | 68 (34.0) | NS |
| Diuretic, n (%) | 79 (32.9) | 78 (32.5) | 83 (34.6) | NS |

Note: ACE = angiotensin-converting enzyme; BMI = body mass index; BP = blood pressure; CAD= coronary artery disease; CRP = C-reactive protein; IGFBP-1 = insulin-like factor binding protein-1; IQR = interquartile range; ECG = electrocardiogram indicative of IHD; EF=ejection fraction; estimated CG GFR<60= estimated Cockcroft-Gault glomerular filtration ratio less than 60 mL/min; IHD= ischemic heart disease; HOMA = homeostatic model assessment NYHA = New York Heart Association functional class; EF = ejection fraction; Hb = hemoglobin; NT-proBNP = N-terminal proBNP.

Table 1b. Post hoc analyses for those population baseline characteristics and cardio-metabolic variables which were significantly different when compared between tertiles 1 to 2, and 1 to 3 of IGFBP.

| | IGFBP-1 | | |
|------------------------------------|-----------------------|-----------------------|-----------------------|
| | tertile 1 vs 2 | tertile 1 vs 3 | tertile 2 vs 3 |
| age | <0.001 | <0.001 | 0.01 |
| current smokers | NS | NS | NS |
| DM | NS | NS | NS |
| BMI | <0.001 | <0.001 | <0.001 |
| blood glucose | NS | NS | NS |
| triglycerides | NS | <0.001 | 0.03 |
| ¹⁰log insulin | 0.001 | <0.001 | 0.009 |
| ¹⁰log proinsulin | NS | 0.001 | 0.005 |
| log NT pro-BNP | 0.03 | <0.001 | <0.001 |
| eGFR<60 | <0.001 | <0.001 | <0.001 |
| Hb | <0.001 | <0.001 | <0.001 |

Note: IGFBP-1 = insulin-like factor binding protein-1, DM=Diabetes Mellitus, BMI= Body Mass Index, eCG GFR<60= estimated Cockcroft-Gault glomerular filtration ratio less than 60 mL/min, Hb = hemoglobin; NT-proBNP = N-terminal proBNP, ¹⁰ log= log 10 transformed, vs = versus.

Table 2. Cardiovascular mortality and all-cause mortality in tertiles 1 to 3 of IGFBP-1, proinsulin, and insulin in the study population during eight years of follow-up.

| | | IGFBP-1 | | |
|-------------------|----------------------|----------------|--------------|--------------|
| | | n (%) | n (%) | n (%) |
| IGFBP-1 | CV mortality | 32 (23.9%) | 40 (29.9%) | 62 (46.3%) |
| | All causes mortality | 57 (24.8%) | 71 (30.9%) | 102 (44.3%) |
| proinsulin | CV mortality | 43 (32.1%) | 30 (22.4%) | 61 (45.5%) |
| | All causes mortality | 68 (29.6%) | 60 (26.1%) | 102 (44.3%) |
| insulin | CV mortality | 43 (32.1%) | 37 (27.6%) | 54 (40.35%) |
| | All causes mortality | 73 (31.7%) | 63 (27.4%) | 94 (40.9%) |
| NT-proBNP | CV mortality | 30 (22.45) | 24 (17.9%) | 80 (59.7%) |
| | All causes mortality | 57 (24.8%) | 53 (23.0%) | 120 (52.2%) |

Note: IGFBP-1 = insulin-like factor binding protein-1, CV mortality = cardiovascular mortality, NT-proBNP = N-terminal proBNP

Table 3a. Univariate Cox proportional hazard regression analysis of prognostic power concerning all causes and cardiovascular mortality for the entire follow-up period for tertiles 2 to 1 and 3 to 1 of IGFBP-1, insulin, and proinsulin.

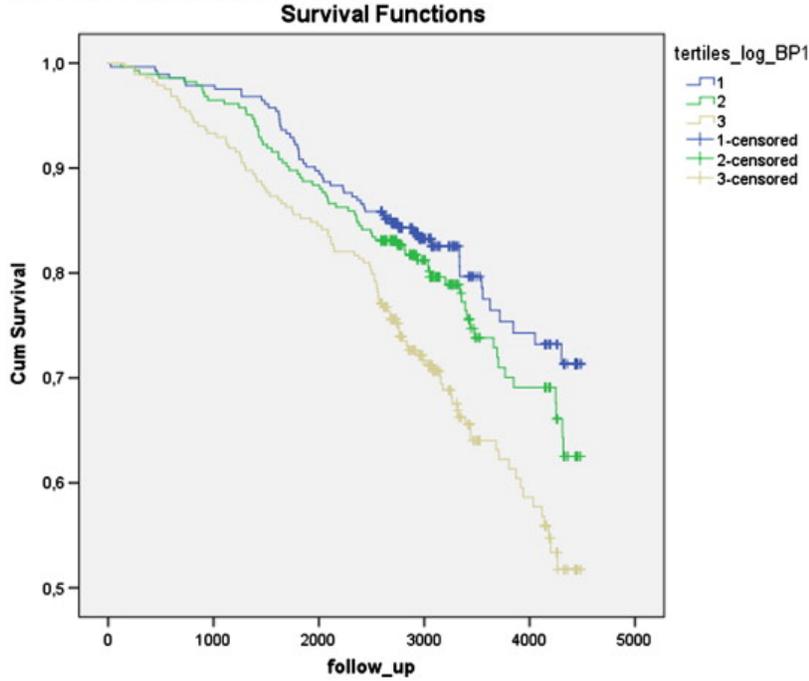
| | All-cause mortality | | | | | | proinsulin | | |
|---------------------------------|----------------------------|---------------|----------------|----------------|---------------|----------------|-------------------|---------------|----------------|
| | IGFBP-1 | | | insulin | | | HR | 95% CI | p value |
| | HR | 95% CI | p value | HR | 95% CI | p value | HR | 95% CI | p value |
| tertile 1 | 1 | | | 1 | | | 1 | | |
| tertile 2 | 1.2 | 0.9-1.7 | 0.2 | 0.8 | 0.6-1.2 | 0.3 | 0.8 | 0.6-1.1 | 0.2 |
| tertile 3 | 1.9 | 1.3-2.6 | <0.001 | 1.2 | 0.9-1.6 | 0.3 | 1.6 | 1.2-2.2 | 0.003 |
| Cardiovascular mortality | | | | | | | | | |
| tertile 1 | 1 | | | 1 | | | 1 | | |
| tertile 2 | 1.2 | 0.8-2.0 | 0.4 | 0.8 | 0.5-1.3 | 0.4 | 0.6 | 0.4-1.0 | <0.05 |
| tertile 3 | 2.0 | 1.3-3.1 | 0.001 | 1.1 | 0.8-1.7 | 0.5 | 1.5 | 1.0-2.2 | 0.04 |

Note: IGFBP-1 = insulin-like factor binding protein-1, CI = confidence interval, HR =hazard ratio.

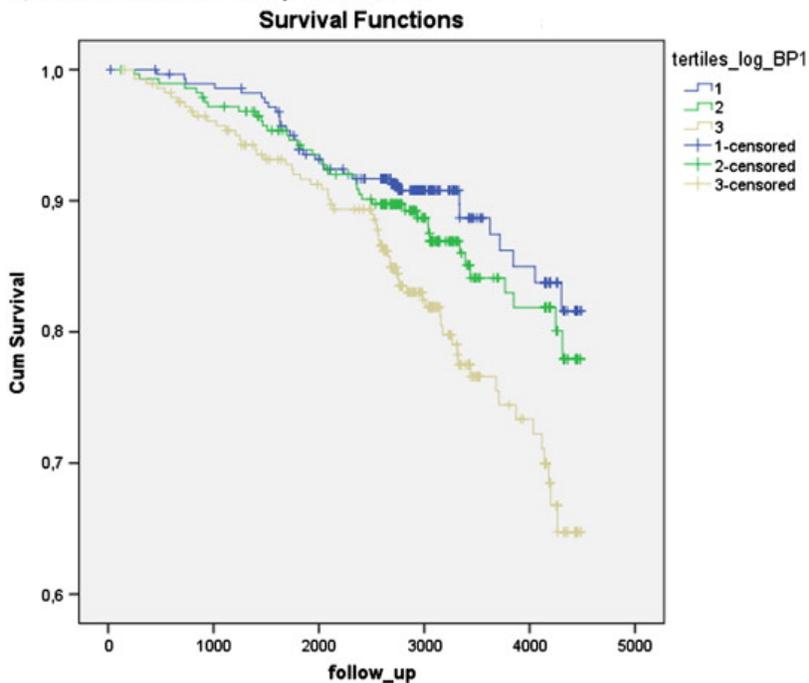
Legend to figure:

Figure 1. Kaplan-Meier analysis comparing all-cause mortality(a) and cardiovascular mortality during a follow-up period up to 12 years (b) for tertiles 1 to 3 of IGFBP-1, as well as analysis of cardiovascular mortality for tertiles 1 to 3 of proinsulin (c) and insulin (d).

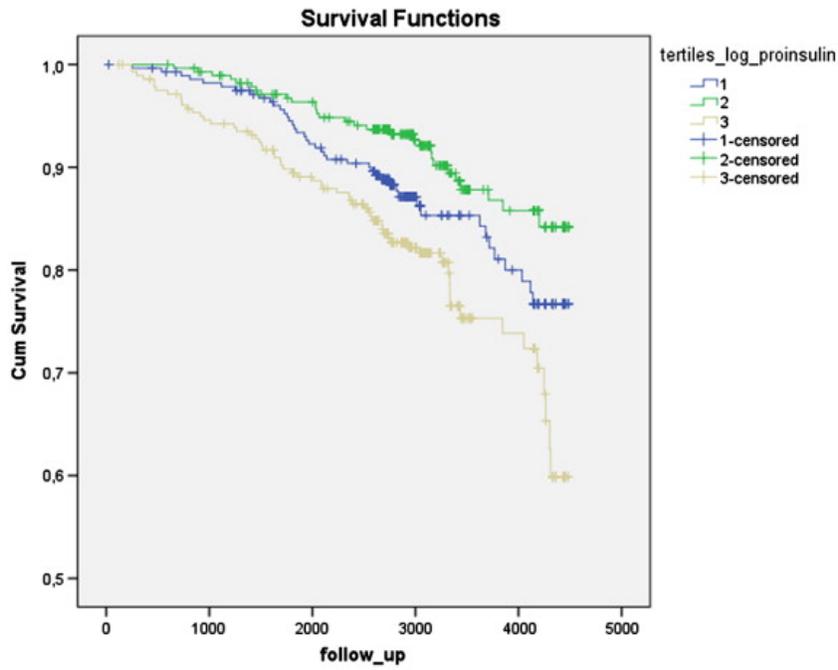
a) All-cause mortality for IGFBP-1



b) Cardiovascular mortality for IGFBP-1



c) Cardiovascular mortality for proinsulin



d) Cardiovascular mortality for insulin

