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Associations between insulin-like growth factor binding protein-2 and insulin sensitivity, metformin, and mortality in persons with T2D

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ABSTRACT

Aims: Serum insulin-like growth factor binding protein-2 (IGFBP-2) is low in persons with type 2 diabetes mellitus (T2D) and possibly regulated by metformin. Counter-intuitively, high IGFBP-2 associates with mortality. We investigated the association between IGFBP-2, metformin-treatment, and indices of insulin sensitivity, and assessed IGFBP-2 in relation to prior comorbidity and mortality during five-year follow-up.
Methods: The study included 859 treatment-naive and 558 metformin-treated persons enrolled in the Danish Centre for Strategic Research in T2D and followed for 4.9 (3.9–5.9) years through national health registries. All proteins were determined in serum collected at enrollment.
Results: Following adjustment for age, metformin-treated and treatment-naive persons has similar IGFBP-2 levels. Low IGFBP-2 level was associated with increased BMI, fasting glucose, and C-peptide. IGFBP-2 was higher in the 437 persons who had comorbidities at enrollment than in those with T2D only (343 (213;528) vs. 242 (169;378) ng/mL). During follow-up, 87 persons died, and IGFBP-2 predicted mortality with an unadjusted HR (95% CI) per doubling in IGFBP-2 concentration of 2.62 (2.04;3.37) and a HR of 2.21 (1.61;3.01) following full adjustment.
Conclusions: In T2D, high IGFBP-2 associates with low glucose and insulin secretion, is unaffected by metformin treatment, and associates with risk of prior comorbidity and mortality.

1. Introduction

Insulin-like growth factor binding protein (IGFBP)-2 participates in physiological processes by regulating the bioavailability of insulin-like growth factor (IGF)-1 and −2. The metabolic actions of the two IGFs mimic those of insulin, being capable of stimulating peripheral glucose uptake, lipogenesis, and glycogen synthesis through activation of the IGF-1 receptor [1,2]. However, as opposed to insulin, IGF bioactivity is regulated by a family of IGFBPs which comprise an intricate regulatory network with immense adaptability [3]. Especially IGFBP-2 is implicated in glucose and lipid metabolism [4], and circulating concentrations in humans are positively correlated to insulin sensitivity, directly downregulated by insulin, and increased following fasting [2]. In addition, IGFBP-2 is inversely associated with body mass index (BMI) and reduced in obese adults, in whom low serum levels are independently associated with the development of type 2 diabetes mellitus (T2D) [2]. This close connection to metabolic status has put forward IGFBP-2 as a potential biomarker in obesity, prediabetes, and diabetes [5,6]. Additionally, in murine models, IGFBP-2 overexpression counteracts obesity and reverses the insulin resistant phenotype in diet-induced obese mice and leptin-deficient mice [7,8]. These findings suggest that IGFBP-2 is a therapeutic target in obesity and insulin resistance. However, its use as a treatment strategy in humans has been sparsely investigated. This is likely due to the lack of a clear understanding of its plethora of pathways and the somewhat contradictory association of IGFBP-2 with various diseases. Notably, despite elevated serum IGFBP-2 appears to be metabolically beneficial, being associated with an increased insulin sensitivity and a lower risk of developing

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diabetes, a high IGFBP-2 level has also been linked to an increased mortality, not only in subjects with specific diseases, but also in relatively healthy populations [9,10]. For example, IGFBP-2 is usually overexpressed in various cancers, and high circulating levels are invariably associated with an increased risk of cancer mortality [11,12] and poor outcomes in cardiovascular diseases [5,9]. The reason that IGFBP-2 appears to possess both beneficial and detrimental functions may relate to the fact that IGFBP-2 besides controlling IGF action also possess IGF-independent effects [13].

Metformin is the undisputed first drug of choice in T2D, and *in vitro* and murine studies have suggested that IGFBP-2 expression is regulated by metformin, but results are contradictory [14,15]. A single human study has shown an increase in IGFBP-2 in 36 T2D persons receiving metformin as compared to 20 persons receiving other glucose-lowering medications [14].

The present study sought to investigate the potential effect of metformin treatment on IGFBP-2 levels in T2D persons enrolled in the nationwide Danish Centre for Strategic Research in Type 2 Diabetes (DD2) study. The study cohort comprised all subjects who were drug-naive at enrollment as well as persons treated with metformin as the only anti-diabetic treatment six months prior to enrollment. We hypothesized that IGFBP-2 associates with insulin sensitivity and metabolic markers, and that levels were elevated in persons with comorbid conditions prior to enrollment. As IGFBP-2 appears to predict mortality, the study also aimed to evaluate the association between IGFBP-2 level at enrollment and all-cause mortality during the available five years of follow-up time.

2. Material and methods

2.1. Study design and data sources

The study included persons and data from the nationwide DD2 cohort. The DD2 project is ongoing and has since 2010 enrolled persons with recent onset T2D from general practitioners and hospital outpatient clinics. The implementation and logistics of the DD2 cohort has been described in detail elsewhere [16,17]. Using the unique personal identifier provided by the Danish Civil Registration System, enrolled person data were linked to several Danish health registers, where subjects were followed prospectively from date of enrollment until death or 22 August 2018, whichever came first. From the Danish National Patient Register (DNPR) [18], information regarding Charlson Comorbidity Index (CCI) conditions at baseline was obtained using ICD-10 disease codes. More detailed cancer data were available from the Danish Cancer Registry. Linkage with the Danish Diabetes Database for Adults (DDDA) provided additional information on routine laboratory measurements, antihypertensive treatment, hypolipidemic treatment, and BMI [16,17,19]. However, for some persons, linkage to the DDDA was not possible, and thus, certain baseline data were missing. Information on drug prescriptions, including the date of dispensing, as well as amount and type of drug prescribed, was obtained from the Danish National Health Service Prescription Database [20]. Because of the high-quality Danish healthcare registers, no persons were lost during follow-up. Variable sources and definitions are summarized in *Supplementary Table S1*.

The study was approved as part of the DD2 study by the National Committee on Health Research Ethics (Denmark) (record number S-20100082) and the Danish Data Protection Agency (record number 2008–58-0035). All cohort participants gave written informed consent to participate.

2.2. Study cohort

The source cohort comprised all individuals with T2D diagnosed in accordance with the World Health Organization definition of T2D. All persons included in this study were enrolled between November 2010 and August 2018, with blood samples stored in the DD2 biobank (n = 8246) [16]. Person follow-up data was extracted from the registers on August 28. Subjects were excluded if the presented with rare subtypes of diabetes, secondary diabetes, glucocorticoid-associated diabetes, or latent autoimmune diabetes of adults (LADA), defined as glutamic acid decarboxylase antibodies (GADA) $\geq$ 20 IU/mL and age $>$ 30 years [19]. Persons were also excluded if they were $\leq$ 30 years and had GADA $\geq$ 20 IU/mL, indicative of type 1 diabetes mellitus (T1D). Finally, persons were excluded if they had no available measurements of GADA, which is necessary to exclude presence of LADA or T1D with certainty. To investigate the association between IGFBP-2 and measures of insulin sensitivity, persons that were non-fasting or without measurement of fasting glucose or C-peptide were also excluded. Persons fulfilling the inclusion criteria and were eligible for analysis.

The present study cohort was restricted to treatment-naive or metformin-treated T2D persons. Although the DD2 cohort recruits recently diagnosed T2D persons, approximately 84 % have already initiated glucose-lowering treatment at enrollment [16]. Thus, of the persons that fulfilled inclusion criteria, we selected all subjects that were drug-naive (receiving no anti-diabetic treatment one year prior to enrollment). The group of metformin-treated T2D persons were defined as subjects receiving metformin as the only anti-diabetic treatment one year prior to enrollment and $\geq$ two prescriptions six months prior to enrollment. From the group of metformin-treated persons, a subgroup was randomly selected to generate the final study cohort. The cohort was used for analysis of IGFBP-2 serum levels.

2.3. Laboratory measurements

Laboratory measurements were performed in an ISO 15189 accredited laboratory (*Supplementary Table S1*). Fasting serum was assessed for IGFBP-2 using an assay developed on the automated Simoa HD-1 Analyzer platform (Quanterix©, Billerica, MA, USA) at Center Hospital Lillebaelt, Region of Southern Denmark. Serum samples were diluted 200-fold before analysis, and four in-house quality controls were included in each run. The seven-point calibration curve was prepared using 3-fold dilutions starting at 10 μg/L and analyzed in duplicates, while the controls and samples were run in single determinations. The calibration curve was generated using a four-parameter logistic regression-fit. The analytical coefficients of variation (CV%) ranged from 8 to 15 %. A through description of the method is given in *Supplementar y methods*.

2.4. Statistics

Calculations of BMI, estimated glomerular filtration rate (eGFR), diabetes duration, homeostatic model assessment 2 of insulin sensitivity (HOMA2-S) and beta cell function (HOMA2-B) are summarized in *Supplementary Table S1*. Based on hospital diagnosis ICD-10 codes, the comorbidity burden of each person within the 10-year period before the DD2 enrollment date was computed using the CCI score, defining three comorbidity levels: low (score of 0), medium (score of 1–2) and high (score of $\geq$ 3) [21]. T2D was not included in the CCI score as it constituted the index disease. We separately described all previous cardiovascular events or cancer diseases prior to enrollment.

Non-normally distributed variables were $\log_2$-transformed prior to statistical analyses. Person groups were compared using Student's *t*-test or Mann-Whitney U-statistics on continuous variables, and χ^2-test on categorical variables. Characteristics of the study population in different IGFBP-2 tertile groups were evaluated for linear trend across ordered groups using linear regression analyses with the ordered group as a continuous explanatory variable or using the Jonckheere-Terpstra test. Associations were analyzed using linear regression, Cox regression, or Pearson correlation.

Receiver operating characteristic (ROC) curves were used to analyse the prognostic performance of IGFBP-2. Cumulative incidence of all-cause mortality according to IGFBP-2 tertiles was plotted using the

Kaplan-Meier method, and incidence distributions were compared using the log-rank test. Survival analyses were performed using Cox proportional-hazards models. Hazard proportionality and linearity assumptions were graphically verified by log–log plots, fitted survival curves, and smoothed Martingale and Schonenfeld residuals plots [22].

Extensive adjustments were performed to ensure robustness of the potential associations, and all candidate confounder variables and blood biomarkers were initially considered for the regression models. In a partially adjusted Cox regression model 1, HRs were adjusted for pre-specified confounders based on clinical judgement, as is recommended. Thus, model 1 included sex, age, BMI, C-peptide, eGFR, and CCI. In the fully adjusted model 2, HRs were adjusted for sex, age, BMI, C-peptide, eGFR, CCI, diabetes duration, fasting blood glucose, HbA_{1c}, diastolic blood pressure, HDL cholesterol, and smoking. To maximize power and avoid selection bias, imputation methods were applied to handle missing covariable data. Multivariate normal imputation was used to impute missing values of continuous variables, generating 20 complete datasets. Variables were assumed to follow a joint multivariate normal distribution and to be missing at random. The categorical variable smoking was used as a binary variable with two categories (0 = never smoking; 1 = smoking [former/current]) and was imputed on a continuous scale and rounded to 0 if the value was smaller than 0.5, or 1 otherwise. The imputed models were validated by comparing the mean, median, and interquartile range (IQR) of the imputed data set with the original data set. Imputations were not performed on IGFBP-2 variables or outcome variables.

Sensitivity analyses were performed to investigate the effect of diabetes duration prior to enrollment. Comparative analyses were made on individuals with T2D diagnosed at the time of enrollment, on individuals with a diagnosis occurring prior to enrollment but within the last 2 years, and on individuals with a diabetes duration exceeding 2 years. Furthermore, sensitivity analyses on the entire cohort, treatment-naive, and metformin-treated persons were performed.

Continuous variables are shown as median (IQR). Categorical variables are indicated as numbers (n) and percentage (%). $P < 0.05$ was considered statistically significant. All statistics were performed using Stata version 17 (College Station, TX, USA).

3. Results

3.1. Study cohort and characteristics

The source cohort included 8246 persons from the DD2 biobank [16], of which 3789 subjects were excluded for one or more reasons (flowchart shown in Fig. 1). Persons with rare subtypes of diabetes, secondary diabetes, glucocorticoid-associated diabetes, LADA, or T1D

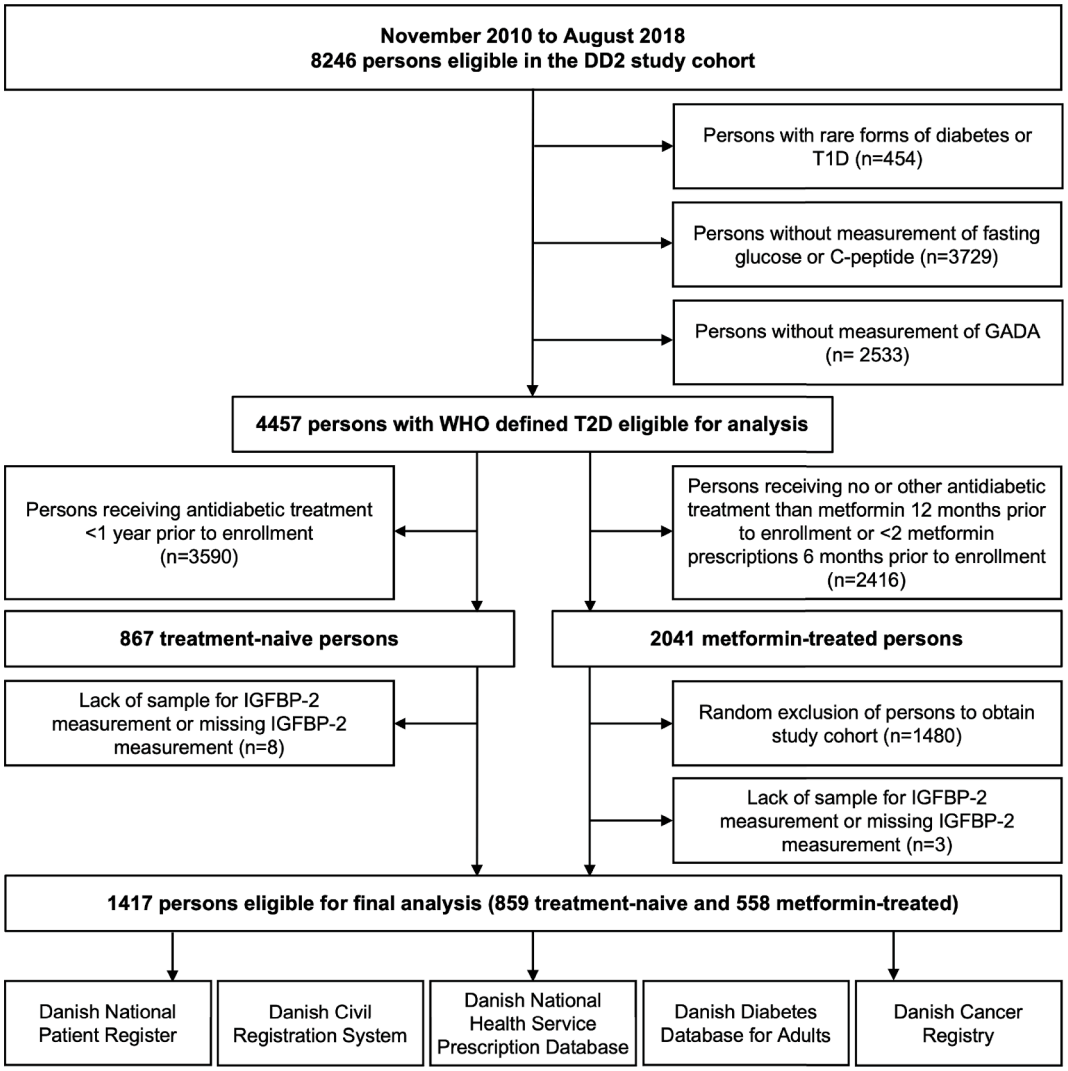

Fig. 1. Flow chart of data collection in the DD2 cohort study. Some excluded patients met multiple exclusion criteria. DD2, Danish Centre for Strategic Research in Type 2 Diabetes; GADA, glutamic acid decarboxylase antibodies; HOMA2, homeostatic model assessment 2; IGFBP-2, insulin-like growth factor binding protein-2; T1D, type 1 diabetes; WHO, World Health Organization.

were excluded (n = 454), as well as persons with no available measurements of GADA (n = 2533). Subjects that were non-fasting or without measurement of fasting glucose or C-peptide were also excluded (n = 3729). Following these initial exclusions, 4457 persons fulfilled the inclusion criteria and were eligible for analysis. The study cohort was then restricted to persons who were drug-naive (n = 867) or had received metformin as the only anti-diabetic treatment one year prior to enrollment (n = 2041). A total of 561 metformin-treated persons were randomly selected to generate the study cohort of 1428 persons. Following analysis of IGFBP-2, measurements were missing in 11 persons. Thus, the final study cohort comprised 859 drug-naive and 558 metformin-treated persons eligible for analyses. For some persons, linkage to the DDDA was not possible, and thus, certain baseline data were missing (n = 2–830; 0.1–58.6 %) (*Supplementary Table S2*).

Treatment-naive persons were older than persons receiving metformin, whereas diabetes duration was longer in metformin-treated persons. BMI and sex distribution were similar in both groups. HbA_{1c} was slightly higher in metformin-treated persons, but no differences were seen in HOMA2-S, HOMA2-B, fasting glucose, or C-peptide between groups. There was no difference in CCI. In all subjects, median IGFBP-2 level was 260 (181;431) ng/mL. Subjects receiving metformin had lower levels of IGFBP-2 as compared to treatment-naive persons. However, treatment with metformin was associated with lower age and following adjustment for age, metformin no longer associated with IGFBP-2 (p = 0.798). Additional regression analyses with adjustment for all potential confounders confirmed this finding. Restricting analyses to those with T2D diagnosed at the time of enrollment, individuals with a diagnosis occurring prior to enrollment but within the last 2 years, or to individuals with a diabetes duration exceeding 2 years did not change the results. Thus, in all subsequent analyses, drug-naive and metformin-treated persons were analyzed as one group. Table 1 presents characteristics of the entire study population and of persons stratified by tertiles of serum IGFBP-2 levels. Characteristics of treatment-naive and metformin-treated T2D persons are shown in *Supplementary Table S3*.

3.2. Associations with metabolic markers

At baseline, IGFBP-2 was negatively associated with BMI (r = -0.361) and waist-hip ratio (r = −0.120) and positively associated with age (r = 0.531). Levels of IGFBP-2 associated with indices of insulin sensitivity, being inversely associated with fasting glucose (p = −0.278) and C-peptide level (r = −0.331) and positively associated with HOMA2-S (r = 0.350). IGFBP-2 was not associated with HOMA2-B. HDL cholesterol and triglyceride level also correlated with IGFBP-2 (r = 0.230 and r = −0.298, respectively). An inverse association was observed between IGFBP-2 and eGFR (r = −0.369), and thus, increased protein

Table 1
Characteristics of T2D persons stratified by tertiles of serum IGFBP-2 levels. Characteristics of the persons in the entire cohort and stratified by IGFBP-2 tertile. Values for continuous variables are shown as median (interquartile range). Categorical variables are indicated as numbers (n) and percentage (%) of persons.

Characteristic	All persons (n = 1417)	IGFBP-2 tertile			p for trend
		Low (n = 473) 74–207 ng/mL	Middle (n = 472) 209–362 ng/mL	High (n = 472) 366–1486 ng/mL	
Age, years	64 (56;70)	56 (49;63)	64 (57;70)	70 (64;75)	<0.001
Male sex, n (%)	802 (56.6)	252 (53.3)	270 (57.2)	280 (59.3)	0.164
BMI, kg/m^2	29.7 (26.7;33.6)	32.2 (28.7;36.6)	29.8 (27.0;33.2)	27.7 (24.8;30.6)	<0.001
Waist-hip ratio	0.97 (0.91;1.03)	0.98 (0.92;1.03)	0.98 (0.91;1.03)	0.95 (0.89;1.02)	0.001
Metformin, n (%)	558 (39.4)	201 (42.5)	187 (39.6)	170 (36.0)	0.124
Diabetes duration, years	0.9 (0.02;2.3)	0.7 (0.02;2.2)	0.8 (0.00;2.2)	1.2 (0.03;2.6)	0.108
HbA_{1c}, %	6.3 (6.0;6.8)	6.6 (6.1;6.9)	6.3 (6.0;6.7)	6.3 (5.9;6.7)	<0.001
HbA_{1c}, mmol/mol	45.7 (42.6;50.9)	47.6 (43.6;51.9)	45.7 (42.7;49.8)	45.4 (41.0;49.8)	<0.001
HOMA2-S: insulin sensitivity	37.0 (28.1;48.7)	31.3 (23.9;39.7)	36.8 (28.6;49.4)	43.8 (34.7;57.6)	<0.001
HOMA2-B: beta cell function	93.7 (72.7;119.4)	96.7 (75.0;123)	93.1 (71.9;119)	91.0 (70.6;116)	0.035
C-peptide, pmol/L	1098 (834;1422)	1267 (1022;1659)	1098 (824;1380)	945 (725;1192)	<0.001
Plasma glucose, mmol/L	7.0 (6.3;7.9)	7.4 (6.7;8.2)	7.0 (6.3;7.8)	6.7 (6.1;7.4)	<0.001
Lipids, mmol/L					
Total cholesterol	4.4 (3.8;5.1)	4.4 (3.7;5.3)	4.5 (3.8;5.2)	4.3 (3.8;5.0)	0.236
LDL cholesterol	2.3 (1.8;2.9)	2.4 (1.8;2.9)	2.3 (1.8;3.0)	2.2 (1.8;2.8)	0.082
HDL cholesterol	1.2 (1.1;1.5)	1.2 (1.0;1.3)	1.2 (1.1;1.5)	1.4 (1.1;1.6)	<0.001
Triglycerides	1.5 (1.1;2.2)	1.8 (1.3;2.6)	1.5 (1.1;2.1)	1.3 (1.0;1.7)	<0.001
Blood pressure, mmHg					
Systolic	130 (124;140)	130 (124;140)	130 (125;140)	130 (124;138)	0.569
Diastolic	80 (74;85)	80 (76;86)	80 (74;85)	78 (66;92)	<0.001
Anti-hypertensive treatment, n (%)	758 (67.4)	221 (60.7)	252 (68.1)	285 (76.0)	<0.001
Hypolipidemic treatment, n (%)	679 (61.2)	215 (59.1)	231 (62.4)	233 (62.1)	0.585
Creatinine, μmol/L	74 (64;86)	71 (62;82)	74 (64;86)	77 (66;90)	<0.001
eGFR, ml/min/1.73 m^2	87 (74;96)	93 (82;101)	87 (74;96)	81 (64;89)	<0.001
Smoking, n (%)					
Never	497 (48.6)	179 (55)	145 (43)	173 (48)	0.036
Previous	339 (33.2)	93 (29)	121 (36)	125 (35)	
Current	186 (18.2)	54 (17)	71 (21)	61 (17)	
Physical activity, days/week*	4 (2;7)	3 (1;6)	4 (2;7)	4 (2;7)	0.013
Weekly alcohol consumption, n (%)					
≤14/21 units (women/men)	1306 (92.2)	438 (92.6)	423 (89.6)	445 (94.3)	0.026
>14/21 units (women/men)	111 (7.8)	35 (7.4)	49 (10.4)	27 (5.7)	
CCI score, n (%)					
0	980 (69.2)	367 (75.3)	345 (73.1)	268 (56.8)	<0.001
1–2	376 (26.5)	93 (19.7)	113 (24.0)	170 (36.0)	
≥3	61 (4.3)	13 (2.8)	14 (3.0)	34 (7.2)	
All previous cardiovascular disease, n (%)	360 (25.4)	76 (16.1)	123 (26.0)	161 (34.1)	<0.001
All previous cancer, n (%)	158 (11.2)	38 (8.0)	49 (10.4)	71 (15.0)	0.002
Mortality, n (%)	87 (6.1)	9 (1.9)	25 (5.3)	53 (11.2)	<0.001

*Days per week with a minimum of 30 min of physical activity.
BMI, body mass index; CCI, Charlson Comorbidity Index; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA2, homeostasis model assessment 2; IGFBP-2, insulin-like growth factor binding protein-2; LDL, low-density lipoprotein.

concentration was associated with a reduced kidney function. IGFBP-2 was not associated with T2D duration at enrollment. Persons with high IGFBP-2 were more likely to receive anti-hypertensive treatment.

3.3. IGFBP-2 and comorbidity

Persons with one or more hospital-diagnosed comorbidities (CCI > 0) within the 10-year period prior to enrollment were older and had longer diabetes durations than persons with no comorbidities (CCI = 0). More men than women suffered from comorbidities, but no differences were seen in BMI. IGFBP-2 level was lower in persons with no comorbidities at enrollment (242 (169;378) ng/mL) as compared to persons with a CCI score of 1–2 (327 (212;514) ng/mL) or ≥ 3 (415 (229;675) ng/mL) (Fig. 2). The characteristics of T2D persons with (CCI > 0) or without (CCI = 0) comorbidities are shown in *Supplementary Table S4*.

IGFBP-2 level was increased in subjects with previous cardiovascular disease or cancer at any timepoint before the enrollment date, both when compared to all other persons and when compared to persons with a CCI score of 0. Additional analyses of the comorbid diseases included in calculation of the CCI showed that levels of IGFBP-2 were elevated in certain conditions as compared to persons with no comorbidities. Levels were higher in persons with previous myocardial infraction (n = 69), congestive heart failure (n = 43), peripheral vascular disease (n = 60), cerebrovascular disease (n = 77), chronic pulmonary disease (n = 99), ulcer disease (n = 27), renal disease (n = 21), and any cancer disease (n = 124). Following adjustment for age, the associations persisted between high IGFBP-2 level and myocardial infarction, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, and ulcer disease.

3.4. Follow-up analysis of all-cause mortality

The persons were followed in the registers for a median of 4.9 (3.9;5.9) years from the date of enrollment, during which 87 died. Mortality was not associated with T2D duration at enrollment. However, death was observed more frequently in persons with a CCI score of 1–2 (n = 45 (11.8 %)) or a CCI score ≥ 3 (n = 11 (17.7 %) than in persons with no preexisting comorbidities (n = 32 (3.2 %)), p < 0.001.

IGFBP-2 level was significantly increased with increasing CCI score

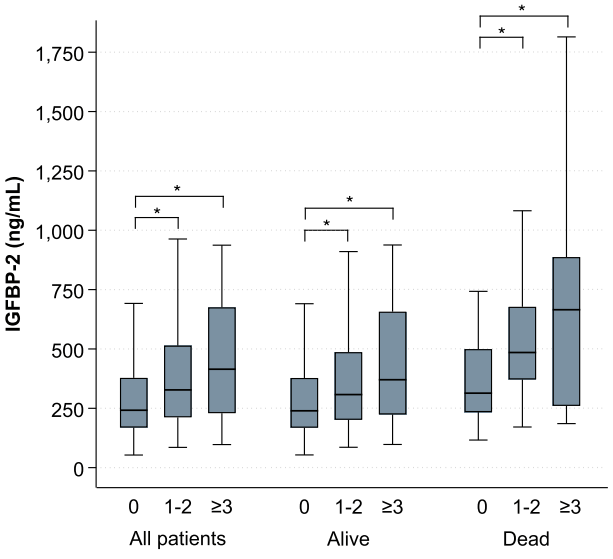

Fig. 2. Baseline level of IGFBP-2 according to CCI and mortality. IGFBP-2 concentrations at baseline in persons with a low (0), medium (1–2), or high (≥3) CCI score according to mortality status at follow-up. Boxes represent median with 25th and 75th percentiles. *p < 0.05. CCI, Charlson Comorbidity Index; IGFBP-2, insulin-like growth factor binding protein-2.

both in persons who were alive and in persons who had died at the end of follow-up (Fig. 2). Since IGFBP-2 was highly associated with comorbidities prior to enrollment, its relation to all-cause mortality was analyzed both in the entire cohort and separately on persons without preexisting comorbidities or with a medium to high CCI score (CCI > 0). ROC area under the curve (AUC) demonstrated that in the entire cohort, IGFBP-2 had a discriminatory capability of 72 (67;77)%. Log-rank analysis showed different incidence distributions according to IGFBP-2 tertile, and increased mortality was associated with increased IGFBP-2 tertile level in the entire cohort. A total of 9, 25, and 53 deaths were observed in the low, middle, and high tertile groups, respectively (p_{trend} < 0.001). The associations persisted when subjects were divided into groups based on CCI score, and the strongest association was observed in persons with one or more comorbidities. Kaplan-Meier survival curves according to tertiles of IGFBP-2 are shown in Fig. 3. The association between mortality and IGFBP-2 was investigated using both the continuous variable and tertiles with the low tertile as reference group (Table 2). With each 2-fold increase in IGFBP-2 concentration, the mortality HR increased by 162 % in unadjusted analysis. In a categorical model using the first tertile as reference, IGFBP-2 was associated with mortality with an increase in HR of 187 % and 523 % for the middle and high tertile, respectively. As a continuous variable, IGFBP-2 remained associated with mortality in the partially and fully adjusted models, whereas the categorical model was significant when comparing the high vs. low tertile. Separate analyses performed on subjects stratified by presence of comorbidities confirmed these findings (*Supplementary Table S5*). In persons with a CCI = 0, IGFBP-2 as a continuous variable was only associated with outcome in the univariable analysis. However, in persons with CCI > 0, IGFBP-2 associated with mortality in all models. Restricting analyses to those treated with metformin or to those with T2D diagnosed at the time of enrollment, prior to enrollment but within the last 2 years, or more than 2 years before enrollment did not change the associations.

4. Discussion

In this cohort of 1417 persons with T2D, low circulating IGFBP-2 was associated with high fasting glucose and insulin secretion and low HOMA2-S, which may indicate insulin resistance. However, levels were not affected by treatment with metformin. Interestingly, IGFBP-2 level at enrollment was higher in persons who suffered from comorbidities, and concentrations associated positively with five-year all-cause mortality. The prognostic ability of IGFBP-2 appeared to rely primarily on its association with comorbidities. This finding may appear contradictory, as subjects with high IGFBP-2 had a more favorable metabolic risk profile.

To our knowledge, this is the first clinical study to demonstrate that metformin does not exert an effect on circulating IGFBP-2 level. Metformin is the most extensively prescribed insulin-sensitizing and glucose-lowering agent [23], and thus, given the association between IGFBP-2 and insulin sensitivity, it is not far-fetched to hypothesize that IGFBP-2 is influenced by metformin actions. So far, the association between IGFBP-2 and metformin has been assessed in two previous studies only, and results were conflicting. In cell cultures and mice, metformin increased gene expression of *IGFBP2* [14]. The opposite was demonstrated in prostate cancer cell lines, with metformin treatment causing a reduction in *IGFBP2* gene expression [15]. In a relatively small study by Kang et al., IGFBP-2 was assessed in 36 T2D persons receiving metformin, 20 persons receiving other glucose-lowering medications, and 53 non-diabetes controls [14]. In T2D persons receiving metformin, IGFBP-2 level was increased compared to T2D persons receiving other medications, and levels were comparable to those found in non-diabetics. Thus, the study contained no strict comparison of drug-naive and metformin-treated T2D persons, as opposed to our study. Therefore, we conclude that metformin does not seem to affect IGF homeostasis by modulating IGFBP-2 in persons with T2D.

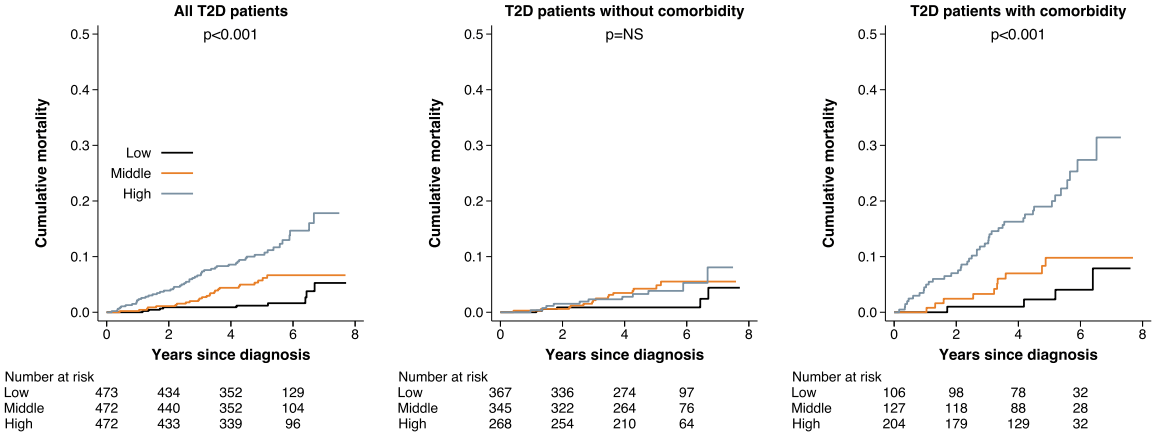

Fig. 3. All-cause mortality in persons according to tertiles of IGFBP-2. P-values: log-rank test for equality of survival between tertile groups. T2D, type 2 diabetes mellitus.

Table 2
Cox regression analyses. IGFBP-2 as a continuous and categorical variable was investigated both in univariable and multivariable analyses. Model 1 was adjusted for sex, age, BMI, C-peptide, eGFR, and CCI. Model 2 was adjusted for sex, age, BMI, C-peptide, eGFR, CCI, diabetes duration, fasting blood glucose, HbA_{1c}, diastolic blood pressure, HDL cholesterol, and smoking. Missing covariables were treated with multiple imputation.

Log_2-IGFBP-2		Univariable		Model 1		Model 2	
Model	Range (ng/mL)	HR (95 % CI)	p	HR (95 % CI)	p	HR (95 % CI)	p
Continuous*		2.62 (2.04;3.37)	<0.001	2.11 (1.55;2.88)	<0.001	2.21 (1.61;3.01)	<0.001
Categorical†							
Low tertile	74–207	Reference		Reference		Reference	
Middle tertile	209–362	2.87 (1. 34;6.16)	0.027	2.02 (0.93;4.45)	0.077	2.09 (0.95;4.61)	0.068
High tertile	366–1486	6.23 (3.07;12.64)	<0.000	3.23 (1.47;7.09)	0.003	3.57 (1.61;7.92)	0.002

* HR per doubling of IGFBP-2; modeled as log(IGFBP-2)/log(2).
BMI, body mass index; CCI, Charlson Comorbidity Index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HR, hazard ratio; IGFBP-2, insulin-like growth factor binding protein-2.
† HR with the low tertile as reference group.

In the present study, low IGFBP-2 was cross-sectionally associated with increased fasting glucose, C-peptide, insulin resistance, and body fat. This finding is in accordance with previous observations and supports the common conception that IGFBP-2 possesses metabolically beneficial and T2D-protective effects, primarily mediated through its leading operator, IGF-1 [24]. Supportive of this, IGFBP-2 transgenic mice show reduced caloric intake and susceptibility to obesity and insulin resistance in response to dietary excess [7]. IGFBP-2 overexpression also results in improved hepatic insulin sensitivity and a reversal of the diabetic phenotype in diet-induced obese mice and leptin-deficient mice [8]. Furthermore, IGFBP-2 is the primary binding protein secreted by differentiating white adipocytes [25], and administration of IGFBP-2 to human adipocytes results in an impairment of adipogenesis and adipocyte size, especially at the visceral level [26]. However, despite a potential benefit of IGFBP-2 in obesity prevention, its use as a therapeutic has yet to be pursued [7,8,13].

Considering the strong inverse association between IGFBP-2 and insulin, IGFBP-2 may also serve as a robust biomarker for the identification of individuals with so-called insulinopenic T2D, who are characterized by relatively high insulin sensitivity but reduced beta cell function [27]. Unlike the hyperinsulinemic T2D person, who is severely insulin resistant and benefits from treatment with insulin-sensitizing agents such as metformin, the ideal path to normoglycemia for the insulinopenic person is to improve insulin secretion or use insulin treatment. IGFBP-2 could potentially identify this subgroup of persons, who may benefit from treatment aiming at increasing insulin levels and thus allow for more individualized treatments. Importantly, the lack of noticeable prandial or diurnal variability makes IGFBP-2 especially attractive as a biomarker in situations where fasting blood samples are not available [28].

Despite the apparent beneficial metabolic effects of IGFBP-2, our study also demonstrate that elevated serum IGFBP-2 concentrations are anything but beneficial as regards morbidity. High IGFBP-2 has been observed in numerous diseases, including cardiovascular disease [29,30], neurodegenerative diseases [31], osteoporosis, and bone diseases [32]. Especially in cancer, IGFBP-2 has been considered to act as an oncogene, and it appears integrally involved in cellular growth and apoptosis through IGF-driven mechanisms [11,33–35]. Blocking of IGFBP-2 efficiently reduce tumor growth and metastasis *in vitro* and in rodents [36,37]. Of note, IGFBP-2 seems to exert intrinsic roles in tumorigenesis through a variety of IGF-independent molecular pathways [36]. IGFBP-2 interacts with transcription factors and cytoplasm-nuclear transporters [36] and it can inactivate the tumor suppressor gene PTEN [38]. Thus, these findings provide direct mechanistic links between IGFBP-2 and cancer disease. In conditions outside of cancer, IGFBP-2 appears to possess a plethora of functions, although the effects are less clear. Its association to morbidities may partly be explained by impaired nutritional status, as IGFBP-2 levels are known to be very high in patients with anorexia nervosa and reduced following refeeding [39]. High levels of IGFBP-2 also associate with various inflammatory mediators, and malnutritional, catabolic states, and inflammatory conditions are all coexistent with many diseases and furthermore, associated with reduced IGF-1. Collectively, our results suggest that there is a U-shaped association between serum IGFBP-2 level and detrimental effects. Low IGFBP-2 associates with high fasting glucose and insulin secretion, high IGFBP-2 associates with several morbidities. Thus, additional studies

may be able to identify an ideal IGFBP-2 range that associates with a healthier phenotype. Interestingly, similar U-shaped relationships are found for IGF-1 and other IGFBPs, especially IGFBP-1 [6,40].

Besides the association between IGFBP-2 and morbidity, we found a strong association between baseline IGFBP-2 level and mortality during follow-up. In the entire cohort, persons in the high IGFBP-2 tertile group died at three times the rate of persons in the low tertile group, despite adjustment for CCI and age. Thus, combined with its association with morbidity, IGFBP-2 possesses prognostic value for mortality, and this relationship appears independent of metabolic status. Consequently, regarding non-diabetes disease monitoring and mortality, IGFBP-2 levels must be interpreted in relation to insulin sensitivity, and analyses should always be adjusted for potential metabolic confounders.

The primary strength of the study is the size and comprehensiveness of the DD2 cohort, and its linkage to several high-quality Danish health registers. However, for this investigation, subjects were selected based on treatment characteristics, and consequently, our cohort does not fully represent the general T2D person in Denmark (n = 8246 at index date for this study). Furthermore, those who did not receive any glucose-lowering therapy at baseline were older than those who received monotherapy or combination therapy, and persons with a high comorbidity level were more likely to initiate glucose-lowering therapy. We also noticed that the likelihood of initiating glucose-lowering therapy during the first year following diagnosis was higher in persons with central obesity, limited physical activity, high blood glucose, and high HbA_{1c} [41]. In addition, the limitations of this study include a possible participation bias when entering the DD2 cohort [42]. We may expect reduced participation of individuals with a severe T2D phenotype or high cardiovascular risk, which may bias results toward the null hypothesis. Furthermore, studying associations with morbidity and mortality is rendered difficult by the complex nature of the T2D patient profile. A variety of other drugs used in this cohort, including antihypertensive and hypolipidemic treatments, likely impede the interpretations. Finally, based on our observations and the study design, we can only speculate on potential causal pathways that link IGFBP-2 to T2D.

5. Conclusions

In persons with T2D, high IGFBP-2 levels associated with increased comorbidity at baseline and all-cause mortality. Concentrations of IGFBP-2 correlated with estimated parameters of insulin sensitivity, including fasting glucose and insulin secretion, but were unaffected by treatment with metformin. Our findings suggest that IGFBP-2 is intimately involved in the metabolic dysregulation that accompanies T2D, possesses a plethora of functions in human health and disease and holds potential both as an indicator of insulin resistance and for the detection and monitoring of various pathologies.

5.1. Duality of Interest

The authors have nothing to declare.

Author contributions

RH, JVS, JSN, and JF conceived and designed the study. JSN is the principal manager of the DD2 project, and MRK is research consultant at DD2. RH, MRK, and JVS were responsible for data collection of the background population and generation of the database for the current cohort. IB and DAO were responsible for the biochemical analyses. Statistical analyses were performed by RH. Data interpretation was performed by RH, MRK, JSN, and JF. All authors participated in the discussion and interpretation of the results. RH organized the writing and wrote the initial draft, which was critically revised and approved by all authors. RH, JSN, and JF are the guarantors of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2023.110977.

References

[1] Clemmons DR. Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. Endocrinol Metab Clin North Am 2012;41:425–43,:vii-viii.
[2] Hjortebjerg R, Flyvbjerg A, Frystyk J. Insulin growth factor binding proteins as therapeutic targets in type 2 diabetes. Expert Opin Ther Targets 2014;18:209–24. https://doi.org/10.1517/14728222.2014.858698.
[3] Hjortebjerg R, Frystyk J. Determination of IGFs and their binding proteins. Best Pract Res Clin Endocrinol Metab 2013;27:771–81. https://doi.org/10.1016/j.beem.2013.08.010.
[4] Haywood NJ, Slater TA, Matthews CJ, Wheatcroft SB. The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes. Mol Metab 2019;19:86–96. https://doi.org/10.1016/j.molmet.2018.10.008.
[5] Heald AH, Kaushal K, Siddals KW, Rudenski AS, Anderson SG, Gibson JM. Insulin-like Growth Factor Binding Protein-2 (IGFBP-2) is a Marker for the Metabolic Syndrome. Exp Clin Endocrinol Diabetes 2006;114:371–6. https://doi.org/10.1055/s-2006-924320.
[6] Brismar K, Hilding A, Ansurudeen I, Flyvbjerg A, Frystyk J, Östenson CG. Adiponectin, IGFBP-1 and -2 are independent predictors in forecasting prediabetes and type 2 diabetes. Front Endocrinol (Lausanne) 2022;13:1092307. https://doi.org/10.3389/fendo.2022.1092307.
[7] Wheatcroft SB, Kearney MT, Shah AM, Ezzat VA, Miell JR, Modo M, et al. IGF-Binding Protein-2 Protects Against the Development of Obesity and Insulin Resistance. Diabetes 2007;56:285–94. https://doi.org/10.2337/db06-0436.
[8] Hedbacker K, Kv B, Wysocki RW, Asilmaz E, Ahima RS, Farooqi IS, et al. Antidiabetic Effects of IGFBP2, a Leptin-Regulated Gene. Cell Metab 2010;11:11–22.
[9] van den Beld AW, Carlson OD, Doyle ME, Rizopoulos D, Ferrucci L, van der Lely AJ, et al. IGFBP-2 and aging: a 20-year longitudinal study on IGFBP-2, IGF-I, BMI, insulin sensitivity and mortality in an aging population. Eur J Endocrinol 2019;180:109–16. https://doi.org/10.1530/eje-18-0422.
[10] van den Beld A, Blum W, Brugts M, Janssen J, Grobbee D, Lamberts S. High IGFBP2 levels are not only associated with a better metabolic risk profile but also with increased mortality in elderly men. Eur J Endocrinol 2012;167:111–7.
[11] Espelund U, Renehan AG, Cold S, Oxvig C, Lancashire L, Su Z, et al. Prognostic relevance and performance characteristics of serum IGFBP-2 and PAPP-A in women with breast cancer: a long-term Danish cohort study. Cancer Med 2018. https://doi.org/10.1002/cam4.1504.
[12] Liou J-M, Shun C-T, Liang J-T, Chiu H-M, Chen M-J, Chen CC, et al. Plasma Insulin-Like Growth Factor-Binding Protein-2 Levels as Diagnostic and Prognostic Biomarker of Colorectal Cancer. J Clin Endocrinol Metab 2010;95:1717–25. https://doi.org/10.1210/jc.2009-2668.
[13] Boughanem H, Yubero-Serrano EM, López-Miranda J, Tinahones FJ, Macias-Gonzalez M. Potential Role of Insulin Growth-Factor-Binding Protein 2 as

Therapeutic Target for Obesity-Related Insulin Resistance. Int J Mol Sci 2021;22:1133. https://doi.org/10.3390/ijms22031133.
[14] Kang HS, Cho H-C, Lee J-H, Oh GT, Koo S-H, Park B-H, et al. Metformin stimulates IGFBP-2 gene expression through PPARalpha in diabetic states. Sci Rep. 2016;6:23665-. 10.1038/srep23665.
[15] Biernacka KM, Persad RA, Bahl A, Gillatt D, Holly JMP, Perks CM. Hyperglycaemia-induced resistance to Docetaxel is negated by metformin: a role for IGFBP-2. Endocr Relat Cancer 2017;24:17–30. https://doi.org/10.1530/ERC-16-0095.
[16] Christensen DH, Nicolaisen SK, Berencsi K, Beck-Nielsen H, Rungby J, Friborg S, et al. Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile. BMJ Open 2018;8:e017273.
[17] Nielsen JS, Thomsen RW, Steffensen C, Christiansen JS. The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) study: implementation of a nationwide patient enrollment system. Clin Epidemiol 2012;4:27–36. https://doi.org/10.2147/clep.S30838.
[18] Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90. https://doi.org/10.2147/clep.S91125.
[19] Christensen DH, Nicolaisen SK, Ahlqvist E, Stidsen JV, Nielsen JS, Hojlund K, et al. Type 2 diabetes classification: a data-driven cluster study of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. BMJ Open Diabetes Res Care 2022;10. https://doi.org/10.1136/bmjdrc-2021-002731.
[20] Johannesdottir SA, Horváth-Puhó E, Ehrenstein V, Schmidt M, Pedersen L, Sørensen HT. Existing data sources for clinical epidemiology: The Danish National Database of Reimbursed Prescriptions. Clin Epidemiol 2012;4:303–13. https://doi.org/10.2147/clep.S37587.
[21] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83. https://doi.org/10.1016/0021-9681(87)90171-8.
[22] Harrell Jr FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361–87. https://doi.org/10.1002/(sici)1097-0258(19960229)15:4<361::aid-sim168>3.0.co;2-4.
[23] Zilov AV, Abdelaziz SI, AlShammary A, Al Zahrani A, Amir A, Assaad Khalil SH, et al. Mechanisms of action of metformin with special reference to cardiovascular protection. Diabetes Metab Res Rev 2019;35:e3173.
[24] Frystyk J, Skjærbæk C, Vestbo E, Fisker S, Ørskov H. Circulating levels of free insulin-like growth factors in obese subjects: the impact of Type 2 diabetes. Diabetes Metab Res Rev 1999;15:314–22. https://doi.org/10.1002/(SICI)1520-7560(199909/10)15:5<314::AID-DMRR56>3.0.CO;2-E.
[25] Boney CM, Moats-Staats BM, Stiles AD, D'Ercole AJ. Expression of insulin-like growth factor-I (IGF-I) and IGF-binding proteins during adipogenesis. Endocrinology 1994;135:1863–8. https://doi.org/10.1210/en.135.5.1863.
[26] Yau SW, Russo VC, Clarke IJ, Dunshea FR, Werther GA, Sabin MA. IGFBP-2 inhibits adipogenesis and lipogenesis in human visceral, but not subcutaneous, adipocytes. Int J Obes 2015;39:770–81. https://doi.org/10.1038/ijo.2014.192.
[27] Stidsen JV, Henriksen JE, Olsen MH, Thomsen RW, Nielsen JS, Rungby J, et al. Pathophysiology-based phenotyping in type 2 diabetes: A clinical classification tool. Diabetes Metab Res Rev 2018;34:e3005.
[28] Rajaram S, Baylink DJ, Mohan S. Insulin-Like Growth Factor-Binding Proteins in Serum and Other Biological Fluids: Regulation and Functions. Endocr Rev 1997;18:801–31.
[29] Hjortebjerg R, Laugesen E, Høyem P, Oxvig C, Stausbøl-Grøn B, Knudsen ST, et al. The IGF system in patients with type 2 diabetes: associations with markers of cardiovascular target organ damage. Eur J Endocrinol 2017;176:521–31. https://doi.org/10.1530/eje-16-0940.
[30] Hoeflich A, David R, Hjortebjerg R. Current IGFBP-related biomarker research in cardiovascular disease – we need more structural and functional information in clinical studies. Front Endocrinol (Lausanne) 2018;9. https://doi.org/10.3389/fendo.2018.00388.
[31] McGrath ER, Himali JJ, Levy D, Conner SC, DeCarli CS, Pase MP, et al. Circulating IGFBP-2: a novel biomarker for incident dementia. Ann Clin Transl Neurol 2019;6:1659–70. https://doi.org/10.1002/acn3.50854.
[32] Amin S, Riggs BL, Atkinson EJ, Oberg AL, Melton 3rd LJ, Khosla S. A potentially deleterious role of IGFBP-2 on bone density in aging men and women. J Bone Miner Res 2004;19:1075–83. https://doi.org/10.1359/jbmr.040301.
[33] Yao X, Sun S, Zhou X, Guo W, Zhang L. IGF-binding protein 2 is a candidate target of therapeutic potential in cancer. Tumour Biol 2016;37:1451–9. https://doi.org/10.1007/s13277-015-4561-1.
[34] Thomsen J, Hjortebjerg R, Espelund U, Ortoft G, Vestergaard P, Magnusson NE, et al. PAPP-A proteolytic activity enhances IGF bioactivity in ascites from women with ovarian carcinoma. Oncotarget 2015;6(32266–78).
[35] Agerholm J, Hjortebjerg R, Espelund U, Rasmussen TR, Folkersen B, Bjerre M, et al. Development of a novel assay for IGFBP-2 complexed with IGF-I and-II in human serum. Growth Horm IGF Res 2020;51:38–45. https://doi.org/10.1016/j.ghir.2020.01.003.
[36] Russo VC, Azar WJ, Yau SW, Sabin MA, Werther GA. IGFBP-2: The dark horse in metabolism and cancer. Cytokine Growth Factor Rev 2015;26:329–46. https://doi.org/10.1016/j.cytogfr.2014.12.001.
[37] Lee E-J, Mircean C, Shmulevich I, Wang H, Liu J, Niemistö A, et al. Insulin-like growth factor binding protein 2 promotes ovarian cancer cell invasion. Mol Cancer 2005;4:7. https://doi.org/10.1186/1476-4598-4-7.
[38] Perks CM, Vernon EG, Rosendahl AH, Tonge D, Holly JMP. IGF-II and IGFBP-2 differentially regulate PTEN in human breast cancer cells. Oncogene 2007;26:5966–72. https://doi.org/10.1038/sj.onc.1210397.
[39] Støving RK, Flyvbjerg A, Frystyk J, Fisker S, Hangaard J, Hansen-Nord M, et al. Low Serum Levels of Free and Total Insulin-Like Growth Factor I (IGF-I) in Patients with Anorexia Nervosa Are Not Associated with Increased IGF-Binding Protein-3 Proteolysis1. J Clin Endocrinol Metab 1999;84:1346–50. https://doi.org/10.1210/jcem.84.4.5622.
[40] Wheatcroft SB, Kearney MT. IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis. Trends Endocrinol Metab 2009;20:153–62. https://doi.org/10.1016/j.tem.2009.01.002.
[41] Mor A, Berencsi K, Svensson E, Rungby J, Nielsen JS, Friborg S, et al. Prescribing practices and clinical predictors of glucose-lowering therapy within the first year in people with newly diagnosed Type 2 diabetes. Diabet Med 2015;32:1546–54. https://doi.org/10.1111/dme.12819.
[42] Rohde C, Nielsen JS, Schöllhammer Knudsen J, Thomsen RW, Østergaard SD. Risk factors associated with mortality among individuals with type 2 diabetes and depression across two cohorts. Eur J Endocrinol 2022;187:567–77. https://doi.org/10.1530/eje-22-0466.