Insulin-like growth factor-binding protein-1 in the prediction and development of type 2 diabetes in middle-aged Swedish men

M. S. Lewitt · A. Hilding · C.-G. Östenson · S. Efendic · K. Brismar · K. Hall

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Abstract

Aims/hypothesis Insulin-like growth factor-binding protein-1 (IGFBP-1) production in the liver is inhibited by insulin, and low circulating levels are associated with the metabolic syndrome. The aim of this study was to evaluate the predictive role and change in IGFBP-1 concentrations during development of abnormal glucose regulation.

Methods IGFBP-1 levels were determined at baseline and at 10 years in an incident case-control prospective study of Swedish white men aged 35–56 years. Individuals with normal glucose tolerance at baseline who developed abnormal glucose tolerance during a 10 year period ($n=355$) according to WHO criteria were pair-matched to controls for age and family history of diabetes.

Results Fasting IGFBP-1 concentrations were lower in individuals who later developed abnormal glucose regulation and correlated inversely with fasting proinsulin values ($r=-0.48$; $p<0.0001$), and both were significant predictors. Individuals in the highest quartile at baseline for an algorithm incorporating fasting IGFBP-1, blood glucose, proinsulin and waist and height had a 40-fold increased risk of developing type 2 diabetes compared with the lowest quartile (95% CI 7.7–214). IGFBP-1 increased 32% (95% CI 17–49%) during the

10 years in those developing diabetes and was increased in relation to insulin levels, suggesting the emergence of hepatic insulin resistance. Moreover, elevated IGFBP-1 levels at follow-up were associated with higher 2 h glucose values during an OGTT.

Conclusions/interpretation Low IGFBP-1 predicts the development of abnormal glucose regulation and, as an inhibitor of the insulin-like actions of insulin-like growth factors, elevated levels of IGFBP-1 after the development of diabetes may also play a pathophysiological role.

Keywords Diabetes prediction · Hepatic insulin resistance · Impaired fasting glucose · Impaired glucose tolerance · Insulin-like growth factor-binding protein-1 · Insulin-like growth factor-I · Type 2 diabetes

Abbreviations

FHD	family history of diabetes
IFG	impaired fasting glucose
IGF-I	insulin-like growth factor-I
IGFBP-1	insulin-like growth factor-binding protein-1
IGT	impaired glucose tolerance
Wa ² HtR	waist ² :height ratio

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M. S. Lewitt (✉) · A. Hilding · C.-G. Östenson · S. Efendic · K. Brismar · K. Hall
The Rolf Luft Research Center for Diabetes and Endocrinology,
Department of Molecular Medicine and Surgery,
Karolinska Institutet, L1:01,
Karolinska University Hospital,
Stockholm, SE-171 76, Sweden
e-mail: Moira.Lewitt@ki.se

Introduction

Insulin-like growth factor-binding protein-1 (IGFBP-1) is secreted by the liver and has an inhibitory effect on the actions of insulin-like growth factor (IGF)-I and IGF-II in peripheral tissues. IGFBP-1 synthesis is regulated by insulin and other factors that are altered in conditions associated with abnormal glucose regulation. The suppression of IGFBP-1 production by insulin

is achieved within the physiological range of insulin concentrations [1–3]. There are multiple stimulators, including glucagon [4] and inflammatory cytokines [5, 6]. The pattern of regulation and its inhibitory effect on the insulin-like activity of the IGFs, suggest that IGFBP-1 has a role as a glucose counter-regulator [7]. Adding to the complexity of IGFBP-1 regulation, IGFBP-1 passage across the endothelium is reported to be enhanced by insulin [8], and we have recently discovered a specific IGFBP-1 protease activity [9].

In cross-sectional studies, low circulating concentrations of IGFBP-1 are associated with the metabolic syndrome and cardiovascular disease [10–14]. In a prospective population study, individuals with low IGFBP-1 had increased risk of cardiovascular disease mortality [15]. Surprisingly, few studies have considered its value as a predictive marker of abnormal glucose tolerance. Low IGFBP-1 concentrations in early pregnancy predict the development of gestational diabetes [16]. In one population study individuals with low fasting IGFBP-1 levels were found to have an inverse relationship between IGF-I concentrations and the later development of glucose intolerance [17].

Identification of individuals at high risk of developing type 2 diabetes mellitus should be given high priority in prevention of the disease. In addition to patients with diabetes, individuals with impaired glucose tolerance (IGT) are also at increased risk of micro- and macrovascular disease and premature cardiovascular death [18, 19]. We have recently shown that family history of diabetes (FHD) is associated with increased prevalence of abnormal glucose tolerance [20] especially in men [21]. The aim of the present study was to determine IGFBP-1 levels in this population, in order to evaluate their predictive role and change in concentrations during the development of abnormal glucose regulation.

Methods

Study population The present study was an incident case-control study of Swedish white men who were part of a 10 year follow-up epidemiological survey, the Stockholm Diabetes Prevention Program (SDPP), which is described elsewhere [20–22]. The SDPP is a population-based study of individuals aged 35–56 years who were without known diabetes at the time of recruitment in 1992–1994. To study the impact of diabetes heredity a sample of individuals without FHD were matched to those with FHD by age and municipality. FHD was defined as known diabetes in at least one first-degree relative or at least two second-degree

relatives and with an onset of diabetes at an age predominantly above 35 years.

In the baseline study group a normal OGTT was found in 1,422 men with and 1,413 without FHD (Fig. 1). After 10 years, 2,746 men not having type 2 diabetes at baseline were invited to participate in a follow-up study and 2,383 accepted (87%). The baseline and follow-up studies consisted of a questionnaire covering lifestyle factors, a health examination and an OGTT. The data on FHD were updated at the follow-up and the frequency increased from 51.8% to 56.7%. Measurements of weight, height and waist were performed with the individuals wearing light indoor clothes and no shoes. Waist was measured in the standing position midway between the lower costal margin and the iliac crest. BP was taken in the supine position. On each occasion a 75 g OGTT was performed after an

Fig. 1 Study design: selection and follow-up of men in the Stockholm Diabetes Prevention Program. The groups from which the present study population was derived were categorised according to 1999 WHO criteria and are shown in bold type. FHD+, family history of diabetes; FHD-, no family history of diabetes; T2DM, type 2 diabetes mellitus. *Exclusion criteria: already known diabetes (2.5%), unclear FHD (27.4%), insufficient FHD, according to inclusion criteria (15.0%), foreign origin (2.1%)

overnight fast. Blood was sampled before and 2 h after glucose ingestion.

Only those in whom heredity was certain at follow-up were included in the selection group ($n=2,150$). The study group comprised 355 individuals who had normal glucose tolerance at baseline and abnormal glucose regulation at follow up. FHD was present in 69%. The controls were pair-matched to cases by random selection of 355 individuals from the group of 1,795 with normal glucose tolerance at baseline and at follow-up, within FHD and age. We did not match for weight or waist measurements in order to determine the effect of these variables. There were 43 men in whom a diagnosis of diabetes mellitus was made during the 10 year period. The diagnosis was made by a physician according to WHO criteria. In those diagnosed before 1999 the 1985 WHO criteria were used and from 1999 the revised criteria were used [23]. Only three in this group had an OGTT in the follow-up study. There were 23 taking oral hypoglycaemic agents, and two on insulin therapy. All other individuals were categorised after OGTT at follow-up according to 1999 WHO criteria. Individuals with a 2 h plasma glucose of 7.8–11.0 mmol/l and fasting values of <6.1 mmol/l were classified as having IGT, while impaired fasting glucose (IFG) was defined as fasting glucose levels of 6.1–6.9 mmol/l and a 2 h glucose <7.8 mmol/l. Those with a fasting value of 6.1–6.9 mmol/l and a 2 h glucose of 7.8–11.0 mmol/l were classified as having combined glucose intolerance (IFG+IGT). There were 64 individuals with a fasting glucose of ≥ 7.0 mmol/l and/or 2 h glucose of ≥ 11.1 mmol/l who were classified as having diabetes mellitus. In this group 30 had isolated post-challenge hyperglycaemia: 19 individuals had fasting glucose values of ≤ 6.0 mmol/l and 11 had fasting glucose levels of 6.1–6.9 mmol/l. The group of 11 individuals had similar baseline characteristics to the rest of the type 2 diabetes group and are included in that group for the analyses.

All participants gave informed consent and the study was approved by the Ethics Committee of Karolinska University Hospital and carried out in accordance with the Declaration of Helsinki.

Assays Serum IGFBP-1 was measured by an in-house RIA using a polyclonal antibody and human IGFBP-1 as standard, as previously described [24]. The intra- and inter-assay CV values were 3% and 10%, respectively. Samples taken from the two OGTT studies 10 years apart from the same individual were analysed in the same assay. IGFBP-1 is stable in samples stored for prolonged periods. When samples were re-analysed after 6 years of

storage at -20°C , the regression line was $y = -4.61 + 1.011x$ ($n=20$), where x was the original measurement in recently obtained samples and y the value measured after prolonged storage. Phosphorylated and non-phosphorylated IGFBP-1 have similar potencies in this assay (data not shown).

IGF-I was measured in serum by RIA after acid-ethanol extraction and cryoprecipitation, using des(1–3)IGF-I as tracer to minimise interference by IGFBPs [25]. Samples from the same individual were measured in the same assay. The intra- and inter-assay CV values were 4% and 11%, respectively.

Venous serum glucose was assayed using the glucose oxidase method with a Yellow Spring Glucose Analyzer (Yellow Springs, Yellow Springs, OH, USA). Immuno-reactive insulin was assayed by an in-house RIA, using a polyclonal antibody and human insulin as a standard, as previously described [26]. Proinsulin cross-reacts in this assay 100%. Baseline insulin values were measured in assays performed in 1992–1994, and the follow-up measurements were made in 2002–2004. Proinsulin was determined in baseline samples using an ELISA as previously described [22]. The limit of detection was 0.25 pmol/l and the inter-assay CV was 7.6–5.3% at 1.1–8.0 pmol/l. In addition to intact proinsulin, the four proinsulin intermediates cross-react with 65–99% efficiency in this assay.

Data analysis The results presented in the tables and figures are means $\pm$ 95% CIs. Waist was dependent on height in both the cases ($r=0.23$; $p<0.0001$) and the controls ($r=0.21$; $p<0.0001$). Waist²:height ratio (Wa^2HtR) ($[\text{waist measurement in cm}/10]^2/[\text{height in m} \times 10]$) was used because it abolished the effect of height ($r=0.01$ for both cases and controls) and allowed comparisons between groups. Correction for height has been used when evaluating the impact of waist measurement in other population studies [27]. Wa^2HtR , serum IGFBP-1, insulin and proinsulin values were not normally distributed and were log transformed before analysis. Differences between IGFBP-1 at baseline and follow-up were analysed by paired Student's t tests. Since the insulin assays were performed 10 years apart, differences between these time-points were not analysed. Comparison of continuous variables between two or more independent groups was assessed by an unpaired t test or ANOVA.

Linear regression analysis was used to examine the relationship between IGFBP-1 and other variables, and to investigate differences between regression lines analysis of covariance was used. Conditional logistic regression was performed to calculate ORs and 95% CIs. Variables were categorised in quartiles according to their distribution in the whole group, amongst cases

Table 1 Baseline data for the control group ($n=355$)

Variables at baseline	Mean (95% CI)
Age (years)	47.4 (46.9–47.9)
Height (m)	1.80 (1.79–1.80)
Weight (kg)	83.0 (81.9–84.2)
BMI (kg/m^2)	25.7 (25.4–26.1)
Waist (cm)	92 (91–92)
Wa ² HtR ^a	4.64 (4.57–4.72)
IGF-I ($\mu\text{g}/\text{l}$) ^a	182 (177–187)
Systolic BP (mmHg)	123 (122–125)
Diastolic BP (mmHg)	79 (78–80)
Glucose (mmol/l)	
0 h	4.6 (4.6–4.7)
2 h	4.4 (4.3–4.5)
Proinsulin (pmol/l) ^a	6.2 (5.8–6.6)
Insulin (pmol/l) ^a	
0 h	102 (102–108)
2 h	270 (252–282)
IGFBP-1 ($\mu\text{g}/\text{l}$) ^a	
0 h	20 (19–21)
2 h	9 (9–10)
2 h/0 h (%)	47 (46–49)

^a Geometric mean

and their respective controls. The lowest quartile (for glucose, BMI, Wa²HtR, insulin, proinsulin and algorithms [see below]) or the highest quartile (for IGFBP-1) were used as the reference in the calculation of OR. Algorithms were derived in order to evaluate the combined contribution of variables in a way that could be useful to other investigators in the field. The equations were as follows:

Algorithm 1: $\text{f-Glucose} - 0.33 \times [^2 \log (\text{f-IGFBP-1})]$

Algorithm 2: $\text{f-Glucose} + \text{Wa}^2\text{HtR}$

Algorithm 3: $\text{f-Glucose} - 0.33$

$\times [^2 \log (\text{f-IGFBP-1}/\text{f-Proinsulin})]$

Algorithm 4: $\text{f-Glucose} + 0.33$

$\times [^2 \log (\text{Wa}^2\text{HtR}) - ^2 \log (\text{f-IGFBP-1}/\text{f-Proinsulin})]$

where f indicates fasting.

Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC, USA) and Statistica StatSoft, version 7 (Tulsa, OK, USA). Statistical significance was set at <0.01 , to take into account the repeated comparisons.

Results

Baseline values Baseline data for the whole control group are shown in Table 1, for the IGT, IFG and IFG+IGT

groups in Table 2 and for the diabetes groups in Table 3. In the diabetes group diagnosed at follow-up 19 men had a normal fasting glucose (mean 5.2 mmol/l, 95% CI 4.9–5.4) but met the diagnostic criteria for diabetes 2 h after a glucose load. This group, shown separately in Table 3, was shorter in stature but otherwise not different from matched controls. They were leaner than the rest of the diabetes group, at both baseline and follow-up (data not shown). In the main diabetes groups ($n=88$) anthropomorphic measures, glucose, insulin and proinsulin concentrations were markedly different compared with controls matched for heredity and age. In addition both systolic and diastolic BPs were higher than controls. This diabetes group included individuals diagnosed during the 10 years before follow-up evaluation ($n=43$) as well as those in whom the diagnosis was made for the first time at the follow-up ($n=45$). These groups did not differ at baseline in the parameters shown in Table 3. The IGT individuals also had higher systolic and diastolic BPs at baseline compared with controls. This was not seen in those with IFG or the IFG+IGT group.

At baseline, IGFBP-1 concentrations fasting and 2 h after the OGTT, were lower in each of the study groups compared with matched controls (Tables 2 and 3). A notable exception is the subgroup of 19 individuals with isolated post-challenge hyperglycaemia. This group did not have lower IGFBP-1 concentrations at baseline. The suppression in IGFBP-1 after an oral glucose load was similar in all groups and not significantly different from matched controls. IGF-I values were not significantly different from controls in any of the groups. The group with diabetes had a higher percentage of individuals with heredity for diabetes (84% compared with 65% for the IGT, IFG and IFG+IGT groups). The pattern in the baseline parameters was similar in those with and without FHD.

In the whole control group, log-transformed fasting IGFBP-1 correlated inversely with log-transformed fasting insulin levels ($r=-0.34$; $p<0.001$) and with log-transformed proinsulin levels ($r=-0.47$; $p<0.001$) as well as Wa²HtR ($r=-0.40$; $p<0.001$), BMI ($r=-0.44$; $p<0.001$) and glucose ($r=-0.19$; $p<0.001$), which all correlated positively to each other (data not shown). Proinsulin levels alone explained 22% of the IGFBP-1 variation, while insulin accounted for only 11%. In multiple regression analyses, 26% of the IGFBP-1 variation could be explained by the variables proinsulin and Wa²HtR ($p<0.001$). Wa²HtR correlated with age ($r=0.16$; $p=0.003$), while IGFBP-1, proinsulin, insulin, BMI and glucose were independent of age. IGF-I showed the expected correlation with age ($r=-0.19$; $p<0.001$).

Factors predicting the development of abnormal glucose regulation Baseline variables and algorithms were categorised into quartiles and ORs for the development of IGT,

Table 2 Baseline data for the group having IGT, IFG or IFG+IGT at 10 year follow-up

Variables at baseline	IGT (<i>n</i> =122)		IFG (<i>n</i> =79)		IFG+IGT (<i>n</i> =47)	
	Mean (95% CI)	<i>p</i> value ^a	Mean (95% CI)	<i>p</i> value ^a	Mean (95% CI)	<i>p</i> value ^a
Age (years)	47.6 (46.7–48.4)	0.978	46.9 (45.9–47.9)	0.901	47.3 (45.8–48.9)	0.938
Height (m)	1.79 (1.78–1.80)	0.374	1.78 (1.76–1.80)	0.081	1.79 (1.77–1.81)	0.362
Weight (kg)	87.3 (85.0–89.6)	0.089	85.1 (82.7–87.6)	0.135	91.1 (87.2–95.1)	<0.001
BMI (kg/m ²)	27.3 (26.7–28.0)	0.020	26.7 (26.1–27.3)	0.002	28.3 (27.3–29.4)	<0.001
Waist (cm)	95 (94–97)	0.042	93 (92–95)	0.083	97 (95–100)	<0.001
Wa ² HtR ^b	5.02 (4.87–5.18)	0.022	4.86 (4.70–5.02)	0.022	5.27 (5.03–5.53)	<0.001
IGF-I (μg/l) ^b	189 (181–197)	0.675	178 (168–189)	0.405	188 (173–203)	0.504
Systolic BP (mmHg)	130 (127–133)	<0.001	124 (122–127)	0.375	131 (127–135)	0.123
Diastolic BP (mmHg)	83 (81–84)	0.007	81 (79–82)	0.494	84 (81–87)	0.067
Glucose (mmol/l)						
0 h	4.7 (4.6–4.8)	0.462	5.1 (4.9–5.2)	<0.001	4.9 (4.8–5.1)	0.002
2 h	5.3 (5.1–5.5)	<0.001	4.8 (4.5–5.1)	0.014	5.1 (4.8–5.5)	<0.001
Proinsulin (pmol/l) ^b	8.2 (7.3–9.3)	0.001	8.5 (7.3–10.0)	0.002	9.1 (7.5–11.1)	<0.001
Insulin (pmol/l) ^b						
0 h	120 (114–132)	0.067	120 (108–126)	0.025	126 (114–138)	0.011
2 h	426 (384–474)	<0.001	294 (258–330)	0.281	378 (312–462)	<0.001
IGFBP-1 (μg/l) ^b						
0 h	14 (13–16)	0.002	16 (14–19)	0.027	13 (10–16)	0.001
2 h	6 (6–7)	<0.001	7 (6–8)	0.031	6 (5–7)	<0.001
2 h/0 h (%)	45 (42–48)	0.148	45 (41–49)	0.990	45 (39–50)	0.112
FHD frequency	68%		59%		64%	

^a*p* value (unpaired *t* test), cases vs matched controls^bGeometric mean**Table 3** Baseline data for the group having type 2 diabetes at 10 year follow-up

Variables at baseline	Type 2 diabetes with fasting glucose at follow-up				
	>6.0 mmol/l (<i>n</i> =88)		≤6.0 mmol/l (<i>n</i> =19)		
	Mean (95% CI)	<i>p</i> value vs matched controls	Mean (95% CI)	<i>p</i> value vs matched controls	<i>p</i> value vs >6.0 mmol/l glucose
Age (years)	47.4 (46.4–48.4)	0.962	48.9 (47.0–50.9)	0.847	0.196
Height (m)	1.79 (1.78–1.80)	0.347	1.76 (1.73–1.79)	0.009	0.077
Weight (kg)	90.3 (87.4–93.1)	<0.001	77.9 (73.3–82.5)	0.053	<0.001
BMI (kg/m ²)	28.2 (27.4–29.0)	<0.001	25.1 (23.8–26.4)	0.392	<0.001
Waist (cm)	97 (95–99)	<0.001	91 (88–93)	0.388	0.005
Wa ² HtR ^a	5.21 (5.01–5.43)	<0.001	4.64 (4.34–4.95)	0.739	0.012
IGF-I (μg/l) ^a	187 (178–197)	0.052	173 (152–197)	0.821	0.306
Systolic BP (mmHg)	131 (128–134)	<0.001	125 (118–132)	0.660	0.137
Diastolic BP (mmHg)	83 (81–85)	<0.001	79 (75–83)	1.000	0.116
Glucose (mmol/l)					
0 h	5.1 (5.0–5.2)	<0.001	4.6 (4.3–5.0)	0.854	0.002
2 h	5.6 (5.3–5.9)	<0.001	5.7 (5.0–6.4)	0.016	0.713
Proinsulin (pmol/l) ^a	12.1 (10.5–13.9)	<0.001	5.6 (4.2–7.5)	0.708	<0.001
Insulin (pmol/l) ^a					
0 h	138 (126–150)	<0.001	114 (96–138)	0.374	0.157
2 h	402 (342–474)	<0.001	354 (294–426)	0.415	0.132
IGFBP-1 (μg/l) ^a					
0 h	14 (12–16)	<0.001	20 (15–27)	0.774	0.036
2 h	6 (5–7)	<0.001	9 (6–13)	0.803	0.071
2 h/0 h (%)	46 (43–50)	0.874	44 (38–52)	0.248	0.584
FHD frequency	84%		58%		

^aGeometric mean

IFG or IFG+IGT and diabetes were determined. (Fig. 2; Electronic supplementary material [ESM] Tables 1, 2 and 3). The 19 cases with diabetes with isolated post-challenge hyperglycaemia and fasting glucose ≤ 6.0 mmol/l were excluded for this analysis. IGF-I was not a significant predictor of abnormal glucose regulation (data not shown). Individuals in the highest quartile for fasting glucose had a five- to tenfold increased risk of developing IFG and type 2 diabetes. When the inverse of IGFBP-1 was used alone, or combined with glucose (Algorithm 1), individuals in the highest quartile had a greater than a tenfold risk of diabetes. There was a trend to an increased prediction if proinsulin was added to IGFBP-1 (Algorithm 3). A similar prediction was obtained for glucose and Wa^2HtR (Algorithm 2), with those in the highest quartile having a 26-fold increased risk of diabetes. When all of the variables were combined (Algorithm 4), individuals in the highest quartile at baseline had a 40-fold increase risk of developing type 2 diabetes compared with the lowest quartile.

When using fasting IGFBP-1 as a continuous variable, the risk of abnormal glucose regulation was increased twofold by each halving of IGFBP-1 (Table 4). In order to evaluate which variables contributed independently in each of the algorithms, models were used where the variables were included separately and as continuous values in the conditional logistic regression analyses (Table 4). When used in Algorithm 1, fasting IGFBP-1 was independently associated with the development of abnormal glucose regulation. When fasting IGFBP-1 and fasting proinsulin were combined (Algorithms 3 and 4) proinsulin was excluded from independent association with the development of IFG or IFG+IGT, and IGFBP-1 was excluded from independent association with the development of diabetes.

Changes in IGFBP-1 at follow-up Overall there was no change in fasting IGFBP-1 concentrations in any of the control groups at 10 year follow-up, compared with baseline (paired *t* tests). In the individuals who had IGT and/or IFG at the 10 year follow-up, the fasting IGFBP-1 concentrations were unchanged and remained lower than matched controls (Fig. 3a). A similar pattern was seen for each group (IFG, IGT, IFG+IGT) when analysed separately. There was no significant change in fasting IGFBP-1 values in the 19 individuals with diabetes diagnosed on the basis of glucose values 2 h after oral glucose (20 μ g/l [95% CI 15–27] and 20 μ g/l [95% CI 13–32] at baseline and follow up, respectively). In those who developed diabetes, fasting IGFBP-1 concentrations were higher at the 10 years follow-up compared with baseline (paired *t* tests; Fig. 3b) so that they were not different from their control group at follow-up. During the OGTT performed at follow-up there was a

Fig. 2 ORs (95% CIs) of variables and algorithms for the development of IGT (a), IFG or IFG+IGT (b) and type 2 diabetes (c). The lowest quartile was used as the reference in the calculation. Note that the inverse of IGFBP-1 was used. f, fasting

similar suppression of IGFBP-1 to about 45% in all groups (data not shown).

In the group developing diabetes there was a parallel upwards shift of the regression line of fasting IGFBP-1 on fasting insulin at follow-up, which was not seen at baseline. Shown in Fig. 4a, the regression line at 10 year follow-up for the diabetes group was 37% higher ($F=16.09$; $p<0.001$) than that of the controls. In the control group, the change in IGFBP-1 was inversely correlated with a change in waist measurement (Fig. 4b). This relationship was not seen in the group developing diabetes with fasting glucose >6 mmol/l, but was preserved in the 19 with fasting glucose ≤ 6.0 mmol/l.

In the whole diabetes group, the percentage change in IGFBP-1 during 10 years was inversely correlated to the fall in IGF-I during 10 years (Fig. 5a) and to the IGF-I levels at follow-up ($r=-0.32$; $p<0.001$). There was no significant relationship between the change in IGFBP-1 and IGF-I in the

Table 4 ORs for the association of baseline variables used in the four algorithms with the development of IGT, IFG or IFG+IGT, and type 2 diabetes at 10 year follow-up: conditional logistic regression analysis is used with variables in continuous values

Included variables	IGT (<i>n</i> =122)		IFG or IFG+IGT (<i>n</i> =126)		Diabetes with fasting glucose >6.0 mmol/l (<i>n</i> =88)	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
Fasting IGFBP-1 ^{a,b}	1.70 (1.23–2.35)	0.001	1.79 (1.31–2.46)	<0.001	1.97 (1.37–2.82)	<0.001
Algorithm 1						
Fasting glucose	1.13 (0.67–1.88)	0.654	4.61 (2.41–8.82)	<0.001	3.56 (1.69–7.48)	<0.001
Fasting IGFBP-1 ^{a,b}	1.69 (1.22–2.35)	0.002	1.76 (1.24–2.48)	0.002	1.70 (1.15–2.51)	0.007
Algorithm 2						
Fasting glucose	1.07 (0.67–1.74)	0.797	4.67 (2.43–8.97)	<0.001	4.44 (1.97–10.01)	<0.001
Wa ² HtR	1.31 (0.99–1.72)	0.058	2.40 (1.48–3.88)	<0.001	2.39 (1.50–3.80)	<0.001
Algorithm 3						
Fasting glucose	1.10 (0.65–1.86)	0.732	4.14 (2.14–8.02)	<0.001	3.75 (1.60–8.79)	0.002
Fasting IGFBP-1 ^{a,b}	1.50 (1.06–2.13)	0.022	1.58 (1.08–2.31)	0.019	1.06 (0.66–1.72)	0.814
Fasting proinsulin ^a	1.44 (1.02–2.04)	0.037	1.27 (0.87–1.85)	0.212	2.33 (1.37–3.96)	0.002
Algorithm 4						
Fasting glucose	1.12 (0.65–1.94)	0.675	4.72 (2.34–9.53)	<0.001	4.06 (1.69–9.77)	0.002
Fasting IGFBP-1 ^{a,b}	1.56 (1.06–2.29)	0.024	1.55 (1.04–2.31)	0.033	0.91 (0.54–1.53)	0.720
Fasting proinsulin ^a	1.49 (1.03–2.15)	0.035	0.99 (0.64–1.52)	0.963	1.96 (1.11–3.46)	0.020
Wa ² HtR ^a	0.76 (0.22–2.62)	0.658	11.25 (1.76–71.87)	0.011	5.35 (0.70–40.70)	0.105

^a ²log-transformed values^b Decreasing values

matched control group. The percentage change in IGFBP-1 also correlated with the 2 h glucose value (Fig. 5b), and with the percentage glucose response during OGTT in the diabetes group ($r=0.32$; $p=0.009$), but not with the fasting glucose level. There was no significant correlation with blood glucose values in the matched control group. In addition, fasting IGFBP-1 at follow-up correlated with the 2 h glucose response during OGTT in the whole diabetes group ($r=0.37$; $p=0.002$) and not in the controls. There was no relationship between IGFBP-1 levels at baseline and the glucose response at follow-up (data not shown).

Fig. 3 Fasting IGFBP-1 concentrations at baseline and at the 10 year follow-up in men (black symbols) developing IGT and/or IFG (**a**; $n=248$) and diabetes (**b**; $n=88$), compared with their matched control group (white symbols). Values are the geometric means and 95% CIs. * $p<0.001$ compared with controls (unpaired t test); † $p<0.001$ compared with baseline (paired t test). The 19 diabetic individuals with fasting glucose ≤ 6 mmol/l and isolated post-challenge hyperglycaemia were excluded

Discussion

In this case–control study of men enrolled in a population-based prospective programme, low fasting IGFBP-1 concentrations predicted the development of abnormal glucose regulation 10 years later. When IGFBP-1 was used in combination with glucose, waist, height and proinsulin, individuals in the highest quartile were observed to have a 40-fold increased risk of developing diabetes compared with the lowest quartile. In individuals developing diabetes mellitus during a 10 year interval, fasting IGFBP-1 concentrations increased to levels greater than expected for the fasting insulin concentration and change in waist measurement. The higher fasting IGFBP-1 levels in this group of individuals with type 2 diabetes were inversely related to the change in IGF-I levels and correlated positively with glucose values 2 h after an oral glucose challenge.

As a result of the study design, a majority of the individuals in this case–control study have a family history of type 2 diabetes. The original cohort was selected so that 50% had FHD, a group in which there was a twofold higher prevalence of disturbed glucose metabolism [20]. Thus, even though the control group was matched for age and heredity, these observations are in a population of men with high genetic and social heredity for diabetes and further studies in more representative populations, including women, are required before the results can be extrapolated. Apart from age and heredity we have not determined whether the predictive value of IGFBP-1 is independent of other known

Fig. 4 Relationship between fasting IGFBP-1 and insulin concentrations (**a**) and the change in IGFBP-1 and change in waist (**b**) in men developing type 2 diabetes at 10 year follow-up. The individuals developing diabetes ($n=107$) are represented by the red and black circles and solid regression lines and are compared with their matched control group (white symbols, dotted regression lines). The 19 individuals with fasting glucose ≤ 6 mmol/l and isolated post-challenge

hyperglycaemia are identified by the black circles and regression line. **a** Regression lines: controls: ${}^2\log(\text{IGFBP-1})=9.099-[0.758\times{}^2\log(\text{insulin})]$; diabetes: ${}^2\log(\text{IGFBP-1})=9.657-[0.765\times{}^2\log(\text{insulin})]$. **b** Correlation coefficients: controls: $r=-0.44$, $p<0.001$; diabetes with fasting glucose >6 mmol/l: $r=-0.12$, $p=0.289$; diabetes fasting glucose ≤ 6.0 mmol/l: $r=0.66$, $p=0.002$

risk factors, such as high triacylglycerol levels. This will also need to be evaluated in future studies.

Fasting glucose levels predicted both diabetes and IFG in this population. Other long-term studies that are more representative of the general population also suggest that fasting blood glucose values below the WHO criteria for IFG strongly predict type 2 diabetes [28–30]. In a study in western Finland in a population enriched for FHD, fasting glucose ≥ 5.6 mmol/l and BMI ≥ 30 kg/m² predicted diabetes 6 years later (HR 3.7 [31]). We suggest that a com-

bination of FHD, high-normal fasting glucose and increased waist measurement (in relation to height) or low fasting IGFBP-1 identifies a group at very high risk.

There are several studies in which IGFBP-1 was shown to be a marker of hyperinsulinaemia [1, 32, 33]. As expected, fasting IGFBP-1 concentrations correlated inversely with fasting insulin levels in our study. However, the relationship of IGFBP-1 with fasting proinsulin, which has low hepatic extraction, was stronger, suggesting that the IGFBP-1 levels reflect portal insulin delivery [34, 35]. Consistent with

Fig. 5 In individuals developing type 2 diabetes, the change in IGFBP-1 concentrations at 10 year follow-up is related to the fall in IGF-I levels (**a**, $n=107$) and the 2 h glucose value in those diagnosed by OGTT (**b**, $n=64$). The individuals are represented by the red and black circles and solid regression lines and are compared with their

matched control group (white circles). The 19 individuals with fasting glucose ≤ 6 mmol/l and isolated post-challenge hyperglycaemia are identified by the black symbols. The regression lines on IGFBP-1 (% baseline) for the whole diabetes group for IGF-I (% baseline) was $r=-0.326$, $p<0.001$ and the 2 h glucose value was $r=0.345$, $p=0.006$

proinsulin being a more stable marker of insulin secretion, it has previously been shown that the risk of progression to type 2 diabetes was more strongly related to fasting 32,33 split proinsulin than to the fasting insulin level [36].

Fasting IGFBP-1 concentrations increased after 10 years in those developing diabetes, but not in those developing IFG and/or IGT. One possible explanation for this is that IGFBP-1 is a marker of declining beta cell function, just as has been observed for the incremental insulin response to glucose [31]. This is not supported, however, by our observation that, although the inverse relationship between IGFBP-1 and insulin was maintained after the development of diabetes, the regression line was shifted upwards so that IGFBP-1 levels were relatively increased. This is consistent with the emergence of hepatic insulin resistance, for which we have previously hypothesised that IGFBP-1 can be used as a marker [37]. If this is the case, hepatic insulin resistance, although an important determinant of blood glucose response during OGTT [38], is not the earliest feature in the development of type 2 diabetes. Hepatic insulin resistance is associated with reduced hepatic insulin extraction [39], which may also contribute to the altered relationship with IGFBP-1. If this is the case, IGFBP-1 may be a more reliable marker of hepatic insulin sensitivity than peripheral insulin levels. An upwards shift in the relationship between IGFBP-1 in relation to insulin is seen in other chronic conditions, such as those associated with low IGF-I levels, including growth hormone deficiency [40]. There are many other factors that have an impact on circulating IGFBP-1 and we can only speculate on the role of other counter-regulatory hormones [41] and of inflammatory cytokines [5, 6], which might also have played a role in the relative increase in IGFBP-1 at 10 years in type 2 diabetes. It has been reported that, in patients with low fasting IGFBP-1, low IGF-I concentrations are associated with the development of IGT or type 2 diabetes 4.5 years later [17]. In that report the IGF-I levels showed the expected decline in relation to increasing age, the tertile of individuals with low IGF-I being older than the other groups. This may explain the relationship with IGT or type 2 diabetes since in our age-matched case-control study IGF-I did not play a role as a predictive marker.

Abnormal glucose regulation is clearly a heterogeneous condition and the groups in our study varied in their baseline characteristics. Only the IGT group had higher systolic and diastolic BPs. It is well documented that this group is at significant risk of both large- and small-vascular complications and all-cause mortality, independently of progression to diabetes [19, 42]. On the other hand, individuals with IFG do not appear to have this degree of risk [42]. The value of low fasting IGFBP-1 concentrations in predicting IGT was of particular interest, since its use in screening may avoid the need for an OGTT in identifying individuals at risk. There was a group of 19 individuals with normal fasting glucose and

post-challenge hyperglycaemia meeting the WHO criteria for diabetes [42]. This group was lean, BP was not increased and, exceptionally amongst the study groups, fasting IGFBP-1 levels were not suppressed 10 years before diagnosis. It is not surprising that we identified this patient group, which is the majority in the category 'isolated post-challenge hyperglycaemia', and therefore represents a significant proportion of cases of newly diagnosed diabetes in population studies, and is characterised by leanness and an increased risk of cardiovascular complications and death [18, 43, 44].

Could the association of low IGFBP-1 with the development of abnormal glucose regulation have causal or pathological roles? A potential role of IGFs in the pathogenesis of cardiovascular disease has long been recognised. In cross-sectional studies low serum IGFBP-1 levels are associated with an unfavourable cardiovascular risk profile in diabetes and in populations without diabetes [10, 11, 45, 46]. Low IGF-I is also associated with cardiovascular risk factors [45, 47], independently of low IGFBP-1 [12, 15]. It is clear, however, that the IGFBP-1 levels change during the emergence of the diabetic state, increasing because of either hepatic insulin resistance or the influence of stimulatory factors such as cytokines. This increase may result in change in its pathological role at the tissue level. There is a lot of evidence that the IGF system plays a role in glucose homeostasis [48]. In animal models high IGFBP-1 concentrations are associated with glucose intolerance and insulin resistance [49] and IGFBP-1 administration leads to inhibition of the insulin activity of endogenous IGFs [50]. Indeed our observations of increased glucose values and lower IGF-I levels in relationship to increased levels of IGFBP-1 would support such a role. The factors responsible for the increase in IGFBP-1 in diabetes and its role in catabolism and glucose intolerance will be further investigated in future studies.

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