Low levels of insulin-like growth-factor-binding protein-1 (IGFBP-1) are prospectively associated with the incidence of type 2 diabetes and impaired glucose tolerance (IGT): The Söderåkra Cardiovascular Risk Factor Study

U. Petersson, C.J. Östgren, L. Brudin, K. Brismar, P.M. Nilsson

Primary Health Care Centre, Kalmar County Council, Kyrkvägen 28, 38551 Söderåkra, Sweden
Department of Medical and Health Sciences, Linköping University, Sweden
Department of Molecular Medicine and Surgery, Karolinska Institutet, Solna, Sweden
Department of Clinical Sciences, Lund University, University Hospital, Malmö, Sweden

Received 16 July 2008; received in revised form 23 November 2008; accepted 28 November 2008
Available online 17 March 2009

Abstract

**Aim.** – To explore the association between baseline levels of insulin-like growth-factor-binding protein-1 (IGFBP-1), a marker of insulin sensitivity, and the development of type 2 diabetes or impaired glucose tolerance (IGT) in a specifically defined middle-aged population.

**Methods.** – This cross-sectional population-based screening study was conducted in 1989–1990 and included baseline data for 664 non-diabetic subjects aged 40–59 years. Clinical data were collected and blood samples analyzed for blood glucose, serum lipids and insulin. Blood specimens were frozen at baseline and later analyzed for IGF-I, IGFBP-1 and C-reactive protein (CRP). At the follow-up in 2006, the incidence of type 2 diabetes and IGT was reported based on primary-care medical records.

**Results.** – During the 17-year observation period, 42 subjects (6.3%) developed type 2 diabetes/IGT. Those in the lowest quintile of IGFBP-1 (≤ 24/H9262 g/L) at baseline had a diabetes incidence of 12.6% while, in the highest quintile of IGFBP-1 (≥ 59/H9262 g/L), the incidence was 1.5%. Cox’s proportional-hazards model regression analyses were used to determine the incidence of type 2 diabetes/IGT, corrected for age and gender, in relation to IGFBP-1, CRP and waist circumference. Subjects in the lowest IGFBP-1 quintile showed an independently increased risk of type 2 diabetes/IGT [hazards ratio (HR): 3.54; 95% CI 1.18–10.6; *P* = 0.024]. For CRP and waist circumference, the corresponding figures were HR: 6.81; 95% CI 2.50–18.6; *P* < 0.001 and HR: 3.33; 95% CI 1.47–7.6; *P* = 0.004, respectively.

**Conclusion.** – Low levels of IGFBP-1 predicted the long-term development of type 2 diabetes or IGT in a middle-aged population. The association was independent of CRP and abdominal obesity.

© 2009 Elsevier Masson SAS. All rights reserved.

**Keywords:** CRP; IGFBP-1; Prediction; Screening; Type 2 diabetes; Longitudinal study

Résumé


**Objectif.** – Étudier les liens éventuels entre l’insulin-like growth-factor-binding protein-1 (IGFBP-1), marqueur de l’insulinosensibilité, à l’inclusion et le développement ultérieur d’un diabète de type 2 (DT2) ou d’une intolérance au glucose (IGT) dans une population d’âge moyen.


**Résultats.** – Au cours de la période de suivi de 17 ans, 42 sujets (6,3 %) développèrent un DT2 ou une IGT. L’incidence du DT2 chez les sujets dont l’IGFBP-1 à l’inclusion était dans le quintile le plus bas (≤ 24 μg/L) était de 12,6 %, alors que l’incidence était de 1,5 % chez ceux dont l’IGFBP-1 à l’inclusion était dans le quintile le plus élevé (≥ 59 μg/L). Des analyses de régression proportionnelles avec le modèle de Cox entre IGFBP-1 et risque de développer DT2 ou IGT, ajustées sur l’âge, le sexe, la CRP et le tour de taille ont été réalisées. Les sujets situés dans le quintile...
1. Introduction

Type 2 diabetes is often preceded by insulin resistance. Waist circumference and the homeostasis model assessment (HOMA) index are well-known surrogate markers of insulin resistance. Also, insulin-like growth-factor-binding protein-1 (IGFBP-1) as part of the insulin-like growth factor (IGF) system, which is involved in the regulation of glucose metabolism, is associated with insulin resistance and glucose intolerance. IGFBP-1 is one of six binding proteins and has been proposed to be an acute regulator of IGF-I bioavailability [1]. Produced in the liver, it peaks at night and in the early morning, as it is highly dependent on insulin concentrations. High levels of insulin are associated with low IGFBP-1 concentrations.

Low levels of IGFBP-1 are also associated with features of the metabolic syndrome such as insulin resistance, obesity and the development of cardiovascular disease [2–6]. It has been suggested that IGFBP-1 facilitates the transport of IGF-I from plasma to tissue, thus potentially increasing the activity of IGF-I in the target tissue [7]. Many of the processes involved in the formation of atherosclerotic lesions are IGF-I-dependent, promoting macrophage chemotaxis and endothelial cell migration [8], as well as vascular smooth muscle cell proliferation and migration [9]. IGFBP-1 also exerts an effect on cellular growth and migration independently of IGF by binding to the cell surface via $\beta_1$ integrins [10,11]. IGFBP-1 reflects free IGF-I [12]. Serum levels of IGFBP-1 (but not IGF-I) correlate with body mass index (BMI), and upper arm fat and muscle areas in the elderly [13], but also vary considerably in healthy individuals [14]. A monozygotic twin study showed that non-genetic factors explained 64% of the variation seen in serum IGFBP-1 levels [15], whereas insulin and IGF-I explained only 28 and 8%, respectively, of such non-genetic variation [16]. This means that approximately 30% of the variation in IGFBP-1 levels remains unexplained, but could be due to dietary and other lifestyle factors, according to a study in healthy men [17]. In addition, one study has shown that high circulating concentrations of IGF-I were associated with a reduced risk of type 2 diabetes/IGT in normoglycaemic individuals [18]. Finally, a recent study found that low levels of IGFBP-1 predicted an increased risk of cardiovascular events [19].

The aim of the present observational study was to explore the association between IGFBP-1 and IGF-I at baseline with the later development of type 2 diabetes and IGT in a specifically defined middle-aged population.

2. Subjects and methods

The Söderåkra Cardiovascular Risk Factor Study was launched in November 1989 and ran until May 1990 as a population-based, cross-sectional cardiovascular risk-factor screening study in which all inhabitants aged 40–59 years, living in the Söderåkra parish in southern Sweden, were invited to participate. Of a total of 782 invited subjects, 705 (90%)—361 men (88%) and 344 women (93%)—agreed to participate. Details of non-participants at baseline have been described elsewhere [20]. At baseline (study entry), participants underwent laboratory tests, a structured health visit with a specially trained nurse, a self-administered questionnaire, and a visit with a physician for clinical examination and completion of the questionnaire. Blood specimens were drawn without venous stasis after an overnight fast with the subject seated after a 15 minutes rest. Blood glucose, serum cholesterol, HDL cholesterol and serum triglycerides were analyzed. LDL cholesterol was calculated using Friedewald's formula. Indeed, only routine laboratory methods were used by the Department of Clinical Chemistry at Kalmar County Hospital. Extra serum samples were frozen and stored for future analyses. Clinical measurements of height (cm), weight (kg), and waist and hip circumferences (cm) were also taken, and the subjects’ BMI calculated (kg/m²).

After analyses of the blood specimens, all but 13 subjects attended a structured clinical visit with a physician who provided feedback information. Three blood-pressure measurements (Korotkoff I and V) were recorded (mmHg) from the right arm, in the sitting position after a five minutes rest, using a mercury sphygmomanometer and the appropriate cuff width. The mean value of the last two measurements was recorded as the heart rate (Beats/minutes). Following the physical examination, a questionnaire containing lifestyle questions and dietary habits, focused on physical activity, smoking and alcohol consumption, was completed. Tobacco use was expressed as the number of cigarettes or packs of tobacco used per day. Alcohol consumption was expressed in centilitres (cl) of beer, wine and hard liquor consumed per week, which was converted into grammes of alcohol per week. Physical activity (cycling, walking, swimming and any other activities engaging the large muscles) was divided into three categories:

- daily exercise for half an hour or more;
- exercise two to three times a week for half an hour;
- less exercise than exercising two to three times a week for half an hour.
Serum insulin was analyzed after being kept for two years in frozen storage at $-20^\circ\text{C}$ using a radioimmunoassay (RIA) technique (Pharmacia Insulin RIA 100) at the Department of Clinical Chemistry in the Kalmar County Hospital. In 2005, frozen serum stored at $-20^\circ\text{C}$ taken at baseline was defrosted, divided into three separate samples from each subject and refrozen at $-70^\circ\text{C}$ for a short period of time before being analyzed for serum C-reactive protein (CRP) and serum creatinine, using routine methods and commercially available kits (Cobas Integra 700 analyzer, Roche Diagnostics Scandinavia AB, Bromma, Sweden). Frozen serum for analyses of serum IGF-I and IGFBP-1 was sent to the Department of Molecular Medicine and Surgery at the Karolinska Institute in Solna, Sweden. Baseline fasting concentrations of IGF-I were determined in serum by RIA after separation of IGF from IGFBP by acid-ethanol extraction and cryoprecipitation. To minimize interference from any remaining IGFBP (1–3), IGF-I was used as a radioligand. The intra- and interassay coefficients of variation (CV) were 4 and 11%, respectively [21]. IGFBP-1 concentrations in serum were determined by RIA, using the methods described by Póvoa et al. [22]. Sensitivity of the RIA was 3 μg/L and was, for the intra- and interassay CV, 3 and 10%, respectively.

There were 33 subjects with a history of hypertension, six with angina pectoris and seven with myocardial infarction. Any history of malignancy was not noted, as the primary aim of the study was to explore the risk of cardiovascular disease and diabetes. A large proportion of the study participants was taking medication at baseline. In all, 186 of our subjects were using some kind of medical treatment either regularly or occasionally and, in eight cases, more than five drugs were being taken. Antihypertensive medication such as beta-blockers and diuretics were the most frequently prescribed drugs. Oestrogen therapy is known to influence levels of IGFBP-1 [23,24], and oestrogen alone or in combination was being used by 19 women.

2.1. Follow-up procedures

A follow-up survey of the study population was conducted in late 2006 by a nurse specializing in diabetes care. Information was collected mainly through primary-care records but, in 25 cases that were missing medical records, telephone interviews with the study subjects were used instead. The vital status of the cohort was obtained through the cause of death register of the National Board of Health and Welfare in Stockholm, Sweden. Subjects with diabetes at the start of the study ($N = 10$) were excluded, as were those whose frozen serum samples were damaged ($N=5$) during storage. In 26 cases, we were unable to obtain adequate information, so these individuals were also subsequently excluded from the follow-up. Thus, 664 subjects (94% of the baseline population) remained for further analyses. The incidence and duration of type 2 diabetes and IGT was recorded, as defined by the most recent World Health Organization (WHO) diagnostic criteria for the conditions [25,26]. In four cases, an oral glucose tolerance test (OGTT) was conducted for clinical reasons and revealed IGT, resulting in these cases also being considered incidences of abnormal glucose metabolism. In these four cases, capillary blood was drawn after the OGTT and diagnosed as IGT if blood glucose was $\geq 7.8–11.0 \text{ mmol/L}$ (according to WHO criteria of 1998).

Insulin resistance was assessed from fasting blood glucose and serum insulin concentrations by the HOMA index [27]. The metabolic syndrome was categorized according to criteria defined by the International Diabetes Federation (IDF) in 2005 [28]. The main IDF criterion for the metabolic syndrome is a waist circumference $\geq 94 \text{ cm}$ in men and $\geq 80 \text{ cm}$ in women. If these conditions are fulfilled, two or more of the following conditions must also be present: triglycerides $> 1.7 \text{ mmol/L}$; HDL $< 0.13 \text{ mmol/L}$ in men or $< 0.129 \text{ mmol/L}$ in women; systolic blood pressure $\geq 130 \text{ mmHg}$ and/or diastolic blood pressure $\geq 85 \text{ mmHg}$; and plasma glucose $\geq 5.6 \text{ mmol/L}$. In the present study, blood glucose was analyzed and the glucose values multiplied by 1.1 to determine the corresponding plasma glucose levels [29].

2.2. Statistical analyses

The study participants were divided into five quintiles based on their baseline IGFBP-1 concentrations. The lowest quintile (group 1) had IGFBP-1 values $\leq 24 \text{ mcg/L}$ (μg/L), whereas the three middle quintiles (group 2) were combined and had IGFBP-1 values of 25–59 μg/L. The fifth quintile (group 3) had an IGFBP-1 $\geq 59 \text{ μg/L}$. These three groups were related to baseline characteristics (Table 1). Gender differences between the IGFBP-1 subgroups were analyzed using a chi-square test, while the baseline characteristics of the three subgroups, without gender separation, were characterized using non-parametric analyses of variance for group differences (Kruskal–Wallis test). The lowest IGFBP-1 quintile versus the highest was analyzed using non-parametric tests (Mann–Whitney U test).

Differences in metabolic, clinical and lifestyle characteristics between individuals, with and without the metabolic syndrome at baseline (Table 2), were analyzed by the Mann–Whitney U test except for the numerical differences between genders in both groups, which were analyzed by a chi-square test.

Waist circumference was categorized into two groups based on the cutoff value given in the IDF definition of the metabolic syndrome. CRP was classified in three subgroups:

- the lowest quartile;
- the two middle quartiles;
- the highest quartile.

The incidence of type 2 diabetes—including the four cases of IGT—were explored using regression of Cox’s proportional-hazards model in relation to IGFBP-1, waist circumference and CRP, with age, gender, IGF-I and lifestyle factors as covariates. A stepwise deletion method was applied. Hazard ratios (HR) were expressed as 95% confidence intervals (CI), and the statistical calculations were considered significant at $P < 0.05$.

The Söderåkra study was approved by the ethics board in Linköping (32/04).
Table 1
Baseline characteristics of study subjects by IGFBP-1 quintiles (Q1–Q5) in the Söderåkra Cardiovascular Risk Factor Study (1989–1990).

<table>
<thead>
<tr>
<th>IGFBP-1 Q1</th>
<th>IGFBP-1 Q2–4</th>
<th>IGFBP-1 Q5</th>
<th>( p^a )</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (women/men)</td>
<td>51/92</td>
<td>205/181</td>
<td>70/65</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 44 53</td>
<td>47 44 52</td>
<td>45 42 52</td>
<td>0.062</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84 78 92</td>
<td>72 64 83</td>
<td>68 60 77</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>95 89 100</td>
<td>86 78 93</td>
<td>81 73 88</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 26 31</td>
<td>25 23 28</td>
<td>23 22 25</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>130 120 140</td>
<td>129 118 140</td>
<td>120 112 131</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>90 82 90</td>
<td>86 78 90</td>
<td>82 76 88</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>0 0 5</td>
<td>0 0 10</td>
<td>0 0 10</td>
<td>0.127</td>
</tr>
<tr>
<td>Physical activity (groups 1–3)</td>
<td>2 1 2</td>
<td>2 1 2</td>
<td>2 1 2</td>
<td>0.499</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>0 0 49</td>
<td>0 0 30</td>
<td>0 0 20</td>
<td>0.009 0.005</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>5.9 5.2 6.9</td>
<td>5.8 5.1 6.7</td>
<td>5.8 5.0 6.5</td>
<td>0.310</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.9 3.1 4.6</td>
<td>3.7 3.1 4.5</td>
<td>3.7 2.9 4.5</td>
<td>0.597</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 1.2 1.7</td>
<td>1.5 1.3 1.8</td>
<td>1.7 1.3 1.9</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.3 0.9 2.1</td>
<td>0.9 0.7 1.4</td>
<td>0.8 0.6 1.1</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>4.6 4.2 5.0</td>
<td>4.4 4.1 4.8</td>
<td>4.3 4.0 4.7</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>9.8 7.1 13.3</td>
<td>7.3 5.7 9.6</td>
<td>6.0 4.6 7.5</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>HOMA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 1.4 2.8</td>
<td>1.5 1.1 1.9</td>
<td>1.1 0.8 1.4</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>IGF-I (µg/L)</td>
<td>155 132 179</td>
<td>146 121 176</td>
<td>135 111 161</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>IGFBP-1 (µg/L)</td>
<td>19 15 21</td>
<td>39 30 48</td>
<td>73 65 88</td>
<td>0.836</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.6 1.0 3.0</td>
<td>1.5 0.9 2.7</td>
<td>1.3 0.9 2.5</td>
<td>0.533</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>72 64 82</td>
<td>68 60 78</td>
<td>71 62 79</td>
<td>0.305</td>
</tr>
</tbody>
</table>

The lowest IGFBP-1 quintile comprises significantly more men (\( P < 0.0004 \)).

<sup>a</sup> Non-parametric analyses of variance for group differences (Kruskal–Wallis) unless otherwise stated.

<sup>b</sup> Lower versus upper IGFBP-1 quintile using non-parametric test (Mann–Whitney U test) in case of Kruskal–Wallis significance.

<sup>c</sup> Chi-square test.

<sup>d</sup> Fasting glucose (mmol/L) × fasting insulin (µU/L).
### Table 2
Characteristics of individuals in quintiles 1 and 3 (Q1 and Q3) with and without the metabolic syndrome (IDF definition).

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome</th>
<th>No metabolic syndrome</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (women/men)</td>
<td>45/67</td>
<td>281/270</td>
<td>0.047&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50</td>
<td>46</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86</td>
<td>71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>97</td>
<td>85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>29</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134</td>
<td>126</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>90</td>
<td>84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cigarettes/day (N)</td>
<td>0</td>
<td>0</td>
<td>0.277</td>
</tr>
<tr>
<td>Physical activity (1–3)</td>
<td>2</td>
<td>2</td>
<td>0.380</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>0</td>
<td>0</td>
<td>0.772</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.2</td>
<td>5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>4.2</td>
<td>3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.2</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.9</td>
<td>0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>4.9</td>
<td>4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μU/L)</td>
<td>10.7</td>
<td>6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.4</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGF-I (μg/L)</td>
<td>149</td>
<td>146</td>
<td>0.204</td>
</tr>
<tr>
<td>IGFBP-1 (μg/L)</td>
<td>26</td>
<td>42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.1</td>
<td>1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>74</td>
<td>69</td>
<td>0.002</td>
</tr>
</tbody>
</table>

IDF: International Diabetes Federation.

<sup>a</sup> Non-parametric test (Mann–Whitney U test) unless otherwise indicated.

<sup>b</sup> Chi-square test.

### 3. Results

A total of 664 consecutive individuals (338 men and 326 women) were included in the 17-year follow-up. Lifestyle factors such as physical activity, alcohol intake and smoking habits can influence levels of IGFBP-1 [17]. Although the present study found no associations between physical activity, smoking habits and IGFBP-1, higher alcohol intakes were associated with lower IGFBP-1 levels (Table 1). However, when we corrected for these lifestyle factors in the Cox regression analyses of the incidence of type 2 diabetes/IGT, there again was no association. A total of 389 study subjects drank no alcohol at all and, although we had no data for ten subjects, 265 reported an intake of 1–260 g/week of alcohol. Also, at baseline, there were 236 smokers, 418 non-smokers and no data for ten subjects.

Table 1 shows the baseline characteristics according to the three IGFBP-1 subgroups. Group 1 (with the lowest values) represented the first quintile of IGFBP-1 (51 women and 92 men). Group 2 comprised the second, third and fourth quintiles (205 women and 181 men) and group 3 represented the fifth quintile (70 women and 65 men). Women had significantly higher values of IGFBP-1 than did men. However, there were no significant age differences between subgroups. Weight, waist, BMI, and systolic and diastolic blood pressures declined statistically significantly from the first to the fifth quintile. As for lifestyle factors, alcohol intake showed significant differences across the three subgroups, with higher consumption in the lowest quintile. However, cigarette-smoking and physical activity did not differ across the subgroups. Nevertheless, an inverse relationship was found between levels of IGFBP-1 and levels of IGF-I, serum triglycerides, blood glucose, serum insulin and CRP.

Table 2 shows the baseline characteristics of those with and without the metabolic syndrome. There was a slight, but significant (P < 0.05), gender difference, with women having the metabolic syndrome less often (14%) than did men (20%). Age, serum insulin, LDL cholesterol, CRP and also IGFBP-1 were all significantly different in subjects with versus without the metabolic syndrome. However, in contrast, neither IGF-I nor the lifestyle factors studied had any association with the metabolic syndrome.

At the time of the follow-up, 38 individuals had developed type 2 diabetes and an additional four cases were found with IGT based on OGTT. These latter cases were considered to be incidences of abnormal glucose metabolism. A multivariate Cox’s proportional-hazards regression analysis (Table 3) was used to explore the development of diabetes/IGT during the 17 years of follow-up. The regression analysis (age- and gender-corrected) included IGFBP-1, waist circumference and CRP, with lifestyle factors, IGF-I and oestrogen medication used as covariates. As seen in Table 3, the lowest quintile of IGFBP-1 was significantly associated with the development of type 2 diabetes/IGT (HR 3.54; 95% CI 1.18–10.6; P = 0.024). Furthermore, both CRP (HR 6.81; 95% CI 2.50–18.6; P < 0.001) and waist size (HR 3.33; 95% CI 1.47–7.6; P = 0.004) proved to be independent predictors, whereas the covariates showed no significant relationships with the incidence of diabetes/IGT. However, when insulin, IGF-I and IGFBP-1 were included in the multivariate Cox’s regression analysis, both insulin (P = 0.0007) and
IGFBP-1 ($P = 0.017$)—but not IGF-I—were statistically significant predictors of the development of type 2 diabetes/IGT. Univariate regression results are also shown in Table 3.

### 4. Discussion

The main finding of this observational follow-up cohort study was the statistically significant association between low levels of IGFBP-1 (quintile 1) at baseline and the development of type 2 diabetes/IGT in a middle-aged population. IGFBP-1, a peptide of hepatic origin, is one of six binding proteins that regulate IGF-I bioavailability, and is believed to play an important role in the glucose counter regulation involved in the more slowly reacting blood-glucose-lowering mechanism compared with the faster effect induced by insulin. IGFBP-1 is also known to be inversely related to insulin resistance and is directly regulated by insulin—thus making it a marker of insulin sensitivity. As low levels of IGFBP-1 indicate hyperinsulinaemia, our finding that a low IGFBP-1 concentration predicted a higher diabetes incidence during the 17-year period of observation was to be expected. In line with our findings, other authors [30] have shown that a low IGFBP-1 is a marker of hyperinsulinaemia in obese menopausal women. In addition, Saitoh et al. [31] studied prepubertal obese children and found that a low IGFBP-1 was a predictor of glucose-stimulated hyperinsulinaemia. Furthermore, it has been shown that fasting IGFBP-1 in healthy men is a better predictor of insulin response to hyperglycaemia than either glucose or insulin [32].

The result that low IGFBP-1 levels predicted an increased risk of future diabetes/IGT was still present in the multivariate regression analyses independent of waist circumference and CRP. In contrast, age and gender were not significantly related to the development of diabetes/IGT in the same model. Not surprisingly, an increased waist circumference at baseline independently predicted a higher incidence of diabetes/IGT. CRP is a well-known predictor of cardiovascular disease [33] and also of type 2 diabetes [34]. When insulin, IGF-I and IGFBP-1 were included in a multivariate Cox’s regression analysis, both insulin and IGFBP-1—but not IGF-I—remained statistically significant predictors of diabetes/IGT. This suggests that the association between IGFBP-1 and the development of diabetes/IGT might be independent of the actual levels of insulin at baseline.

To the best of our knowledge, these results are the first to show a significant independent association between low serum concentrations of IGFBP-1 and the development of type 2 diabetes/IGT in a population-based study including both men and women. In a population study of Swedish normoglycaemic men, low levels of IGFBP-1 predicted the development of abnormal glucose regulation [35]. Sandhu et al. [18] conducted a prospective (4.5-year) observational study of 615 middle-aged normoglycaemic men and women from a randomized population-based cohort. The odds ratio of the risk of IGT or type 2 diabetes with IGF-I above versus below the median was 0.5, and this inverse association was independently modified by IGFBP-1. We could, however, find no similar associations for IGF-I in the present study.

#### 4.1. Study limitations

In all, 38 (5.7%) subjects developed type 2 diabetes in the present study, although the diagnosis was based on primary-care records and not OGTT in general. In addition, in only four cases (0.6%) was IGT diagnosed. It is likely that additional cases of type 2 diabetes as well as IGT would have been detected if OGTT had been performed in all study participants. Such case underestimation may have resulted in a weaker association than would have been found with OGTT results for all study subjects. In the similar study by Sandhu et al. [18] of 615 normoglycaemic subjects aged 45–65 years, 44 (7%) subjects developed IGT and 7 (1%) subjects developed type 2 diabetes after a 4.5-year follow-up. As our study had a follow-up period of 17 years—almost four times longer—the fact that IGT had progressed to type 2 diabetes in most of our subjects compared with those of Sandhu et al. [18] was only to be expected.
5. Conclusion

Low levels of IGFBP-1 predicted the development of type 2 diabetes/IGT in a specifically defined middle-aged population. The association was independent of an inflammatory marker (CRP) and abdominal obesity, an indication that impaired insulin sensitivity is important in the prediction of future abnormal glucose metabolism and diabetes.

Conflicts of Interest

The authors have none to declare.

Acknowledgements

The authors wish to thank Ann-Kristin Lundström, RN, for her skilful assistance in the collection of data for the incidence of type 2 diabetes, and Elsi Sandberg and Inga-Lena Wivall for their excellent laboratory work. The present study was supported by grants from the Kalmar County Council, the Medical Research Council of Southeast Sweden (FORSS-5691), the Family Er ling Persson Foundation and the Swedish Diabetes Association.

References


