Available online at ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com
Diabetes & Metabolism
Diabetes & Metabolism xxx (2009) xxx–xxx

Original article

Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients

E. Vandemeulebroucke [a], F.K. Gorus [a], K. Decochez [a], I. Weets [a], B. Keymeulen [a], C. De Block [b], J. Tits [c], D.G. Pipeleers [a], C. Mathieu [d,*], the Belgian Diabetes Registry

[a] *Diabetes Research Centre, Brussels Free University, Brussels, Belgium*
[b] *Department of Endocrinology, University of Antwerp, Antwerp, Belgium*
[c] *Department of Endocrinology, Ziekenhuis Oost Limburg Campus St.-Jan, Genk, Belgium*
[d] *Department of Endocrinology, Catholic University of Leuven, Herestraat 49, 3000 Leuven, Belgium*

Received 26 August 2008; received in revised form 5 February 2009; accepted 8 February 2009

Abstract

Aims. – We examined whether parenteral regular insulin can prevent diabetes in IA-2 antibody-positive (IA-2A+) relatives of type 1 diabetic patients, using a trial protocol that differed substantially from that of the Diabetes Prevention Trial-1.

Methods. – Twenty-five IA-2A+ relatives received regular human insulin twice a day for 36 months, during which time they were followed (median [interquartile range; IQR]: 47 [19–66] months) for glucose tolerance, HbA_{1c} and islet autoantibodies, together with 25 IA-2A+ relatives (observation/control group) who fulfilled the same inclusion criteria, but were observed for 52 [27–67] months ($P = 0.58$).

Results. – Twelve (48%) insulin-treated relatives and 15 (60%) relatives in the control group developed diabetes. There was no difference in diabetes-free survival between the two groups ($P = 0.97$). Five-year progression (95% confidence interval) was 44% (25–69) in the insulin-treated group and 49% (29–70) in the observation group. At inclusion, progressors tended to have a higher pro-insulin/C-peptide ratio than non-progressors when measured 2 hours after a standardized glucose load (median [IQR]: 2.7% [1.8–4.3] vs. 1.6% [1.1–2.1]; $P = 0.01$). No major hypoglycaemic episodes or significant increases in body mass index or diabetes autoantibodies were observed.

Conclusion. – Prophylactic injections of regular human insulin were well tolerated, but failed to prevent type 1 diabetes onset in IA-2A+ relatives.

Keywords: Type 1 diabetes; First-degree relatives; Prevention; Insulin prophylaxis; Autoantibodies

Résumé

Traitement par l'insuline d'apparentés au premier degré de IA-2 positifs patients diabétiques.

Objectifs. – Cette étude visait à examiner si des injections d'insuline d'action rapide étaient capables de prévenir le diabète chez des apparentés au premier degré de patients diabétiques, à haut risque de la maladie du fait de la présence d'anticorps IA-2 (IA-2A+). Le protocole utilisé était considérablement différent de celui de *Diabetes Prevention Trial-1*.

Méthodes. – Vingt-cinq apparentés IA-2A+ ont reçu de l'insuline d'action rapide (deux fois 0,5 unités/kg par jour) pendant 36 mois et ont été suivis en termes de tolérance au glucose, d' HbA_{1c} et d'auto-anticorps pendant une durée médiane (écart interquartile) de 47 (de 19 à 66 mois). Le groupe témoin comprenant 25 apparentés IA-2A+ avec un suivi de 52 (de 27 à 67) mois répondait aux mêmes critères d'inclusion que le groupe actif et ne différait pas de celui-ci en termes d'âge et de rapport hommes/femmes.

Résultats. – Douze apparentés traités par l'insuline (48 %) et 15 témoins (60 %) ont développé un diabète. La survie sans diabète était semblable dans les deux groupes ($P = 0{,}97$). La progression vers un diabète en cinq ans (intervalle de confiance à 95 %) était de 44 % (25–69) dans le groupe traité et de 49 % (29–70) dans le groupe témoin. À l'inclusion, on notait chez les progresseurs une tendance à l'augmentation du rapport insuline/C-peptide, mesuré deux heures après une injection de glucose standardisée (médiane [écart interquartile] : 2,7 [1,8–4,3%] vs 1,6 [1,1–2,1%] ; $P = 0{,}01$). Nous n'avons pas observé d'épisodes majeurs d'hypoglycémie, ni d'augmentation de IMC, ou des taux d' auto-anticorps.

Abbreviations: BMI, Body Mass Index; BMI-SDS, Body Mass Index Standard Deviation Score; GADA, Glutamate decarboxylase antibodies; IA-2A, Insulinoma-associated protein-2 antibodies; IAA, Insulin autoantibodies; ICA, Islet cell cytoplasmic antibodies; JDF, Juvenile Diabetes Foundation; OGTT, Oral Glucose Tolerance Test; Clinical Trials Gov. Identifier, NCT00654121.

* Corresponding author.
E-mail address: chantal.mathieu@uz.kuleuven.ac.be (C. Mathieu).

1262-3636/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.diabet.2009.02.005

Conclusions. – Les injections prophylactiques d'insuline d'action rapide sont bien tolérées, mais ne permettent pas de prévenir le développement d'un diabète chez les apparentés IA-2A+.

Mots clés : Diabète de type 1 ; Parents proches ; Prévention ; Prophylaxie par l'insuline ; Auto-anticorps

1. Introduction

Type 1 diabetes is caused by selective T-cell-mediated destruction of pancreatic beta cells [1,2]. When the disease becomes clinically overt, most of the beta cells have already been destroyed [3,4], and there is, at present, no lasting treatment to completely avoid the chronic complications [5–8]. The identification of individuals at high risk of developing hyperglycaemia (>50% in 5 years) on the basis of circulating multiple types of islet antibodies and/or IA-2A [9–14] creates a time window during which pharmacological interventions may be tested, with the aim of stopping or slowing the subclinical disease process at a stage when the functional beta-cell mass is higher than at disease diagnosis. If effective, such a strategy may be able to prevent or delay the development of hyperglycaemia and its chronic complications [15].

On the basis of promising results in animal models [16–18] and pilot studies in humans [19,20], the DPT-1 investigated the potency of prophylactic injections of low doses of slow-acting insulin to prevent diabetes in the autoantibody-positive relatives of a type 1 diabetic proband in a larger randomized human trial, but could find no beneficial effects [21]. Neither did a separate French study [22], using an approach based on the hypothesis that parenteral administration of insulin – the primary beta-cell-specific diabetes autoantigen [23,24] – may allow the islets to rest, thereby reducing the synthesis of insulin and other autoantigens and, thus, the immunogenicity of islet cells.

The observed lack of benefit in the DPT-1 does not, however, rule out prophylactic insulin injections as a potential secondary preventative strategy, as variables in the study protocol – including the selected study population and treatment regimen – may have influenced the outcome. In the DPT-1 trial, long-acting NPH insulin was administered twice a day whereas we hypothesized that administration of short-acting regular human insulin at the time of greatest need – around carbohydrate-rich meals – might be better suited to induce beta-cell rest.

For this reason, we designed an interventional trial using regular human insulin, injected twice daily around main meals, in a group of IA-2A-positive first-degree relatives and compared their progression to diabetes with a group of relatives who fulfilled the inclusion criteria, but did not participate in the trial. Positivity for IA-2A, in the absence of a protective genotype, confers the highest risk for rapid progression to diabetes, irrespective of the number of autoantibodies present [25].

2. Population and methods

2.1. Subjects

First-degree relatives of type 1 diabetic patients were recruited through the Belgian Diabetes Registry (BDR) as part of a national screening program for diabetes risk assessment. In November 2000, the BDR launched a prevention study using subcutaneous regular insulin in high-risk relatives. Twelve centres agreed to participate in the trial and two centres did not. High-risk relatives in the participating centres were offered the choice of insulin treatment or close observation while, in the other centres, only observation was offered (Fig. 1).

Insulin treatment was started in 28 relatives, but three stopped their insulin treatment after three, nine and 15 months, respectively. The remaining 25 relatives continued treatment for 36 months and were analyzed for the development of hyperglycaemia. Median (interquartile range, IQR) follow-up time from the start of insulin treatment was 47 months (IQR 19–66). Twenty-five out of 34 relatives agreed to be followed in an observation group (no placebo treatment). Twelve of these 25 subjects refused to inject insulin or were considered to be unable to inject insulin, and 13 were followed in centres that did not offer insulin treatment. None of those in the observation group was lost to follow-up (median: 52 months [27–62]).

Subjects were eligible for inclusion in either the insulin-treated or observation group if they were between five and 40 years of age, lacked the protective *HLA-DQA1** or *-DQB1** haplotypes (*01-0602* or *05-0301*) [26], were shown to be IA-2A-positive (≥ 0.44% tracer-bound) in at least two blood samples [25], were siblings or offspring of an index patient and had a normal OGTT, according to American Diabetes Association (ADA) criteria [27].

The ethics committee did not agree to having a placebo arm, as it was deemed unethical to allow young people and often small children to inject themselves with a non-active substance.

2.2. Study design and treatment protocol

Insulin-treated relatives injected themselves twice daily with regular human insulin subcutaneously (Actrapid®, Novo Nordisk Pharma, Brussels, Belgium) before their most carbohydrate-rich meals. The starting dose was $2 \times 0.05\ \text{U} \times (\text{kg body weight})^{-1} \times \text{day}^{-1}$. The insulin dose was later adjusted to maintain 1-h postprandial blood glucose values of 5.6–7.8 mmol/L. Patients monitored their blood glucose twice daily during the last week before each visit. In cases of suspected hyperglycaemia or intercurrent illness, additional glucose monitoring was performed. When hypoglycaemia (glycaemia < 3.3 mmol/L) occurred, the insulin dose was decreased. Severe hypoglycaemia was defined as a hypoglycaemic event where the assistance of another person was required to correct the hypoglycaemia. Subjects were seen every three months to adapt insulin dosages and assess glucose tolerance status (by OGTT). Unless diabetes was diagnosed, all participants continued this treatment scheme for 36 months. Afterwards, subjects

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

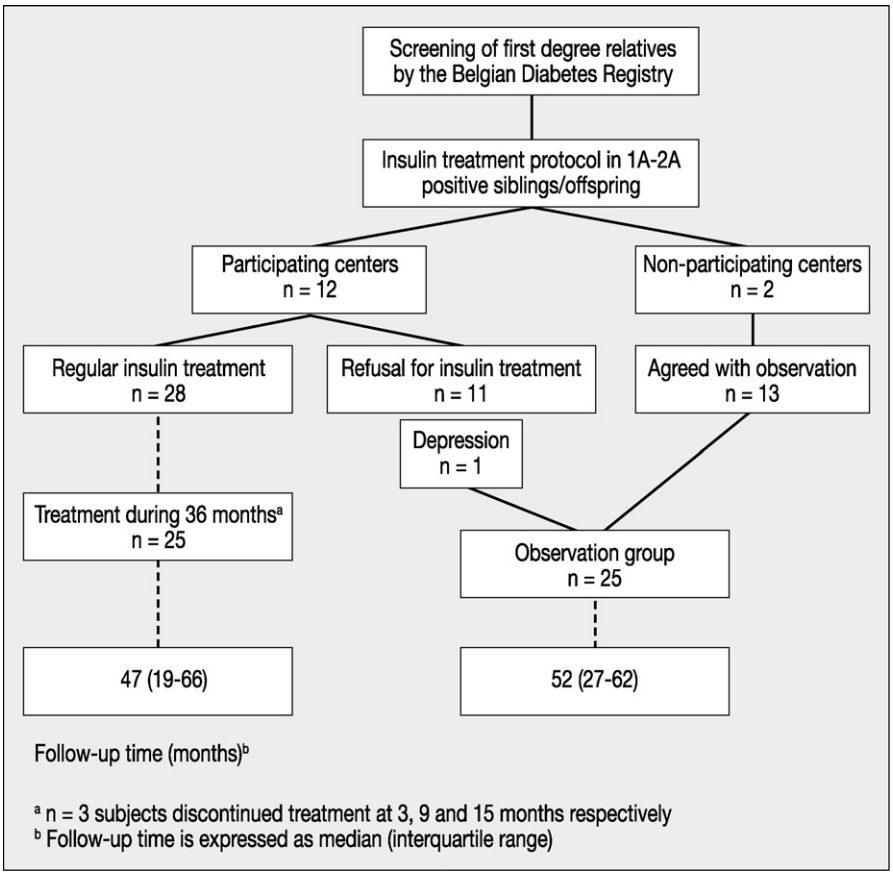

Fig. 1. Study design.

were monitored by OGTT on a 3- to 6-month basis for at least 12 months. At each visit, blood was sampled for autoantibodies and HbA_{1c}, a routine physical examination performed and a medical history taken. If diabetes was diagnosed, the subject was put on intensified insulin treatment and excluded from further participation in the trial.

BMI was recorded and analyzed after transformation into a standard deviation score (BMI–SDS) for comparison with data from a reference cohort, comprising 15,636 male and 14,899 female subjects recruited between 1978 and 1990 [28].

All relatives and – in case of minors – their parents signed informed-consent forms approved by the ethics committees of the BDR and the four participating universities. The study was conducted in accordance with the guidelines of the Declaration of Helsinki, as revised in 2000 (http://www/wma.net/e/policy/b3.htm). In the observation group, no OGTT tests were performed. Every 12 months – or more often in the presence of insulinopenic symptoms – blood was drawn for determination of glucose, C-peptide, pro-insulin and HbA_{1c}. The present study was initiated before the results of the DPT-1 and ENDIT were published, and the ethics committees opposed against randomization of relatives to insulin treatment or no treatment. Thus, the relatives who accepted prophylactic treatment were compared with matching controls who had refused intervention. No relatives not taking insulin injections accepted the more intensified metabolic follow-up with OGTTs.

2.3. Laboratory tests

2.3.1. Metabolic and hormonal parameters

Plasma glucose levels were measured with a Vitros 950 IC analyzer (Ortho Clinical Diagnostics, Rochester, NY, USA). HbA_{1c} was measured with the use of high-performance liquid chromatography [29]. Plasma C-peptide was determined by a two-step time-resolved fluoroimmunoassay (TRFIA) using a commercial kit (AutoDELFIA C-peptide Kit B081-101, Wallac Oy, PerkinElmer Life Sciences, Turku, Finland). Plasma insulin was measured with a radioimmunometric assay (BI-INS-IRMA, CIS Bio International, Gif-sur-Yvette, France), and plasma pro-insulin with a two-sided (sandwich) enzyme-linked immunosorbent assay (ELISA) [30]. HOMA indices for insulin sensitivity (HOMA2-%S) and beta-cell function (HOMA2-%B) were determined with an updated HOMA computer model [31], using fasting glucose and insulin for HOMA2-%S, and fasting glucose and C-peptide for HOMA2-%B.

2.3.2. Antibody assays and HLA-DQ genotyping

ICA were determined by indirect immunofluorescence and endpoint titres expressed as JDF units [32]. IA-2A, GADA and IAA were measured by liquid-phase radiobinding assays and expressed as the percent tracer-bound in haemolysis-free sera [32]. Cutoff values for antibody positivity were determined as the 99th percentile of antibody levels obtained in 790

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

Table 1
Baseline characteristics of insulin-treated and non-treated IA-2A-positive relatives.

	Insulin-treated group ($n = 25$)	Observation group ($n = 25$)	P
Age (years)	16 (11–23)	12 (10–19)	0.128
Male/female ratio	14/11	12/13	0.778
IA-2A level (% tracer-bound)	262 (73–475)	72 (12–296)	0.082
Relationship to index patient			0.142
Sibling	8 (32)	13 (52)	
Offspring father	12 (48)	11 (44)	
Offspring mother	5 (20)	1 (4)	
HLA-DQ genotype			0.202
DQ2+/DQ8+	3 (12)	9 (36)	
Non-DQ2+/DQ8+	15 (60)	12 (48)	
DQ2+/non-DQ8+	5 (20)	2 (8)	
Non-DQ2+/non-DQ8+	2 (8)	2 (8)	
Number (%) of subjects positive for ICA	24 (96)	21 (84)	0.350
ICA titre[a] (JDFU)	100 (50–200)	100 (38–400)	0.579
Number (%) of subjects positive for GADA	23 (92)	21 (84)	0.670
GADA level[a] (% tracer-bound)	64 (10–438)	98 (50–585)	0.307
Number (%) of subjects positive for IAA	10 (40)	12 (48)	0.390
IAA level[a] (% tracer-bound)	1.4 (0.9–4.0)	1.7 (0.9–4.9)	0.720
Number (%) of subjects positive for			0.530
IA-2A only	1 (4)	1 (4)	
Two autoantibodies	1 (4)	4 (16)	
Three autoantibodies	13 (52)	10 (40)	
Four autoantibodies	10 (40)	10 (40)	

Age and autoantibody levels are expressed as medians (IQR); other characteristics are *n* (%).
[a] For median ICA, GADA and IAA levels, only those positive for the respective antibody were taken into account; threshold for significance: $P < 0.05/12$ or $P < 0.004$ (Bonferroni adjustment).

non-diabetic control subjects after dropping the outlying values, and amounted to ≥ 12 JDF units for ICA, $\geq 0.6\%$ for IAA, $\geq 2.6\%$ for GADA and $\geq 0.44\%$ for IA-2A [32]. In the 1995 Immunology of Diabetes Workshop, diagnostic sensitivity adjusted for 99% specificity amounted to 73% for ICA, 85% for GADA and 36% for IAA [33]. In the DASP 2002, sensitivity and specificity were 36 and 98% for IAA, 88 and 96% for GADA, and 62 and 97% for IA-2A, respectively [34]. cDNA samples for preparation of the radiolabelled GAD and the intracellular domain of IA-2 were kindly donated by Professor Å. Lernmark, of the University of Washington in Seattle, WA, USA, and by Dr M. Christie, of the King's College School of Medicine and Dentistry in London, UK, respectively. DNA polymorphisms at the *HLA-DQA1* and *-DQB1* gene loci were determined as described elsewhere [26].

2.4. Statistical analysis

Statistical differences between groups were assessed by the Mann–Whitney U test or the Kruskal–Wallis analysis for continuous variables and by the Chi^2 test, using Yates' correction or Fisher's exact test for categorical variables. The present study was powered to detect a 45% reduction in diabetes risk – from 75% in the control group to 30% in the insulin-treated group – by assuming a power ($1 - \beta$) of 80% and a significance (α) of 0.05. Differences in diabetes-free survival were analyzed using Kaplan–Meier curves and log-rank tests. All statistical tests were two-tailed, using the SPSS for Windows 11.0 (SPSS, Chicago, IL, USA) statistical software package, and results considered significant for $P < 0.05$ or, in case of k comparisons, for $P < 0.05/k$ (Bonferroni adjustment). Urinary C-peptide excretion was measured in 24-h urine collections as an estimate of beta-cell rest [35] and expressed as C-peptide (μg) per g creatinine. During follow-up, an increase in antibody level was considered significant if it exceeded 2.8 times the coefficient of variation of the value at study entry (45% above the cut-off for conversion to positivity and a 30% increase for already positive patients). For ICA, an increase of two titre steps (from 0 to 12 JDF units or a fourfold increase in end titre for initially positive relatives) was considered significant [32].

3. Results

3.1. Baseline characteristics of both study groups

The insulin-treated group did not differ from the observation group in terms of baseline characteristics such as age, gender, type of relationship to the diabetic proband, *HLA-DQ* genotype, or prevalence or levels of autoantibodies. Most relatives were also positive for ICA and GADA. There was a non-significant tendency towards higher IA-2A levels in the insulin-treated group compared with the observation group ($P = 0.083$) (Table 1).

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

3.2. Insulin treatment

The aim of insulin treatment was to provide postprandial beta-cell rest. Capillary glucose values measured 1 h after the most consistent meal were lower than baseline at three, six and 12 months after initiation of insulin treatment (Fig. 2; $P < 0.05$). However, 24-h urinary C-peptide excretion was not significantly lower at three and six months (34 [23–44] and 38 [32–57] μg/g creatinine, respectively; $P = 0.198$ and $P = 0.671$, respectively) than before starting insulin treatment (40 [31–54] μg/g creatinine). The median (IQR) daily insulin dose at study entry was 0.10 (0.09–0.10) U/kg. During the first six months, clinical symptoms suggestive of hypoglycaemia were reported in 12/25 subjects (48%) and their insulin dose was subsequently reduced. After 12 months, the median (IQR) insulin dose was 0.08 (0.07–0.10) $U \times kg^{-1} \times day^{-1}$ ($P = 0.005$ vs. initial dose) and did not change significantly thereafter.

No episodes of severe hypoglycaemia were reported during the 36-month insulin-treatment period. BMI–SDS did not increase during insulin treatment (median [IQR]: −0.17 [−0.7–0.57] at study entry vs. 0.13 [−0.59–0.84] at 36 months; $P = 0.256$). Insulin antibodies either developed or increased in 15/25 insulin-treated relatives, but in only 7/25 relatives in the observation group ($P = 0.046$).

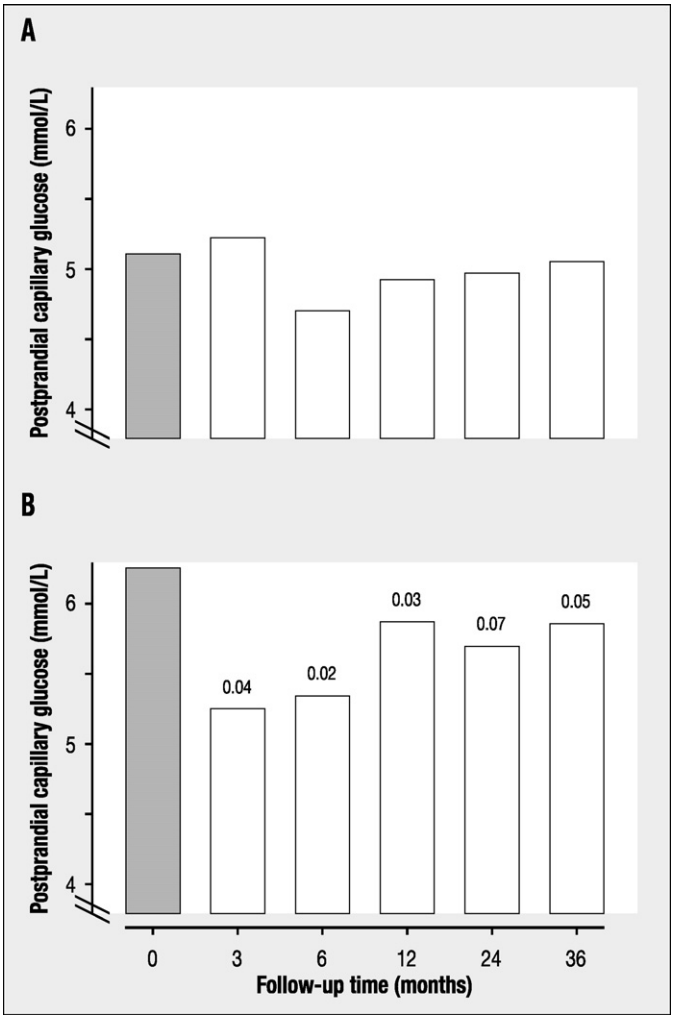

Fig. 2. Pre- and postprandial capillary glucose values before and after the initiation of insulin treatment. Postprandial glucose values were significantly lower after starting insulin treatment (B), while no significant differences in preprandial glucose values were observed (A).

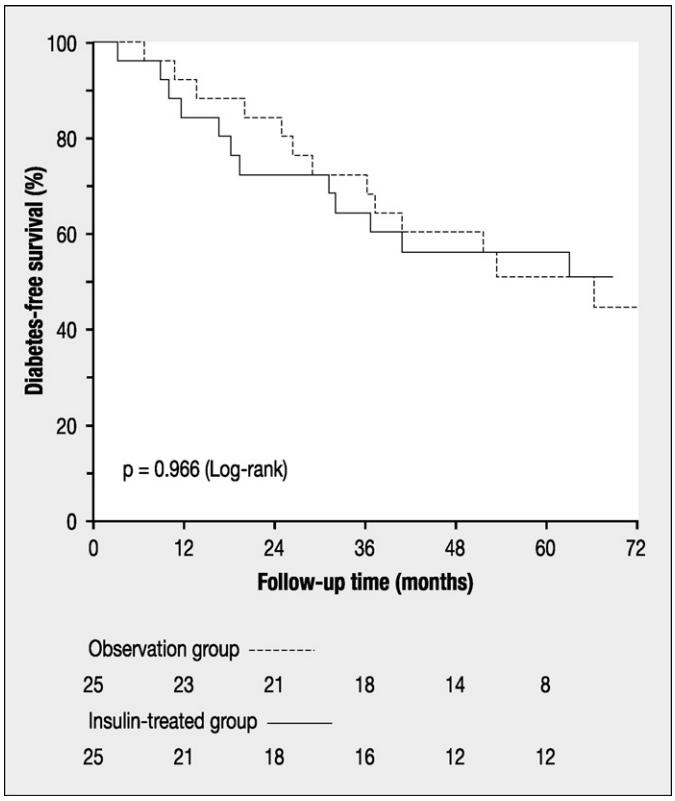

Fig. 3. Diabetes-free survival (%) in the insulin-treated group (solid line) and in the observation group (dotted line). The 5-year diabetes-free survival (95% confidence interval [CI]) was 56.0% (36.6–75.4) in the insulin-treated group and 50.6% (30.4–70.8) in the observation group. The number of subjects in each category (at 12-month intervals) is indicated beneath the time scale. All subjects in the insulin-treated group received insulin during the first 36 months.

3.3. Effect of insulin treatment on development of diabetes

In the observation group, 15 individuals developed diabetes vs. 12 in the insulin-treated group (10 of the latter during insulin treatment and two after stopping insulin treatment). The median (IQR) time between study inclusion and diabetes onset was 19 (10–35) months in the insulin-treated group and 36 (20–53) months in the observation group ($P = 0.11$). Kaplan–Meier analysis revealed no difference in diabetes-free survival between both groups (log-rank test: $P = 0.97$; Fig. 3). Five-year progression (95% confidence interval [CI]) was 44% (25–69) in the insulin-treated group and 49% (29–70) in the observation group (Fig. 3).

3.4. Differences between progressors and non-progressors

In this study, a total of 27 subjects progressed to hyperglycaemia. Progressors and non-progressors did not significantly differ in terms of baseline characteristics except for the presence

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

Table 2
Baseline characteristics of progressors and non-progressors in insulin-treated and observation groups combined.

	Non-progressors ($n=23$)	Progressors ($n=27$)	P
Age (years)	11 (11–20)	16 (10–21)	0.559
Male/female ratio	14/9	12/15	0.247
IA-2A level (% tracer-bound)	45 (9–262)	215 (72–453)	**0.030**
Relationship to index patient			0.103
Sibling	10 (43)	11 (41)	
Offspring father	8 (35)	15 (56)	
Offspring mother	5 (22)	1 (4)	
HLA-DQ genotype			0.753
DQ2+/DQ8+	4 (17)	8 (30)	
Non-DQ2+/DQ8+	14 (61)	13 (48)	
DQ2+/non-DQ8+	3 (13)	4 (15)	
Non-DQ2+/non-DQ8+	2 (9)	2 (7)	
Number (%) of subjects positive for ICA	19 (83)	26 (96)	0.108
ICA titre[a] (JDFU)	50 (25–200)	100 (50–400)	**0.036**
Number (%) of subjects positive for GADA	20 (87)	24 (89)	0.834
GADA level[a] (% tracer-bound)	93 (31–450)	91 (10–539)	0.689
Number (%) of subjects positive for IAA	12 (52)	10 (37)	0.283
IAA level[a] (% tracer-bound)	1.8 (0.9–5.0)	1.4 (1.1–3.3)	0.674
Number (%) of subjects positive for			0.273
IA-2A only	2 (9)	–	
Two autoantibodies	1 (5)	4 (15)	
Three autoantibodies	10 (43)	13 (48)	
Four autoantibodies	10 (43)	10 (37)	

Age and autoantibody levels are expressed as medians (IQR); other characteristics are *n* (%).
[a] For median ICA, GADA and IAA levels, only those positive for the respective antibody were taken into account; threshold for significance: $P<0.05/12$ or $P<0.004$ (Bonferroni adjustment).

of higher IA-2A and ICA levels at study entry in those who progressed (Table 2).

Among the insulin-treated group, no significant differences in insulin dose were measured at six and 12 months between progressors and non-progressors. At study inclusion (Table 3), progressors and non-progressors had similar fasting glucose, C-peptide, insulin and pro-insulin levels. At 2-h post-glucose load (75 g), glycaemia, insulin and pro-insulin levels were similar in both groups, but C-peptide tended to be lower in the progressors than in non-progressors ($P=0.07$). The 2-h pro-insulin/C-peptide ratio was higher in progressors than in non-progressors ($P=0.01$). HOMA indices for insulin secretion and insulin sensitivity did not differ between the groups. As in the combined group, progressors had higher IA-2A levels compared with non-progressors (median [IQR] 166% tracer-bound [46–453] vs. 36% [6–91], respectively; $P=0.003$) and higher ICA titres (median [IQR] 300 [100–400] JDFU) vs. 25 [12–50] JDFU, respectively; $P=0.002$). After starting insulin treatment, we observed no significant changes in levels of insulin antibodies or autoantibodies between progressors and non-progressors.

Table 3
Oral glucose tolerance test results at study entry in progressors and non-progressors in the insulin-treated group.

	Non-progressors ($n=13$)	Progressors ($n=12$)	P
Fasting			
Glucose (mmol/L)	4.3 (4.0–4.8)	4.8 (4.3–5.2)	0.10
C-peptide (pmol/L)	343 (215–693)	365 (214–841)	0.80
Insulin (μU/mL)	7.2 (4.1–15.5)	4.4 (3.7–7.1)	0.22
Proinsulin (pmol/L)	5.0 (2.8–11.1)	6.5 (3.4–9.8)	0.54
PI/C ratio (%)	1.3 (1.0–1.9)	1.5 (1.0–3.1)	0.38
2-h post-glucose load			0.753
Glucose (mmol/L)	5.8 (5.3–6.2)	5.5 (4.8–6.8)	0.59
C-peptide (pmol/L)	1574 (985–2301)	1188 (954–1431)	0.07
Insulin (μU/mL)	37 (17–56)	25 (20–30)	0.17
Pro-insulin (pmol/L)	25 (14–42)	30 (25–42)	0.36
PI/C ratio (%)	1.6 (1.1–2.1)	2.7 (1.8–4.3)	0.01
HOMA2-%S	103 (47–190)	181 (109–206)	0.11
HOMA2-%B	109 (88–145)	109 (81–129)	0.65

Data are expressed as medians (IQR); PI/C, pro-insulin/C-peptide; threshold for significance: $P<0.05/13$ or $P<0.003$ (Bonferroni adjustment).

4. Discussion

Prophylactic parenteral insulin treatment has been proposed as a preventative intervention in subjects at risk of type 1 diabetes based on its effectiveness in preventing the development of diabetes in BB rats [16,17] and NOD mice [17]. Small-scale pilot trials of antibody-positive first-degree relatives of type 1 diabetic patients were promising [19,20]. However, when the results of the first large-scale trial (DPT-1) were published in 2002, no beneficial effects in the ICA-positive first-degree relatives receiving slow-acting insulin were found [21]. The present study – launched in 2000 – also failed to show any

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

preventative effects using a different protocol from that of the DPT-1 [21]. In contrast to earlier reports [13,20–21], we limited our study to only the IA-2A-positive siblings and offspring of type 1 diabetic patients who did not carry the dominant protective *HLA-DQA1** or *-DQB1** haplotypes (*01-0602* or *05-0301*) [26]. Thus, we selected only high-risk relatives whose overall estimated risk for diabetes was higher than the observed risk seen in the ENDIT trial (30% over 5 years) [34], but was comparable to the estimated risk in the DPT-1 trial (≥ 50% over 5 years) [21]. In addition, while previous trials used slow-acting insulin with or without intermittent intravenous insulin administration [13,19–21], we opted for only subcutaneous injections of regular human insulin at main meals, with the intention of inducing beta-cell rest when insulin needs are highest.

To test the potential relevance of the selected protocol conditions, we set up a pilot study of 25 IA-2A-positive first-degree relatives, who were compared with controls who fulfilled the same inclusion criteria, but did not inject insulin. The two groups did not differ in general characteristics such as age and BMI, or in antibody prevalence or concentrations. IA-2A levels tended to be higher in the treatment group compared with the observation group, but this difference was not statistically significant. Nevertheless, we doubt that this is of clinical importance, as previous studies have demonstrated no effects of IA-2A levels on the risk of diabetes [9].

The lack of a protective insulin effect suggests that the disease process was neither arrested nor delayed, but it does not rule out the possibility of such actions [2,36]. It is, for example, conceivable that insufficient beta-cell rest was induced, as suggested by the lack of significant suppression of the 24-h urinary C-peptide excretion after initiation of insulin treatment. Also, as the insulin doses used in human trials are substantially lower than those used in animal studies – to avoid hypoglycaemia – they may therefore have been too low. On the other hand, insulin treatment in the present study resulted in significantly less postprandial glycaemia.

However, it could also be argued that the intervention came too late in the disease process, and might have more beneficial effects in relatives at moderate risk for diabetes. Indeed, the high-risk relatives studied may have had such a high risk of diabetes that only potent immunomodulating agents such as cyclosporin A or monoclonal anti-T-cell antibodies could have changed the course of the preclinical degenerative process [37]. An immune-modulating intervention might, however, benefit from concomitant insulin prophylaxis, as its protective effects in recently diagnosed type 1 diabetic patients are also dependent on the size of the functional beta-cell mass at the start of treatment [8].

The present study had several limitations. First, this was a small-scale study compared with the ENDIT [38] and DPT-1 [21]. Also, as it was only powered to detect a 45% reduction in progression rates, any smaller effects of the prophylactic insulin treatment might have gone unnoticed. In addition, the treated relatives were compared with an observation group rather than true controls. We are also aware that the lack of OGTT in the observation group may have delayed the diagnosis of diabetes. However, this was unlikely, as most subjects in the observation group were sampled for determinations of random glycaemia and HbA_{1c} annually and, in some cases, every six months.

The present study confirms the safety of low doses of regular human insulin in high-risk first-degree relatives. Hypoglycaemic events were noted in 48% of relatives, especially during the first six months of insulin treatment, but were invariably mild and disappeared after carbohydrate intake. When the insulin dosage was decreased, only a few hypoglycaemic events were reported. Our findings also further confirm that prophylactic insulin treatment at the doses used in our trial does not lead to an increase in body weight or BMI [39]. During insulin treatment, there was a non-significant tendency towards seroconversion to insulin antibody positivity in initially IAA-negative relatives and towards increasing insulin antibody levels in initially IAA-positive relatives, as would be anticipated, as these antibodies are most likely directed against the administered insulin.

In most cases, the onset of diabetes was diagnosed during an OGTT rather than on the basis of clinical symptoms, and no ketoacidosis was reported. This illustrates the importance of intensive follow-up in high-risk relatives for early diagnosis of the disease [40]. This could, of course, have influenced the comparison of survival curves as OGTTs were not performed in the observation group.

The pro-insulin/C-peptide ratio might qualify as a "preclinical" marker of the disease: in the preclinical phase, this value – when measured 2 h after glucose ingestion (OGTT) – was significantly higher in progressors than in non-progressors; the fasting pro-insulin/C-peptide ratio appears to increase later in the preclinical disease process, becoming significantly elevated at diagnosis [41]. This conforms with earlier findings of an increased fasting pro-insulin/C-peptide ratio in ICA-positive first-degree relatives with decreased FPIR during IVGTT [42–43] and at onset of the disease [42], and an increased random pro-insulin/C-peptide ratio in antibody-positive, prediabetic first-degree relatives [30].

In conclusion, subcutaneous low-dose injections of regular human insulin did not prevent or delay the development of type 1 diabetes in first-degree relatives who were IA-2A-positive, which is in line with the results of the DPT-1, wherein long-acting insulin was administered to ICA-positive relatives. In addition, the insulin-treated group did not present with any major side-effects. Given these observations, insulin prophylactic trials in individuals at risk should not be abandoned, but may have to be further evaluated for any additional protective effects they may confer to immunomodulating interventions [8,44,45].

5. Conflicts of interest

None.

Acknowledgements

The present trial was financially supported by Novo Nordisk Pharma in Brussels, Belgium. The recruitment, follow-up and laboratory analyses of the study participants was made possible thanks to grants from the Belgian Fund for Scientific Research (FWO; Vlaanderen projects G.0319.01, G.0517.04

and G.0311.07: research fellowship to K. Decochez, B. Keymeulen, C. Mathieu and I. Weets) and the research council of Brussels Free University (VUB; projects OZR1150 and OZR1449, and research fellowship to E. Vandemeulebroucke).

The Belgian Diabetes Registry (BDR) is supported by the Belgian National Lottery, the Ministries of Public Health of the Flemish and French Communities of Belgium, Weightwatchers, Ortho Clinical Diagnostics, Novo Nordisk Pharma, Lifescan, Roche Diagnostics, Bayer and Eli Lilly.

The expert technical assistance of co-workers at the central unit of the BDR (A. Delchambre, A. Demarré, V. Baeten, V. Claessens, T. Demesmaeker, L. De Pree, N. Diependaele, S. Exterbille, P. Goubert, C. Groven, A. Ivens, D. Kesler, F. Lebleu, M. Lichtert, E. Quartier, G. Schoonjans, M. Van der Linden and S. Vanderstraeten) is here gratefully acknowledged. We would also like to thank the following university teams of co-workers for their excellent assistance in collecting samples and performing the follow-ups of the relatives in the trial: J. Van Elven, J. Vertommen (Antwerp); C. Devisscher (Brussels); S. De Neve, A. Hutse (Ghent); and M. Carpentier, A. Ceusters, C. Lauwers, H. Morobé (Leuven). We are particularly indebted to U. Van de Velde for the invaluable assistance in coordinating the central BDR unit. We also sincerely thank the following members of the BDR who followed and treated the participants in the present trial: D. Beckers (Yvoir), K. Casteels (Leuven), W. Coucke (Roeselare), R. Craen (Ghent), I. De Leeuw (Antwerp), J. Deschepper (Brussels), L. Derdelinckx (Bouge-Namur), L. Emsens (Knokke), M. Giri (Ghent), Ph. Jopart (Haine St Paul), G. Lamberigts (Brugge), F. Nobels (Aalst), F. Pfeiffer (Antwerp), D. Rocour-Brumioul (Liège), R. Rooman (Antwerp), R. Rottiers (Ghent), J. Van Besien (Brussels), P. Van Crombrugge (Aalst), L. Van Gaal (Antwerp) and S. Van Imschoot (Brugge). Last, but not least, we gratefully acknowledge the contribution of all members of the BDR (who cannot be listed because of space limitations) to the programme screening for all relatives at risk of type 1 diabetes.

References

[1] Martin S, Wolf-Eichbaum D, Duinkerken G, Scherbaum WA, Kolb H, Noordzij JG, et al. Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N Engl J Med 2001;345:1036–40.

[2] Slover RH, Eisenbarth GS. Prevention of type I diabetes and recurrent beta-cell destruction of transplanted islets. Endocr Rev 1997;18: 241–58.

[3] Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965;14:619–33.

[4] Pipeleers D, Ling Z. Pancreatic beta cells in insulin-dependent diabetes. Diabetes Metab Rev 1992;8:209–27.

[5] The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trials. A randomized controlled trial. Ann Intern Med 1998;128:517–23.

[6] Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet 2001;358:1749–53.

[7] Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, et al. Correlation between beta cell mass and glycamic control in type 1 diabetic recipients of islet cell graft. Proc Natl Acad Sci U S A 2006;103:17444–9.

[8] Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med 2005;352:2598–608.

[9] Decochez K, De Leeuw IH, Keymeulen B, Mathieu C, Rottiers R, Weets I, et al. IA-2 Autoantibodies predict impending type I diabetes in siblings of patients. Diabetologia 2002;45:1658–66.

[10] Gorus FK, Goubert P, Semakula C, Vandewalle C, De Schepper J, Scheen A, et al. IA-2-autoantibodies complement GAD65-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. The Belgian Diabetes Registry. Diabetologia 1997;40:95–9.

[11] Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, et al. Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. Diabetes 1994;43: 1304–10.

[12] Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AE, Bingley PJ, et al. Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. Diabetes 2004;53:384–92.

[13] Schatz D, Cuthbertson D, Atkinson M, Salzler MC, Winter W, Muir A, et al. Preservation of C-peptide secretion in subjects at high risk of developing type 1 diabetes mellitus–a new surrogate measure of non-progression? Pediatr Diabetes 2004;5:72–9.

[14] Staeva-Vieira T, Peakman M, von Herrath M. Translational Mini-Review Series on Type 1 Diabetes: Immune-based therapeutic approaches for type 1 diabetes. Clin Exp Immunol 2007;148:17–31.

[15] Gorus FK, Pipeleers DG. Prospects for predicting and stopping the development of type 1 diabetes. Best Pract Res Clin Endocrinol Metab 2001;15:371–89.

[16] Gotfredsen CF, Buschard K, Frandsen EK. Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. Diabetologia 1985;28:933–5.

[17] Gottlieb PA, Handler ES, Appel MC, Greiner DL, Mordes JP, Rossini AA. Insulin treatment prevents diabetes mellitus but not thyroiditis in RT6-depleted diabetes resistant BB/Wor rats. Diabetologia 1991;34: 296–300.

[18] Atkinson MA, Maclaren NK, Luchetta R. Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. Diabetes 1990;39:933–7.

[19] Füchtenbusch M, Rabl W, Grassl B, Bachmann W, Standl E, Ziegler AG. Delay of type I diabetes in high risk, first-degree relatives by parenteral antigen administration: the Schwabing Insulin Prophylaxis Pilot Trial. Diabetologia 1998;41:536–41.

[20] Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type I diabetes. Lancet 1993;41:927–8.

[21] Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;246:1685–91.

[22] Carel JC, Landais P, Bougnères P. Therapy to prevent type 1 diabetes mellitus. N Engl J Med 2002;247:1115–6.

[23] Wegmann DR, Eisenbarth GS. It's insulin. J Autoimmun 2000;15:286–91.

[24] Muir A, Peck A, Clare-Salzler M, Song YH, Cornelius J, Luchetta R, et al. Insulin immunization of non-obese diabetic mice induces a protective insulitis characterized by diminished intra-islet interferon-gamma transcription. J Clin Invest 1995;95:628–34.

[25] Decochez K, Truyen I, Van der Auwera B, Weets I, Vandemeulebroucke E, De Leeuw I, et al. Combined positivity for *HLA DQ2/DQ8* and IA-2 antibodies defines population at high risk of developing type 1 diabetes. Diabetologia 2005;48:687–94.

[26] Van der Auwera B, Schuit F, Lyaruu I, Falorni A, Svanholm S, Vandewalle CL, et al. Genetic susceptibility for insulin-dependent diabetes mellitus in Caucasians revisited: the importance of diabetes registries in disclosing interactions between *HLA-DQ*- and insulin gene-linked risk. Belgian Diabetes Registry. J Clin Endocrinol Metab 1995;80: 2567–73.

[27] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2005; 28 suppl. 1: S37-42.

[28] Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the UK, 1990. Arch Dis Child 1995;73:25–9.

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005

[29] Gerlo E, Gorus F. Calibration of ion-exchange HPLC measurements of glycohaemoglobin: effect on inter-assay precision. Clin Chem 1997;43:2353–7.
[30] Truyen I, De Pauw P, Jørgensen PN, Van Schravendijk C, Ubani O, Decochez K, et al. Pro-insulin levels and the pro-insulin: C-peptide ratio complement autoantibody measurement for predicting type 1 diabetes. Diabetologia 2005;48:2322–9.
[31] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modelling. Diabetes Care 2004;27:1487–95.
[32] Decochez K, Tits J, Coolens JL, Van Gaal L, Krzentowski G, Winnock F, et al. High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age. The Belgian Diabetes Registry. Diabetes Care 2000;23:838–44.
[33] Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, et al. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 1998;47:1857–66.
[34] Bingley PJ, Bonifacio E, Mueller PW. Diabetes antibody standardization program: First assay proficiency evaluation. Diabetes 2003;52:1128–36.
[35] Rodriguez-Vilar CI, Conget JM, Gonzalez-Clemente JM, Vidal J, Navarro P, Casamitjana R, et al. Effects of insulin administration on beta-cell function in subjects at high risk for type 1 diabetes mellitus. Metabolism 1996;45:873–5.
[36] Schloot N, Eisenbarth GS. Isohormonal therapy of endocrine autoimmunity. Immunol Today 1995;16:289–94.
[37] Schatz D, Gale EA, Atkinson MA. Why can't we prevent type 1 diabetes? Maybe it's time to try a different combination. Diabetes Care 2003;26:3326–8.
[38] Gale EA, Bingley PJ, Emmett CL, Collier T. (European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet 2004;263: 925–31.
[39] Rhodes ET, Wolfsdorf JI, Cuthbertson DD, Feldman HA, Ludwig DS. Effect of low-dose insulin treatment on body weight and physical development in children and adolescents at risk for type 1 diabetes. Diabetes Care 2005;28:1948–53.
[40] Gale EA. Can we change the course of beta-cell destruction in type 1 diabetes? N Engl J Med 2002;346:1740–2.
[41] Snorgaard O, Hartling SG, Binder C. Pro-insulin and C-peptide at onset and during 12 months cyclosporin treatment of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1990;33:36–42.
[42] Røder ME, Knip M, Hartling SG, Karjalainen J, Akerblom HK, Binder C. Disproportionately elevated pro-insulin levels precede the onset of insulin-dependent diabetes mellitus in siblings with low first phase insulin responses. The Childhood Diabetes in Finland Study Group. J Clin Endocrinol Metab 1994;79:1570–5.
[43] Rodriguez-Villar C, Conget I, Casamitjana R, Vidal J, Manzanares JM, Gomis R. High proinsulin levels in late pre-IDDM stage. Diabetes Res Clin Pract 1997;37:145–8.
[44] Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002;346:1692–8.
[45] Leech NJ. When will immunomodulation for the prevention of type 1 diabetes be a reality? Diabet Med 2003;20:10–3.

Please cite this article in press as: Vandemeulebroucke E, et al. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab (2009), doi:10.1016/j.diabet.2009.02.005