

Preliminary Communication

Effects of High-Dose Oral Insulin on Immune Responses in Children at High Risk for Type 1 Diabetes

The Pre-POINT Randomized Clinical Trial

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IMPORTANCE Exposing the oral mucosa to antigen may stimulate immune tolerance. It is unknown whether treatment with oral insulin can induce a tolerogenic immune response in children genetically susceptible to type 1 diabetes.

OBJECTIVE To assess the immune responses and adverse events associated with orally administered insulin in autoantibody-negative, genetically at-risk children.

DESIGN, SETTING, AND PARTICIPANTS The Pre-POINT study, a double-blind, placebo-controlled, dose-escalation, phase 1/2 clinical pilot study performed between 2009 and 2013 in Germany, Austria, the United States, and the United Kingdom and enrolling 25 islet autoantibody-negative children aged 2 to 7 years with a family history of type 1 diabetes and susceptible human leukocyte antigen class II genotypes. Follow-up was completed in August 2013.

INTERVENTIONS Children were randomized to receive oral insulin (n = 15) or placebo (n = 10) once daily for 3 to 18 months. Nine children received insulin with dose escalations from 2.5 to 7.5 mg (n = 3), 2.5 to 22.5 mg (n = 3), or 7.5 to 67.5 mg (n = 3) after 6 months; 6 children only received doses of 22.5 mg (n = 3) or 67.5 mg (n = 3).

MAIN OUTCOMES AND MEASURES An immune response to insulin, measured as serum IgG and saliva IgA binding to insulin, and CD4⁺ T-cell proliferative responses to insulin.

RESULTS Increases in IgG binding to insulin, saliva IgA binding to insulin, or CD4⁺ T-cell proliferative responses to insulin were observed in 2 of 10 (20% [95% CI, 0.1%-45%]) placebo-treated children and in 1 of 6 (16.7% [95% CI, 0.1%-46%]) children treated with 2.5 mg of insulin, 1 of 6 (16.7% [95% CI, 0.1%-46%]) treated with 7.5 mg, 2 of 6 (33.3% [95% CI, 0.1%-71%]) treated with 22.5 mg, and 5 of 6 (83.3% [95% CI, 53%-99.9%]) treated with 67.5 mg ($P = .02$). Insulin-responsive T cells displayed regulatory T-cell features after oral insulin treatment. No hypoglycemia, IgE responses to insulin, autoantibodies to glutamic acid decarboxylase or insulinoma-associated antigen 2, or diabetes were observed. Adverse events were reported in 12 insulin-treated children (67 events) and 10 placebo-treated children (35 events).

CONCLUSIONS AND RELEVANCE In this pilot study of children at high risk for type 1 diabetes, daily oral administration of 67.5 mg of insulin, compared with placebo, resulted in an immune response without hypoglycemia. These findings support the need for a phase 3 trial to determine whether oral insulin can prevent islet autoimmunity and diabetes in such children.

TRIAL REGISTRATION isrctn.org Identifier: ISRCTN76104595

JAMA. 2015;313(15):1541-1549. doi:10.1001/jama.2015.2928

← Editorial page 1520

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A few precisely defined proteins are often the trigger for immune responses that cause autoimmune diseases.¹ This has led to the experimental use of antigen-specific therapies to prevent, stabilize, or reverse immune-related diseases.²⁻⁴ In humans, these have had some success in allergy⁵ and multiple sclerosis⁶ but not type 1 diabetes.⁷⁻⁹

Type 1 diabetes is an autoimmune disease that can be detected in asymptomatic individuals by the presence of islet autoantibodies¹⁰ that develop in children, with a peak incidence at around 1 year of age.¹¹ Autoantibodies against insulin are the first to appear, followed by the expansion of autoimmunity to other antigens before the onset of diabetes.¹ Interferon- γ -producing CD4⁺ T cells directed against proinsulin¹² and CD8⁺ T cells directed against insulin¹³ are also found in patients with type 1 diabetes. Insulin autoimmunity is closely linked to the HLA DR4-DQ8 haplotype, which is found in most children who develop type 1 diabetes.

Oral administration of insulin reduces the development of diabetes in an animal model.¹⁴ The protective mechanism is thought to involve the induction of insulin-specific regulatory T cells.¹⁵ Antigen-specific therapy with insulin before the development of autoantibodies may induce protective immune responses that prevent the emergence of autoimmunity and type 1 diabetes in genetically at-risk children. Therefore, we assessed whether oral insulin in children without prior overt autoimmunity can induce a potentially protective immune response to an autoantigen without causing adverse effects.

Methods

Participants

The Pre-POINT study was a double-blind, placebo-controlled, dose-escalation, phase 1/2 clinical pilot study conducted at 4 outpatient sites in Germany, Austria, the United States, and the United Kingdom. Participants were recruited between September 2009 and April 2013. Follow-up visits were completed in August 2013.

Children were eligible if they were aged 2 to 7 years; seronegative for autoantibodies to insulin, glutamic acid decarboxylase 65 (GAD65), and insulinoma-associated antigen 2 (IA-2); and at high risk of developing type 1 diabetes. High risk was defined in 2 ways: first, if the child had 2 first-degree relatives with type 1 diabetes and the HLA haplotypes DR4-DQB1*0302 or DR4-DQB1*0304 but not HLA alleles or haplotypes DR11, DR12, DQB1*0602, DR7-DQB1*0303, or DR14-DQB1*0503¹⁶; and second, if the child had a sibling with type 1 diabetes and HLA genotype DRB1*04, DQA1*0301, DQB1*0302/DRB1*03, DQA1*0501, DQB1*0201 identical to the HLA genotype of the sibling with type 1 diabetes.¹⁷

The study protocol (available in Supplement 1) was approved by the Ethikkommission der Bayerischen Landesärztekammer (05135); Colorado Multiple Institutional Review Board (05-1043); Ethikkommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien (341/2007); and National Health Service Health Research Authority, National Research Ethics Service Committee South

West-Central Bristol (10/H0106/33). The parents or legal guardians of each child provided written informed consent.

Randomization and Masking

A computer-generated randomization list was prepared with an allocation ratio of 2:3 (placebo to oral insulin) without stratification. Randomization was performed in study blocks of 5 participants (2:3 placebo to insulin) (eFigure 1 in Supplement 2), using a web-based system (<https://www.wapp.ibe.med.uni-muenchen.de/randoulette/>). Randomization to the study blocks was performed sequentially with increasing dose to detect hypoglycemic events at lower doses before administering higher insulin doses, a requirement imposed by the US Food and Drug Administration. All investigators and participants were masked to the treatment allocation. Unblinding was not necessary during the study.

Interventions

Study medication was provided as identical capsules containing either insulin crystals (2.5, 7.5, 22.5, or 67.5 mg) (Lilly Pharmaceuticals) in microcrystalline cellulose (total content of capsule, 200 mg) or a 200-mg microcrystalline cellulose placebo. Parents were instructed to sprinkle the contents of 1 capsule onto 1 teaspoon of glucose-containing food for administration once daily.

Children in the insulin group were randomized to receive insulin in 1 of 5 study blocks. Children in block 1 were assigned to receive 2.5 mg of insulin for 6 months, followed by 7.5 mg for 3 to 12 months; those in block 2 received 2.5 mg for 6 months, followed by 22.5 mg for 3 to 12 months; those in block 3 received 7.5 mg for 6 months, followed by 67.5 mg for 3 to 12 months; those in block 4 received 22.5 mg for 3 to 12 months; and those in block 5 received 67.5 mg for 3 to 12 months. The doses were not adjusted for body weight.

Follow-up visits were scheduled at 2 weeks, 3 months, and 6 months after starting treatment and at 2 weeks, 3 months, 6 months, and 12 months after dose escalation.

Procedures

Blood samples were collected at study visits for complete blood cell count and liver function tests; measurement of levels of blood urea, electrolytes, blood glucose, IgE, and islet autoantibodies; quantification of lymphocyte subsets; and measurement of T-cell responses to insulin. Saliva was collected for measurement of salivary IgA-insulin antibodies.

Medication adherence was assessed by family self-reporting of daily capsule administration using adherence sheets. The protocol mandated that the children should stop treatment if they developed antibodies to GAD65 or IA-2 or if they developed diabetes.

Main Outcomes

The primary outcome was a positive immune response to insulin, defined as an increase in serum IgG antibodies to insulin, salivary IgA antibodies to insulin, or a CD4⁺ T-cell response to insulin. The outcome was assigned to the treatment and dose at the follow-up visit at which the immune response was detected. A secondary mechanistic outcome mea-

sure was the gene expression profile of CD4⁺ T cells responding to insulin.

Insulin autoantibody levels were measured using a competitive radiobinding assay.^{18,19} IgG binding to insulin was measured by a noncompetitive radiobinding assay with protein-G capture of IgG.¹⁸ A positive response was defined as an increase greater than 10 counts per minute from baseline. IgA binding to insulin was measured in saliva using a radiobinding assay (eMethods in Supplement 2).¹⁸

CD4⁺ T-cell antigen responses were measured using frozen peripheral blood mononuclear cells obtained at baseline and at the 3-, 6-, 9-, 12-, and 18-month visits using a dye (Cell Proliferation Dye eFluor 670; eBioscience) dilution assay, quantifying proliferation (eFluor670^{dim} cells) and activation (CD25⁺CD45RO⁺) after 5 days of culture without or with the antigens insulin, GAD65, and proinsulin (eMethods in Supplement 2). The number of (CD4⁺eFluor670^{dim}CD25⁺CD45RO⁺) cells per 50 000 acquired live CD4⁺ T cells was determined, and the stimulation index was calculated for each antigen. Increases in CD4⁺ T-cell responses were defined as a stimulation index of 3 or greater and a 2-fold or greater increase in the stimulation index over the baseline value.

Gene expression of sorted, stimulated single cells was performed as previously described,²⁰ with modification (eMethods in Supplement 2).

Adverse Events

Venous blood glucose level was measured at 0, 30, 60, and 120 minutes after the first administration of oral insulin or placebo and after each dose escalation. All families were issued a glucose meter and instructed to measure capillary blood glucose level 60 minutes after each dose in the first week of treatment or after a dose increase, and monthly thereafter. Hypoglycemia was defined as blood glucose level less than 50 mg/dL (2.78 mmol/L).

IgE against insulin was measured using a radiobinding assay.²¹ Serum antibodies to GAD65 and IA-2 were measured using harmonized radiobinding methods.²²

Statistical Analysis

The sample size of this pilot study was based on 2 assumptions: that 0 or 1 (95% CI, 0-3) of 10 placebo-treated participants followed up for 1 year would develop GAD65 or IA-2 autoantibodies (safety) or responses to insulin (immune efficacy)^{16,17} and that responses to insulin would be incremental with dose increase. The sample size of 6 per dose of oral insulin was chosen to detect a difference between placebo-treated and insulin-treated participants if a response to insulin was observed in more than 50% of children in the highest-dose group. Because Pre-POINT was a pilot study, no attempts were made to adapt sample sizes to reject the null hypothesis of no immune response to study drug.

The numbers of children with antibody or T-cell responses to the study drug in each dose group were compared with the number in the placebo group using the χ^2 test for trends. $P < .05$ (2-tailed) was considered statistically significant. The gene expression data of insulin-responsive CD4⁺ T cells were analyzed by t -distributed stochastic neighbor em-

bedding (eMethods in Supplement 2), a method for visualizing high-dimensional data able to capture nonlinear relationships and reveal otherwise hidden subpopulations of cells.

Repeated-measures analysis of variance was used to compare blood glucose concentrations between each dose and placebo. IgE concentrations were compared between each follow-up visit and baseline using t test. The incidences (number per month of treatment) of all adverse events and of specific types of adverse event for each dose group and for all doses combined were compared with the incidences in the placebo group using Fisher exact test and the χ^2 test for trends. $P < .05$ (1-tailed) was considered statistically significant for comparisons of blood glucose concentrations, IgE concentrations, and incidence of adverse events. These P values were not corrected for multiple comparisons.

The Clopper-Pearson method was used to calculate 95% CIs of proportions having values of 0. The Wald method was used to calculate 95% CIs for other proportions. SAS version 9.2 (SAS Institute Inc) was used for all statistical analyses.

Results

Participants

In total, 84 children with a multiple family history of type 1 diabetes, 279 children with 1 sibling or family member with type 1 diabetes, and 6 children with an unknown family history of diabetes were screened (Figure 1). Of these, 33 were eligible based on their HLA DRB1-DQA1-DQB1 genotype and lack of antibodies to insulin, GAD65, and IA-2. Consent to participate was provided for 25 children. Sixteen children were randomized in Germany, 5 in Colorado, 3 in Austria, and 1 in the United Kingdom. The mean age at randomization was 5.1 (SD, 1.8) years; 15 children were girls (Table 1).

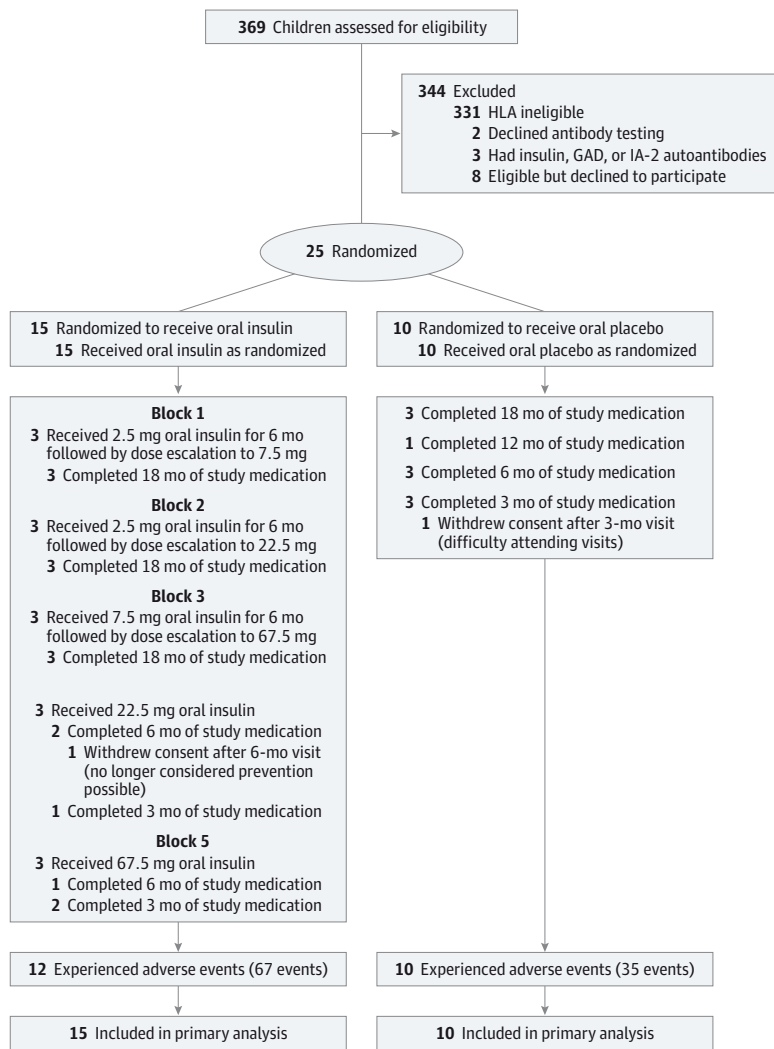
Ten children received oral placebo for 2.5 to 20.1 months (median, 7.3 months; cumulative exposure, 102 months). One child in the placebo group withdrew after the 3-month visit, expressing difficulty in organizing study visits. Fifteen children received oral insulin; 9 received a dose escalation, and each dose group comprised 6 children. One child in the insulin group withdrew after the family became disillusioned about the prospect of preventing diabetes. The cumulative duration of insulin treatment and median per-participant duration are reported in Table 2. The median family-reported adherence to administering medication in children is also shown in Table 2. Of 152 scheduled study visits, 144 (95%) were completed.

Immune Responses to Insulin

Antibody or T-cell responses to insulin were observed in 9 of 15 (60% [95% CI, 35%-85%]) insulin-treated children (Table 3, Figure 2). Responses were also observed in 2 of 10 (20% [95% CI, 0.1%-45%]) children in the placebo group ($P = .02$ for trend).

Six children had increases in serum IgG binding to insulin (Figure 2A), including 0 of 6 (0% [95% CI, 0%-46%]) children treated with 2.5 mg of insulin, 1 of 6 (16.7% [95% CI, 0.1%-46%]) treated with 7.5 mg, 1 of 6 (16.7% [95% CI, 0.1%-46%]) treated with 22.5 mg, 3 of 6 (50% [95% CI, 10%-90%]) treated with 67.5 mg, and 1 of 10 (10% [95% CI, 0.1%-29%]) who re-

Figure 1. Flow of Pre-POINT Randomized Clinical Trial



Abbreviations: GAD, glutamic acid decarboxylase; IA-2, insulinoma-associated antigen 2.

ceived placebo ($P = .051$). In addition, strong IgA binding to insulin at the 3-month visit was detected in the saliva of a second child who received 22.5 mg of insulin (eFigure 2 in Supplement 2).

Nineteen children had sufficient viable cells to measure their T-cell responses to insulin. A response to the study drug during treatment was observed for 5 children (Figure 2B), including 1 of 6 (16.7% [95% CI, 0.1%-46%]) treated with 2.5 mg of insulin, 0 of 5 (0% [95% CI, 0%-52%]) treated with 7.5 mg, 1 of 5 (20% [95% CI, 0.1%-55%]) treated with 22.5 mg, 2 of 4 (50% [95% CI, 1%-99%]) treated with 67.5 mg, and 1 of 7 (14% [95% CI, 0.1%-40%]) who received placebo ($P = .24$). Increases in T-cell responses (>2-fold) to proinsulin accompanied the responses to insulin in 3 of 5 (60% [95% CI, 17%-99.9%]) responders (2 children in the 67.5-mg dose group and 1 in the placebo group) (Figure 2C).

The placebo-treated child with a transient CD4⁺ T-cell response to insulin and proinsulin at the 3-month visit also had a marked increase in effector memory CD8⁺ T cells at this visit (eFigure 3 in Supplement 2). This child had viral gastritis at the

3-month visit. Effector memory CD4⁺ T cells and total CD4⁺FOXP3⁺CD25⁺CD127^{lo} Treg cells were relatively stable during follow-up (eFigure 3 in Supplement 2).

Analysis of the gene expression profiles under insulin-stimulated and proinsulin-stimulated proliferation of single-cell-sorted CD4⁺ T cells from insulin-treated children showed a cluster of cells that expressed *FOXP3* (forkhead box P3) without CD127 or cytokines (FOXP3 signature cells) (Figure 3A and C; eResults in Supplement 2), a typical profile of FOXP3⁺ regulatory T cells.²³ The ratio of FOXP3 signature cells to interferon γ signature cells in samples obtained during oral insulin treatment was 1.05 (95% CI, 0.71-1.56) for insulin-responsive cells and 1.15 (95% CI, 0.59-2.27) for proinsulin-responsive cells, as compared with 0.26 (95% CI, 0.17-0.39) in proinsulin-responsive cells from untreated islet autoantibody-positive children enrolled in the BABYDIET study²⁴ and 0.04 (95% CI, 0.01-0.14) for tetanus toxoid-responsive cells (Figure 3B and C; eResults and eTable 1 in Supplement 2). Analysis of the quantitative gene expression data from all genes examined discretely clustered the insulin-responsive CD4⁺ T cells from the

children who received 67.5 mg of insulin away from the remainder of the insulin-responsive CD4⁺ T cells (eFigure 4 in Supplement 2).

Adverse Events

No families reported signs of hypoglycemia after receiving oral insulin. All blood glucose concentrations determined within 2 hours after the first dose of placebo or oral insulin, or the first dose after a dose escalation, were greater than 50 mg/dL (Figure 4). All 751 reported home-measured blood glucose concentrations were greater than 50 mg/dL (eFigure 5 in Supplement 2).

No child developed autoantibodies to GAD65 or to IA-2. No allergic symptoms were reported after starting treatment. IgE concentrations were above the reference limits in 10 children before starting treatment but did not increase in any of the insulin dose groups or the placebo group (eFigure 6 in Supplement 2). No child had IgE to insulin.

No changes in blood cell counts or blood chemistry values were observed (eTable 2 in Supplement 2). There were 35 adverse events reported over a cumulative exposure period of 102 months in the 10 children who received placebo and 67 adverse events reported over a cumulative exposure period of 186 months in 12 of the 15 children who received insulin (*P* > .90). Infections were the most frequently reported adverse events (eTable 3 in Supplement 2). The median frequency of adverse events was 0.58 events/mo (interquartile range [IQR], 0.13-0.87) for children treated with 2.5 mg of insulin, 0.29 events/mo (IQR, 0.07-0.46) for those treated with 7.5 mg, 0.25 events/mo (IQR, 0.0-0.37) for those treated with 22.5 mg, 0.58 events/mo (IQR, 0.25-0.60) for those treated with 67.5 mg, and 0.33 events/mo (IQR, 0.23-0.47) for those receiving placebo (all *P* > .10). Two serious adverse events were reported. One participant treated with 67.5 mg of insulin had recurring otitis me-

dia throughout childhood and was hospitalized for an elective tonsillectomy. A second participant treated with 22.5 mg of insulin was hospitalized after fracturing the left ulna and radius while playing sport. Neither event was considered related to the study drug.

Table 1. Baseline Characteristics of Children Enrolled in the Pre-POINT Study

Characteristic	Placebo (n = 10)	Insulin (n = 15)
Age, median (range), y	4.5 (2.1-7.2)	6.1 (2.2-7.7)
Sex, No.		
Boys	2	8
Girls	8	7
Family history, No. ^a		
Multiplex	7	9
Siblings	3	6
Clinical site		
Germany	4	12
United States	3	2
Austria	3	0
United Kingdom	0	1
GAD65 or IA-2 autoantibodies	0	0
Elevated total IgE at baseline	2	8
Immune response to insulin at baseline (95% CI) ^b	1 (0.1-29)	2 (0.1-31)

Abbreviations: GAD65, glutamic acid decarboxylase 65; IA-2, insulinoma-associated antigen 2.

^a Multiplex indicates that a child has 2 first-degree relatives with type 1 diabetes; siblings indicates that a child has a sibling sharing HLA DR 3/4.

^b One placebo-treated child and 2 insulin-treated children had a stimulation index greater than 3.0 in the CD4⁺ T-cell response to insulin in their baseline sample. No child had an antibody response to insulin at baseline.

Table 2. Treatment Duration and Adherence in the Pre-POINT Study

	Placebo	Insulin, mg			
		2.5	7.5	22.5	67.5
Treatment duration, mo					
Median (range) per participant	7.3 (2.5-20)	6 (5.7-6.3)	8.9 (6.2-11.8)	8.5 (2.9-12.4)	8.7 (3.3-12.3)
Cumulative	102	36	54	48	48
Family-reported adherence to medication, median (IQR), %	91 (66-94)	98 (98-99)	98 (96-99)	86 (80-96)	99 (98-100)

Abbreviation: IQR, interquartile range.

Table 3. Immune Responses to Insulin According to the Study Drug and Dose Received at the Time of the Response

Immune Response to Insulin	No./Total (%) [95% CI]					<i>P</i> Value ^b
	Placebo	Insulin, mg ^a				
Serum IgG ^c	1/10 (10)[0.1-29]	0/6 (0)[0.0-46]	1/6 (17)[0.1-46]	1/6 (17)[0.1-46]	3/6 (50) [10-90]	.05
Salivary IgA ^d	0/10 (0)[0.0-31]	0/6 (0)[0.0-46]	0/6 (0)[0.0-46]	1/6 (17)[0.1-46]	0/6 (0)[0.1-31]	.40
CD4 ⁺ T cells ^e	1/7 (14)[0.1-40]	1/6 (17)[0.1-46]	0/5 (0)[0.0-52]	1/5 (20)[0.1-55]	2/4 (50) [1-99]	.24
Antibody or CD4 ⁺ T-cell response	2/10 (20)[0.1-45]	1/6 (17)[0.1-46]	1/6 (17)[0.1-46]	2/6 (33)[0.1-71]	5/6 (83)[53-99.9]	.02

^a Responses are assigned according to the insulin dose at first observation.

^b From χ^2 test for trend.

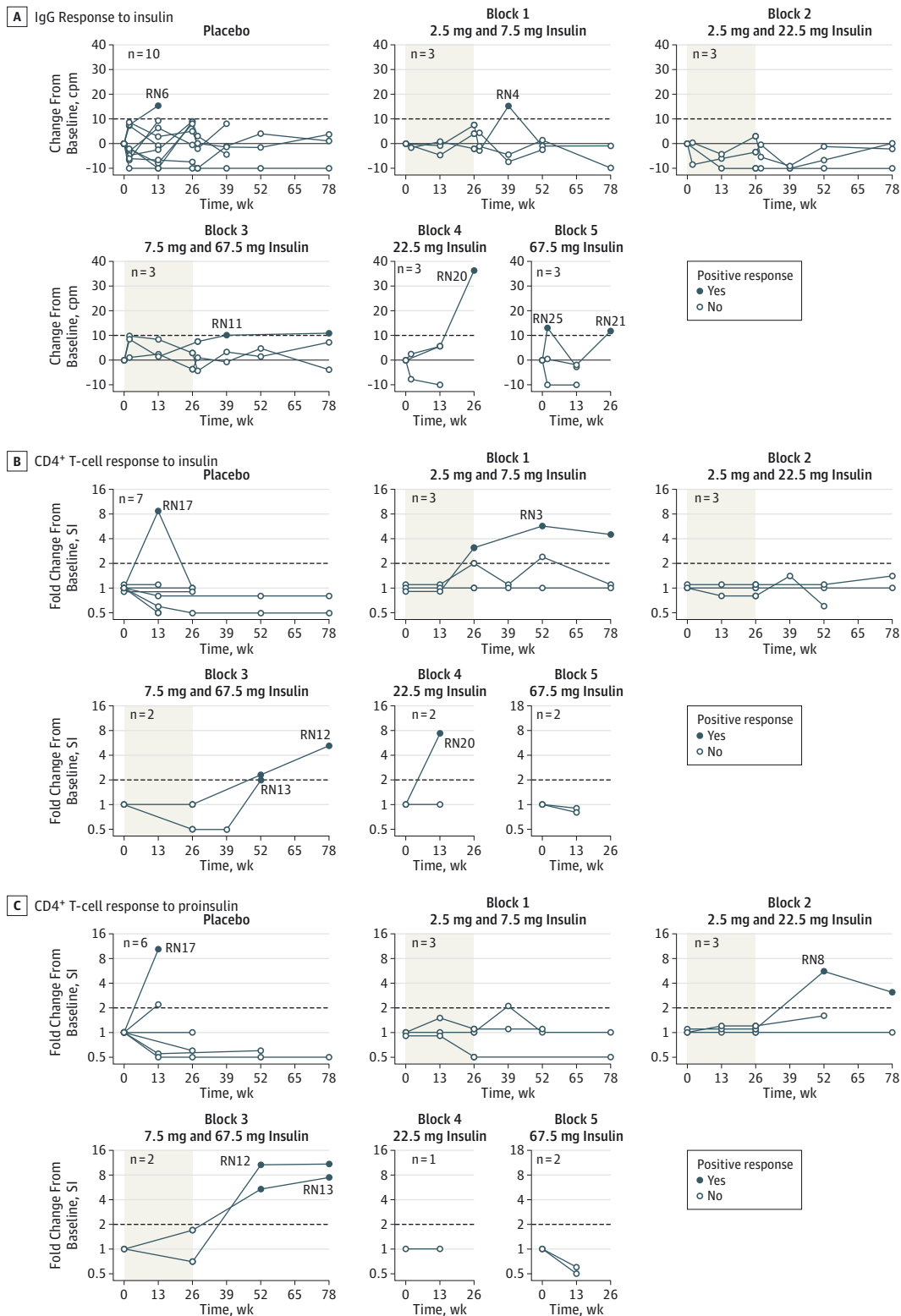
^c A positive response for serum IgG binding to insulin was defined as a change from baseline of more than 10 counts per minute.

^d A positive salivary IgA response to insulin was defined as an increase of more

than 5 SDs from the mean counts per minute of islet autoantibody-negative children.

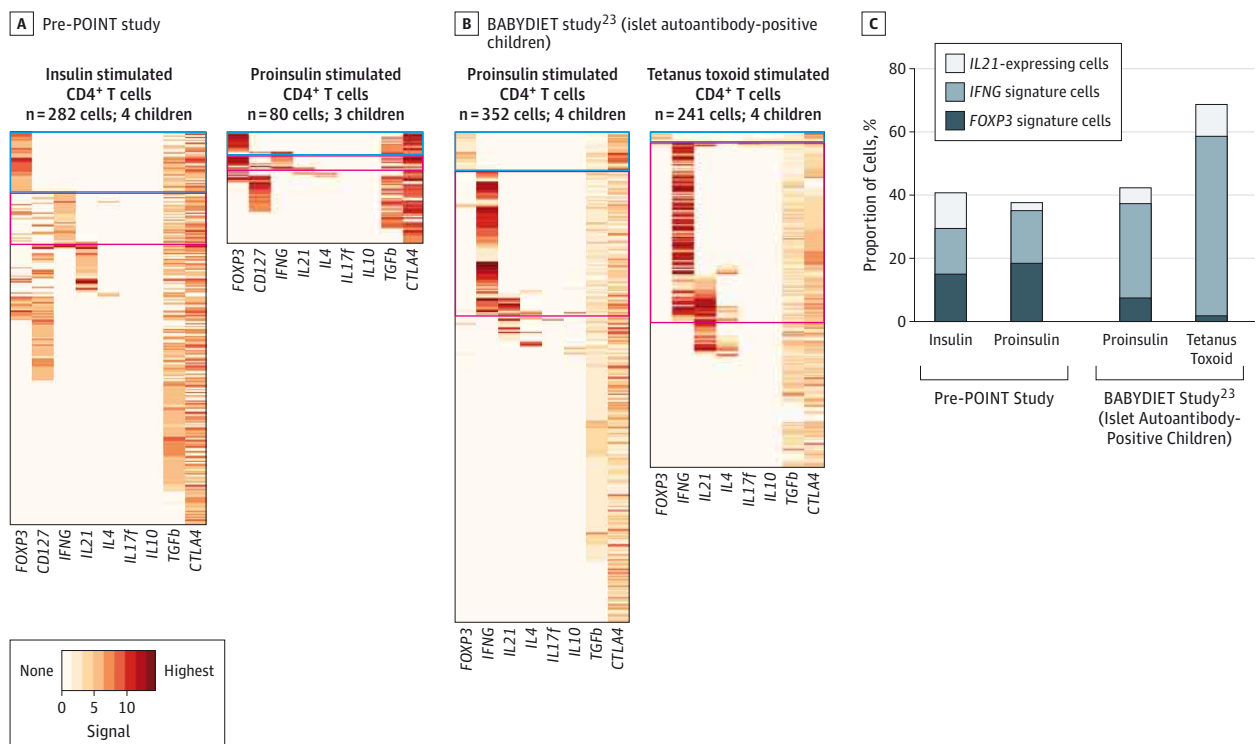
^e A positive CD4⁺ T-cell response to insulin was defined as a stimulation index of 3 or greater and a 2-fold or greater increase in the stimulation index over the baseline value.

Figure 2. Serum IgG Binding to Insulin, CD4⁺ T-Cell Responses to Insulin, and CD4⁺ T-Cell Responses to Proinsulin



Shading indicates period of treatment with the lower starting dose, before dose escalation; dashed lines indicate the threshold for a positive response in each assay. Samples with a positive response are indicated with solid symbols. One participant who received placebo and 1 child in block 1 had CD4⁺ T-cell responses that were

2-fold greater than baseline, but with stimulation index (SI) less than 3, and were therefore not defined as positive responses. The children with positive responses are indicated by their randomization number (RN; see eFigure 5 for randomization number sequence in trial). cpm indicates counts per minute.

Figure 3. Transcription Profiles of Single-Antigen Responsive CD4⁺ T Cells

The gene transcription profile was determined following single-cell sorting. Each cell is represented by a horizontal bar with an intensity ranging from white (no signal) to dark brown (highest signal). A, The profiles of cells from children treated with oral insulin in the Pre-POINT study with T-cell responses to insulin (left; n = 282 cells) or proinsulin (right; n = 80 cells). Boxes indicate cells with *FOXP3* (blue boxes) or *IFNG* (red boxes) signatures. B, For comparison, the gene transcription profiles are shown for proinsulin-responsive CD4⁺ T cells (left; n = 352 cells) and tetanus toxoid-responsive CD4⁺ T cells (right; n = 241 cells) obtained from islet autoantibody-positive children enrolled in the BABYDIET

study.²⁴ Boxes indicate cells with *FOXP3* (blue boxes) or *IFNG* (red boxes) signatures. C, Frequency of antigen-responsive cells with a *FOXP3* signature (*FOXP3* positive, *CD127* negative, and cytokine negative; dark blue), an *IFNG* signature (*IFNG* positive; medium blue), and *IL21* signature (*IL21* positive and *IFNG* negative; light blue) in each of the groups. CTLA4 indicates cytotoxic T-lymphocyte-associated protein 4; *FOXP3*, forkhead box P3; *IFNG*, interferon-gamma; IL, interleukin; Tet Tox, tetanus toxoid; TGFβ, transforming growth factor beta.

Discussion

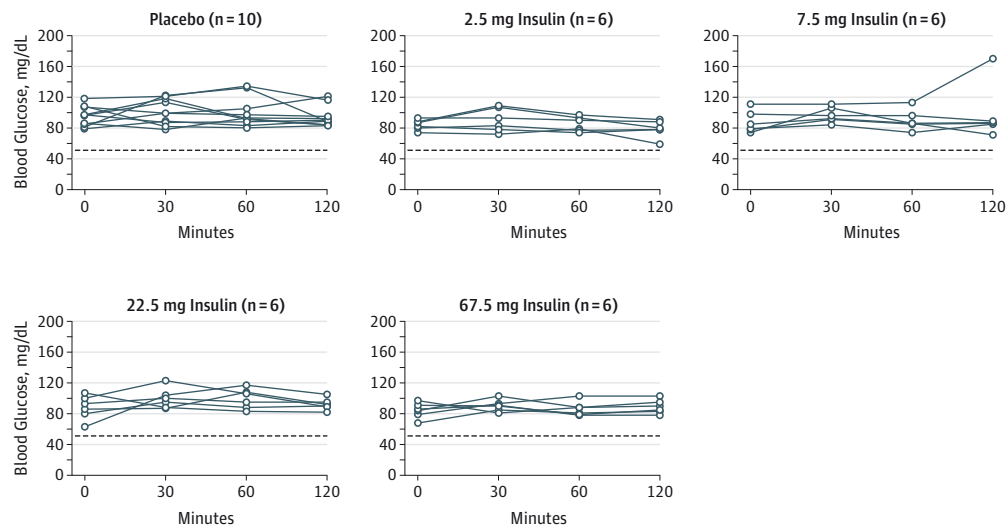
The Pre-POINT pilot study demonstrated that daily oral administration of 67.5 mg of insulin to genetically at-risk healthy children without signs of islet autoimmunity resulted in an immune response without hypoglycemia. The immune response observed in insulin-treated children did not display the features typically associated with type 1 diabetes, such as a dominant proinflammatory *IFNG* CD4⁺ T-cell response.

The incidence and type of adverse events were not different between children who received placebo and children who received oral insulin, regardless of the insulin dose. We did not observe hypoglycemia at any of the tested doses during cumulative treatment of more than 2000 days at each dose. Less than 1% of an oral insulin dose is absorbed and has glucose-lowering activity.²⁵ Nevertheless, hypoglycemia has been reported in an adult following oral ingestion of 3000 units of injectable insulin.²⁶ Therefore, we recommend that future studies administer insulin with glucose-containing foods. Like the DPT-1 (Diabetes Prevention Trial-Type 1) investigators,⁹ we observed no signs of allergy to oral insulin, no insulin-related ad-

verse events, and no evidence that oral insulin induced type 1 diabetes in genetically at-risk children.

Previous studies that administered oral insulin did not search extensively for immune responses and were unable to show that oral insulin administered at a modest dose engages the immune system in humans.^{9,27} Our study did not have confounding autoimmunity at baseline and used higher doses of insulin than in the previous studies. Moreover, the immune outcomes included novel measures of antibody and T-cell responses, and the immune effect of the highest oral dose of insulin was characterized by the composite analysis of the T- and B-cell responses to insulin. The antibody responses were not the high-affinity insulin autoantibodies observed in children who develop type 1 diabetes.²⁸ Instead, we observed IgG binding to insulin in an assay that also detects low-affinity IgG binding, or a strong IgA response to insulin in saliva without a serum IgG response. We previously reported that IgA against insulin were found in cases not associated with type 1 diabetes,¹⁸ but to our knowledge, the Pre-POINT study was the first study to document the presence of insulin antibodies in saliva. Therefore, we suggest that this response was generated within the oral mucosa.

Figure 4. Blood Glucose Concentrations Measured After the First Dose of Placebo or Insulin



Dashed line indicates the threshold for hypoglycemia (50 mg/dL). To convert glucose values to mmol/L, multiply by 0.0555.

In our study, the T-cell responses to insulin were complementary to the antibody responses at the highest insulin dose. The treatment with oral insulin appeared to induce insulin-responsive and proinsulin-responsive regulatory T cells, and responses seen with oral insulin were not the dominant proinflammatory interferon- γ responses associated with type 1 diabetes¹² and observed in islet autoantibody-positive children. The findings appear to be consistent with the notion that oral exposure to an antigen can promote regulatory T-cell responses to the antigen.^{15,29} We also detected a moderate number of *IL21*-expressing insulin-responsive CD4⁺ T cells, but not proinsulin-responsive cells, in insulin-treated children. IL-21⁺ T cells promote B-cell responses to antigens,³⁰ and we observed *IL21* gene-expressing CD4⁺ T cells responding to the vaccine antigen tetanus toxoid, supporting the notion that orally administered insulin acted as a vaccine, as intended, in this study.

This study is encouraging but has a number of limitations. Although no adverse events were attributed to the administration of oral insulin, the sample size was small and the follow-up duration was short. The trial was the first to administer an autoantigen to genetically at-risk children without signs of autoimmunity and therefore only included children with the highest measurable genetic risk. These children represent less than 1% of children who ultimately develop type 1 diabetes, and it is possible that the immune efficacy findings may not

be the same in children with lower genetic risk. The enrolled children (aged 2-7 years) were older than the peak incidence age of islet autoantibody seroconversion (9 months to 2 years).¹¹ Because Pre-POINT was a pilot study with small numbers in each group, the findings of immune efficacy should be interpreted with caution. Furthermore, we were not expecting to observe immune responses against insulin that were typical of type 1 diabetes and therefore included a combination of assays designed to detect weak responses to determine efficacy. However, these assays have not been externally validated. In addition, because we used a dose-escalation design, it was not possible to determine whether prior treatment at a lower dose is necessary to obtain a response at the highest dose (ie, 67.5 mg). Thus, future trials should consider including a dose-escalation phase.

Conclusions

In this pilot study of children at high risk for type 1 diabetes, daily oral administration of 67.5 mg of insulin, compared with placebo, resulted in an immune response without hypoglycemia. These findings support the need for a phase 3 trial to determine whether oral insulin can prevent islet autoimmunity and diabetes in such children.

ARTICLE INFORMATION

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Obtained funding: Bonifacio, Ziegler, Eugster, Achenbach.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This study was supported by grant 8-2006-1091 from the Juvenile Diabetes Research Foundation (Bonifacio), grant O1 KG 0704 from the Bundesministerium für Bildung und Forschung in Germany (Bonifacio), grant FZ-111 from the Deutsche Forschungs Gemeinschaft, and funding from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD). Insulin crystals were provided by Lilly Pharmaceuticals.

Role of the Funder/Sponsors: The funders/sponsors had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation or review of the manuscript. Lilly Pharmaceuticals reviewed the manuscript and agreed with the decision to submit the manuscript for publication. None of the other funders or sponsors had a role in the approval of the manuscript or the decision to submit the manuscript for publication.

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Additional Contributions: We especially thank George Eisenbarth, MD, who brought the United States into the Pre-POINT Study. He was a founding member of the Pre-POINT steering committee, strongly contributed to the Pre-POINT study design, and strongly supported antigen-specific vaccination as a way to prevent type 1 diabetes. In many ways, Pre-POINT fulfilled his ambitions. We regret that he was unable to see the outcome of the Pre-POINT study before he passed away on November 13, 2012.

We thank Denise Kühn, Dipl Hum Biol (DFG Center for Regenerative Therapies Dresden, Technische Universität Dresden), Sevina Dietz, BSc (DFG Center for Regenerative Therapies Dresden, Technische Universität Dresden), and Claudia

Matzke, Biol Tech Assist (Forschergruppe Diabetes e.V.), for laboratory assistance. None of these contributors received additional compensation for their contributions.

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