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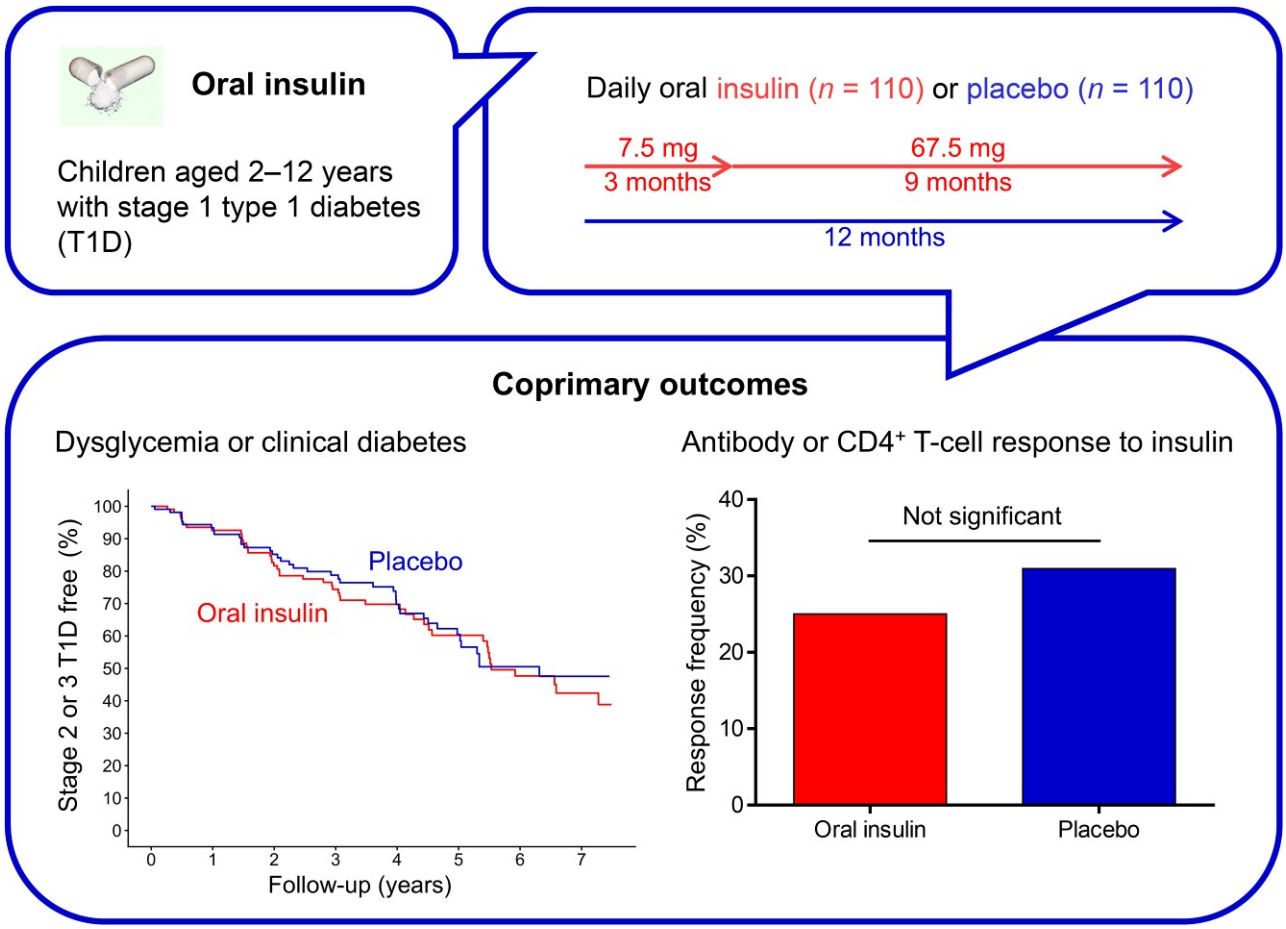

ARTICLE HIGHLIGHTS

- **Why did we undertake this study?**
  Oral antigen exposure can protect against immune-mediated diseases. Insulin is a key autoantigen in childhood type 1 diabetes (T1D).
- **What is the specific question(s) we wanted to answer?**
  Does oral insulin at doses up to 67.5 mg per day for 12 months delay progression to dysglycemia or clinical diabetes or alter immune response to insulin?
- **What did we find?**
  In a randomized clinical trial of 220 children aged 2–12 years with stage 1 T1D, oral insulin did not delay or prevent dysglycemia or clinical diabetes and did not affect immune response to insulin.
- **What are the implications of our findings?**
  Our results do not support using 12 months of high-dose oral insulin for disease modification in stage 1 T1D.

Effect of High-Dose Oral Insulin in Children With Stage 1 Type 1 Diabetes: The Fr1da Insulin Intervention Randomized Controlled Trial

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OBJECTIVE

Oral administration of an antigen can induce immunological tolerance. Insulin is an autoantigen in childhood type 1 diabetes (T1D). We tested the effect of treatment with high-dose oral insulin on disease progression and immune response to insulin in children with stage 1 T1D.

RESEARCH DESIGN AND METHODS

We conducted a phase 2 randomized placebo-controlled double-blind trial in children with stage 1 T1D who received daily oral insulin (7.5 mg/day for 3 months and 67.5 mg/day for 9 months; *n* = 110) or placebo (*n* = 110) for 12 months. The coprimary outcomes were *1*) time from baseline to dysglycemia or clinical diabetes and *2*) increased immune response to insulin within 12 months of treatment (assessed in the first 90 participants).

RESULTS

Of 220 participants (*n* = 112 girls; median age 4.8 years; interquartile range 3.6, 6.2), 179 completed the trial. Dysglycemia or diabetes developed in 87 participants (*n* = 46 receiving oral insulin and *n* = 41 receiving placebo; hazard ratio 1.07; 95% CI 0.66–1.73; *P* = 0.74). The 5-year progression rate was 40% (95% CI 30–51%) in each group. A modest treatment interaction was found with the *INS* rs689 genotype (*P* = 0.03). Increased immune response to insulin was observed in 11 (25%) of 44 participants in the oral insulin group and 13 (31%) of 42 in the placebo group (*P* = 0.63). Oral insulin was well tolerated. No significant study-related adverse events occurred.

CONCLUSIONS

In children with stage 1 T1D, 1 year of high-dose oral insulin did not alter progression to dysglycemia or diabetes or immune response to insulin.

Type 1 diabetes (T1D) has a presymptomatic period defined by the presence of circulating autoantibodies directed against β-cell antigens (1, 2). The progression of clinical T1D in this period is delineated into stages based on the presence of multiple islet autoantibodies with (stage 2) or without (stage 1) dysglycemia (3, 4), which provides a framework for implementing disease-modifying interventions. Teplizumab, an anti-CD3

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monoclonal antibody, is approved in several countries for the treatment of dysgylcemia (stage 2), where it delays progression to clinical diabetes (5). However, critical unmet needs persist, particularly in the development of interventions for stage 1 T1D and combinatory approaches to enhance and extend the efficacy of treatments like teplizumab.

Antigen-based therapies are an appealing strategy because of their targeted approach to modulating the autoimmune response. Oral antigen exposure has demonstrated protective effects against immune-mediated diseases such as childhood allergies (6, 7) and in animal models of autoimmunity (8). Insulin is a key early autoantigen in childhood T1D (9, 10). Loss of immune tolerance to insulin is influenced by the *HLA DRB1*04-DQB1*0302* haplotype (11, 12) and allelic variations in *INS* (13, 14), the gene that encodes insulin, via mechanisms involving thymic T-cell deletion (15, 16) and pancreatic islet endoplasmic reticulum stress (17).

Efforts to mitigate T1D risk in individuals with established autoimmunity have included oral administration of insulin at a dose of 7.5 mg per day (18, 19). Although these trials did not achieve their primary outcome of diabetes prevention, exploratory subgroup analyses within the oral insulin immunotherapy trials revealed potential beneficial effects (18–20). It is unknown whether 7.5 mg insulin is the optimal dose for protection. Antigen doses used in food allergies are considerably higher (6). Furthermore, in small mechanistic studies, we previously found that daily administration of high-dose (67.5 mg/day) but not lower-dose or oral insulin in children at genetic risk of T1D was associated with insulin antibody responses and insulin-responsive $CD4^+$ T cells with regulatory features (21). However, it remains unclear whether high-dose oral insulin can alter immune response or modify disease progression in children with established autoimmunity.

The Fr1da Insulin Intervention trial was conducted in children with stage 1 T1D to determine whether daily administration of oral insulin at doses up to 67.5 mg for 12 months could delay progression to dysglycemia or clinical diabetes and whether such administration was associated with change in antibody or T-cell response to insulin.

RESEARCH DESIGN AND METHODS

Trial Design and Participants

The Fr1da Insulin Intervention randomized clinical trial was conducted at the clinical trial center at the Institute for Diabetes Research in Munich, Germany (ClinicalTrials.gov identifier NCT02620072). The clinical trial protocol was approved by the Ethikkommission der Fakultät für Medizin der Technischen Universität München (420/15) and the Bundesinstitut für Arzneimittel und Medizinprodukte. The trial protocol and statistical analysis plan are provided in the Supplementary Materials. Written informed consent was obtained from both custodial parents. An independent data and safety monitoring board provided trial oversight. Eligible participants were aged 2–12 years with stage 1 T1D. Participants were identified through general population or family screening programs for islet autoantibodies (22, 23). Stage 1 disease was defined by seropositivity for more than one autoantibody to insulin (IAA), GAD (GADA), islet antigen 2 (IA-2A), or zinc transporter 8 (ZnT8A) and confirmed in a second blood sample, and normoglycemia was assessed by oral glucose tolerance test (OGTT) and defined as fasting glucose <110 mg/dL (<6.1 mmol/L), glucose level 2 h after ingestion of oral glucose <140 mg/dL (<7.8 mmol/L), and intermediate glucose levels at 30, 60, and 90 min after ingestion of oral glucose <200 mg/dL (<11.1 mmol/L). The time between the OGTT and random assignment could not exceed 90 days.

Intervention and Procedures

Children were randomly assigned at a one-to-one ratio to receive oral insulin (dose escalation: 7.5 mg for 3 months and increased to 67.5 mg for 9 months) or placebo daily (Fig. 1). Parents were instructed on how to administer and store the study drug. The study drug was to be administered orally as a powder (content of one capsule per day) with a small meal, preferably in the morning (between 7:00 and 10:00 A.M.). Randomly assigned participants were treated for 12 months, and follow-up continued for at least 24 months after the last administration of the study drug (Supplementary Fig. 1). The investigational medicinal product was recombinant human insulin provided as bulk crystals filled in capsules (32 capsules per bottle). Insulin was formulated at doses of 7.5 mg (215.3 IU) and 67.5 mg (1,937.3 IU), with microcrystalline cellulose as the filling substance. The reference placebo was composed of the filling substance (microcrystalline cellulose) at 200 mg. Adherence was assessed with pill counts based on the bottles dispensed and returned.

Follow-up visits were scheduled at 3, 6, 9, and 12 months after starting treatment and every 6 months thereafter until end of study or diagnosis of clinical diabetes (Supplementary Fig. 1). During the 12-month intervention phase, blood samples and saliva were collected at each visit to determine autoantibody and $CD4^+$ T-cell responses to insulin, salivary IgA antibodies to insulin, and flow cytometry for lymphocyte and monocyte subsets as previously described (21, 24, 25) (Supplementary Methods). Serum vitamin D (25OHD) was assessed, and vitamin D supplementation of 1,000 IU per day was recommended for those with vitamin D concentrations <30 ng/mL (75 nmol/L). *HLA DR-DQ* and *INS* (rs689) genotyping was performed on DNA isolated from participants' blood (Supplementary Methods).

To assess progression to dysglycemia or clinical diabetes, fasting blood glucose and hemoglobin A_{1c} were measured centrally at each study visit, and OGTTs were performed every 6 months. Families were instructed to perform home measurements of urine glucose and blood glucose. At baseline and 3-month visits, blood samples were collected before (−10 min) and 30, 60, and 120 min after study drug intake to measure blood glucose, insulin, and C-peptide (Supplementary Methods). Weight, height, and BMI were measured at each visit. Blood cell counts, flow

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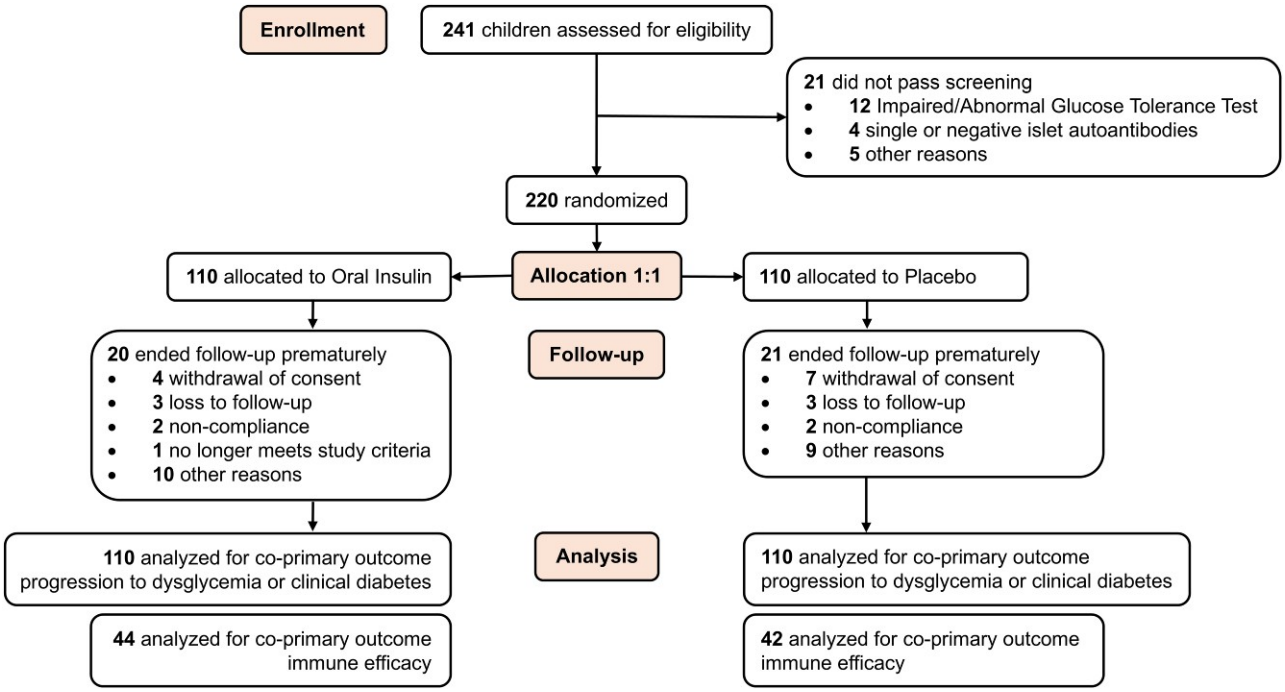

Figure 1—Schematic representation of the distribution of participants in the treatment groups. During the enrollment period, 116,687 children were screened for islet autoantibodies as part of the Fr1da general population screening study; 150 participants were enrolled through the Fr1da study, and 70 participants were enrolled through other family screening programs.

analyses of peripheral blood mononuclear cells, blood chemistry, and electrolytes were measured at baseline and 12 months (Supplementary Methods). Adverse events were recorded up to 6 months after the end of treatment.

Outcomes

The coprimary outcomes included *1*) progression to dysglycemia (stage 2) or clinical diabetes (stage 3), assessed in all participants, and *2*) immune response to insulin. Dysglycemia was confirmed if the criteria previously described and used by TrialNet (3, 26) were met: impaired fasting plasma glucose ≥110 to 125 mg/dL (≥6.1 to 6.9 mmol/L), impaired 2-h glucose ≥140 to 199 mg/dL (≥7.8 to 11.0 mmol/L), or high glucose levels at intermediate time points in an OGTT (30-, 60-, and 90-min levels ≥200 mg/dL [≥11.1 mmol/L]) on two consecutive occasions or on one occasion followed by clinical diabetes on the next occasion. Criteria for clinical diabetes were fasting blood glucose ≥126 mg/dL (≥7 mmol/L) or 2-h plasma glucose ≥200 mg/dL (≥11.1 mmol/L) in an OGTT or symptoms of diabetes and a random plasma glucose value ≥200 mg/dL (≥11.1 mmol/L). The outcome of clinical diabetes as a coprimary outcome was reached if these criteria were observed once. Immune response to insulin was defined as an increase in any one or more of the following parameters during the treatment period: serum antibodies to insulin (positive and greater than twofold increase over baseline), salivary IgA antibodies to insulin (positive and greater than threefold increase over baseline), or a $CD4^+$ T-cell response to insulin (positive [stimulation index >3.0] and greater than twofold increase over baseline). The coprimary outcome of immune response to insulin was assessed in the first 90 participants.

Secondary outcomes included progression to clinical diabetes (the outcome clinical diabetes as a secondary outcome was reached if the above criteria were observed on two occasions [27]); antibody response to insulin on follow-up; $CD4^+$ and $CD8^+$ T-cell responses to autoantigens proinsulin, insulin, and GAD65; frequency of HLA DQ8 insulin tetramer–positive T cells with a regulatory T-cell phenotype at 6 months (performed in participants who were HLA DQ8 positive); and peripheral blood T-cell or monocyte population. Safety assessments included blood glucose, insulin, and C-peptide after study drug intake at baseline and 3-month visits. All data and measurements were submitted to the database before unblinding.

Statistical Analysis

Efficacy analyses were conducted in the intention-to-treat population, which included all randomly assigned children who received at least one dose of study medication, according to the treatment they were randomly assigned to receive. Safety analyses included all participants who received at least one dose of study medication, according to the treatment they received. The complete statistical analysis plan is included in the Supplementary Materials.

To maintain an overall two-tailed α level of 0.05, individual α levels for testing the coprimary outcomes were calculated in accordance with the fallback procedure (28). For the coprimary outcome of dysglycemia or clinical diabetes, a 48.75% progression rate at 3.75 years in the placebo group was expected. Assuming an exponential distribution, random assignment of 220 children at a ratio of one to one over 55 months, with an additional follow-up of 36 months and a dropout rate of 13%, had 86.8% power to detect a 50% reduced hazard ratio (HR) in the insulin group in the Cox model with the Wald test at a two-tailed α level of 0.033. For the coprimary outcome of immune response to insulin during the treatment period, a 20% responder status

in the placebo group was assumed. Random assignment of 90 children at a one-to-one ratio had 91.4% power to detect a threefold increase (60%) in the insulin group with the Fisher exact test at a two-tailed α level of 0.016. Sample size and power estimations were performed using PS power and sample size calculation software (version 3.1.2) (29).

The difference between treatment groups was estimated by the HR from the Cox model and the Wald test. The censoring time for children who did not reach the coprimary outcome of dysglycemia or clinical diabetes was defined as the time of their last OGTT, whereas for children who did not reach the secondary outcome of clinical diabetes, it was defined as the time of their last contact with the trial center. The hazard rate of annualized progression from the primary outcome to clinical diabetes was estimated assuming an exponential survival model.

Comparisons between the placebo and insulin groups for secondary outcomes of the immune response were performed using Mann-Whitney *U* and Fisher exact tests. Changes in $CD4^+$ and $CD8^+$ T-cell responses from baseline to end of treatment (month 12) were analyzed using the Wilcoxon matched pairs signed rank test. Subanalyses were planned for stratification by HLA DR4-DQ8 haplotype, *INS* rs689 genotype, age, and first-degree relative with T1D status.

There were no expected adverse events in this trial. The number and percentage of all adverse events are presented for each treatment arm, categorized according to MedDRA and by severity. Glucose, insulin, and C-peptide values before and after study drug intake at baseline (7.5 mg oral insulin or placebo) and 3 months (67.5 mg oral insulin or placebo), as well as height (*z* score), weight (*z* score), and BMI (*z* score) during and after treatment, were compared between treatment arms. Other clinical and blood measurements were described and assessed to determine whether the study drug caused unexpected changes or adverse events.

Apart from the confirmatory testing of the coprimary end points, all testing was explorative. For the coprimary outcomes of progression to dysglycemia or clinical diabetes and immune response to insulin, simultaneous 95% CIs were calculated, taking adjustments for multiple testing into account. For all other point estimations, unadjusted 95% CIs are provided. All statistical analyses were performed using SAS software (version 9.4).

RESULTS

Participants and Follow-up

Between 11 December 2015 and 4 June 2021, 220 children (n = 112 girls; median age 4.8 years; interquartile range [IQR] 3.6, 6.2) were randomly assigned to the oral insulin group (*n* = 110) or the oral placebo group (*n* = 110) (Fig. 1). Randomly assigned groups were reasonably balanced with respect to baseline characteristics (Table 1).

Follow-up for dysglycemia or clinical diabetes was completed in June 2024. Follow-up for the coprimary outcome of immune response to insulin, determined in the first 90 participants, was completed in September 2018. Immune response status was missing for one participant in the oral insulin group and three participants in the oral placebo group. Follow-up to end of study was completed by 90 (82%) of the 110 participants in the insulin group and 89 (81%) of the 110 participants in the placebo group. The median follow-up of participants from random assignment to the coprimary outcome of dysglycemia or clinical diabetes or end of follow-up was 3.6 years (IQR 2.0, 5.5).

The median relative drug adherence, assessed through pill counts, was 98% (IQR 94%, 99%) in the oral insulin group and 98% (IQR 95%, 100%) in the placebo group. In total, 100 children (91%) in the oral insulin group and 104 (95%) in the placebo group met the per-protocol criterion with a drug adherence level $\geq$85%.

Progression to Dysglycemia or Clinical Diabetes

The coprimary outcome of dysglycemia or clinical diabetes developed in 87

Table 1—Baseline characteristics of children enrolled in the Fr1da Insulin Intervention study

Characteristic	Oral insulin	Placebo	*P*
Participants, *n*	110	110	
Girls, *n* (%)	59 (53.6)	53 (48.2)	0.50
Age, years, median (IQR)	4.8 (3.7, 6.4)	4.9 (3.4, 6.2)	0.32
Range	2.4–12.0	2.3–11.0	
Weight, *z* score, median (IQR)	0.53 (−0.23, 1.21)	0.61 (−0.14, 1.13)	0.82
Range	−1.16 to 3.30	−1.64 to 3.15	
Height, *z* score, median (IQR)	0.55 (−0.19, 1.58)	0.61 (0.05, 1.08)	0.93
Range	−3.00 to 3.81	−1.94 to 2.87	
BMI, *z* score, median (IQR)	0.12 (−0.36, 0.79)	0.24 (−0.26, 0.75)	0.95
Range	−1.53 to 3.24	−2.11 to 2.98	
25OHD, ng/mL, median (IQR)	32.3 (26.4, 40.7)	30.8 (24.4, 38.3)	0.25
Range	13.8–97.3	8.7–88.0	
First-degree relative with T1D, *n* (%)	37 (33.6)	32 (29.1)	0.56
Mother	13 (11.8)	12 (10.9)	1.00
Father	9 (8.2)	10 (9.1)	1.00
Sibling	11 (10.0)	10 (9.1)	1.00
Mother and father	4 (3.6)	0	0.12
HLA genotype, *n* (%)			
HLA-DR3/DR4-DQ8	31 (28.2)	28 (25.5)	0.72
HLA-DR4-DQ8/DR4-DQ8	1 (0.9)	7 (6.4)	0.07
HLA-DR4-DQ8/x*	34 (28.2)	30 (27.3)	0.66
HLA-DR3/y†	13 (11.8)	23 (20.9)	0.10
HLA-DRx/x	19 (17.3)	21 (19.1)	0.86
n.d.	12 (10.9)	1 (0.9)	0.003
INS rs689 genotype, *n* (%)			
A/A	77 (70.0)	70 (63.6)	0.39
A/T	22 (20.0)	33 (30.0)	0.12
T/T	3 (2.7)	6 (5.5)	0.50
n.d.	8 (7.3)	1 (0.9)	0.035

n.d., not determined. *x indicates non-DR3, non-DR4-DQ8. †y indicates non-DR4-DQ8.

participants (n = 46 in insulin group and n = 41 in placebo group). The 5-year progression rate was 40% (95% CI 30–51%) in both the oral insulin and placebo groups (Fig. 2*A*). The annualized rate of progression was 10.9% in the insulin group and 10.0% in the placebo group. The HR of insulin related to placebo was 1.07 (simultaneous 95% CI 0.66–1.73; P = 0.74).

The secondary outcome of clinical diabetes developed in 74 participants (n = 38 in insulin group and n = 36 in placebo group). The 5-year progression rate was 34% (95% CI 25–46%) in the insulin group and 31% (95% CI 22–43%) in the placebo group, corresponding to annualized rates of 8.4% and 8.2%, respectively (Fig. 2*B*). The HR of insulin related to placebo was 1.02 (95% CI 0.65–1.62; P = 0.92).

No significant treatment effects on the coprimary outcome of dysglycemia or clinical diabetes were observed in subgroup analyses of HLA DR4-DQ8, *INS* rs689 genotype, age, IAA-positive status, IA-2A–positive status, and T1D first-degree relative status (Fig. 3). An interaction was observed between treatment and *INS* rs689 genotype for the secondary outcome of clinical diabetes (P = 0.03). However, no significant differences between the oral insulin and placebo groups were observed when participants were stratified for *INS* rs689 genotype (Supplementary Fig. 2). In the per-protocol analysis, defined as drug adherence level ≥85%, the results were consistent with those of the primary analysis for the coprimary and secondary outcomes.

Immune Response Outcomes

Responder status for the coprimary outcome of immune response to insulin was observed in 11 (25%) of 44 participants in the oral insulin group and 13 (31%) of 42 participants in the placebo group (P = 0.63), with a 6% difference in the estimated response probabilities (simultaneous 95% CI −17 to 28) (Fig. 4 and Supplementary Table 1). Responses were observed for IAAs in nine participants in each group, salivary IgA IAAs in two participants in each group, and $CD4^+$ T cells against insulin in two participants in the oral insulin and three in the placebo group (Fig. 4 and Supplementary Table 1). Responses to insulin did not always persist to end of treatment (Supplementary Fig. 3).

Secondary Outcomes

$CD4^+$ T-cell response to GAD65 increased from baseline in both the oral insulin and placebo groups during the treatment phase (Supplementary Fig. 4). $CD8^+$ T-cell response to proinsulin increased from baseline in the placebo group during the treatment phase but did not change significantly in the oral insulin group (Supplementary Fig. 5). No significant changes in $CD4^+$ or $CD8^+$ T-cell response to insulin were observed in the insulin or placebo group (Supplementary Fig. 6). The frequency of insulin tetramer–positive $CD4^+$ T cells with a regulatory T-cell phenotype did not differ between the groups after 6 months of treatment (Supplementary Fig. 7). No other changes or differences in T-cell responses to autoantigen were observed (Supplementary Fig. 8).

IAA and $CD4^+$ T-cell responses to insulin and proinsulin were examined with respect to *INS* rs689 genotype (Supplementary Fig. 9). At baseline, participants with a T1D-susceptible *INS* rs689 genotype had increased IAA (median titer 3.9 vs. 2.5 units; P = 0.007) as compared with participants without a susceptible genotype. No differences were observed for $CD4^+$ T-cell response to insulin or proinsulin.

Adverse Events

Blood glucose concentrations did not decrease below 50 mg/dL (2.8 mmol/L) in the insulin or placebo group after ingestion of study drug at baseline or when the dose was increased after 3 months. At the baseline visit, participants in the oral insulin group had higher insulin concentrations (median [IQR] 24.9 μU/mL [14.3, 33.4]) than participants in the

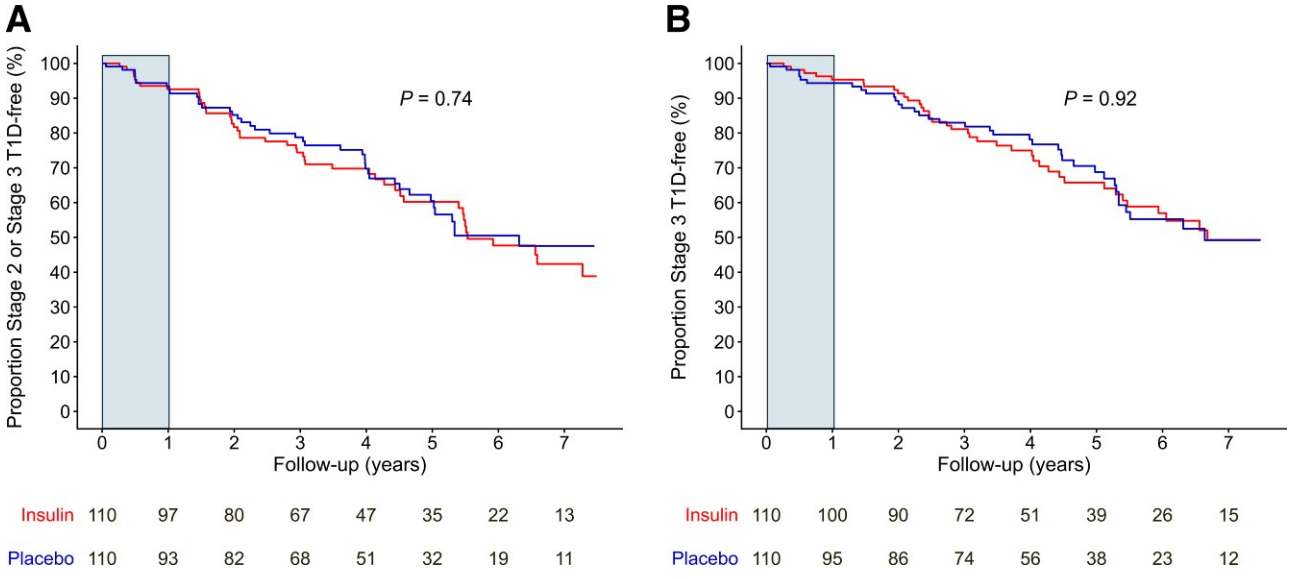

Figure 2—Effects of treatment with oral insulin on the development of dysglycemia (stage 2) or clinical diabetes (stage 3) and clinical T1D. Kaplan-Meier estimates of the proportions of participants who received oral insulin (red) or placebo (blue) and who were not diagnosed with the coprimary outcome of dysglycemia or clinical diabetes (*A*) or the secondary outcome of clinical diabetes (*B*) from random assignment. Participants not reaching the coprimary outcome were censored at the time of their last OGTT (*A*), and those not reaching the secondary outcome were censored at the time of their last contact with the study center (*B*). Shaded area indicates the treatment phase of the study. There were no significant differences in progression to either study end point between the treatment groups.

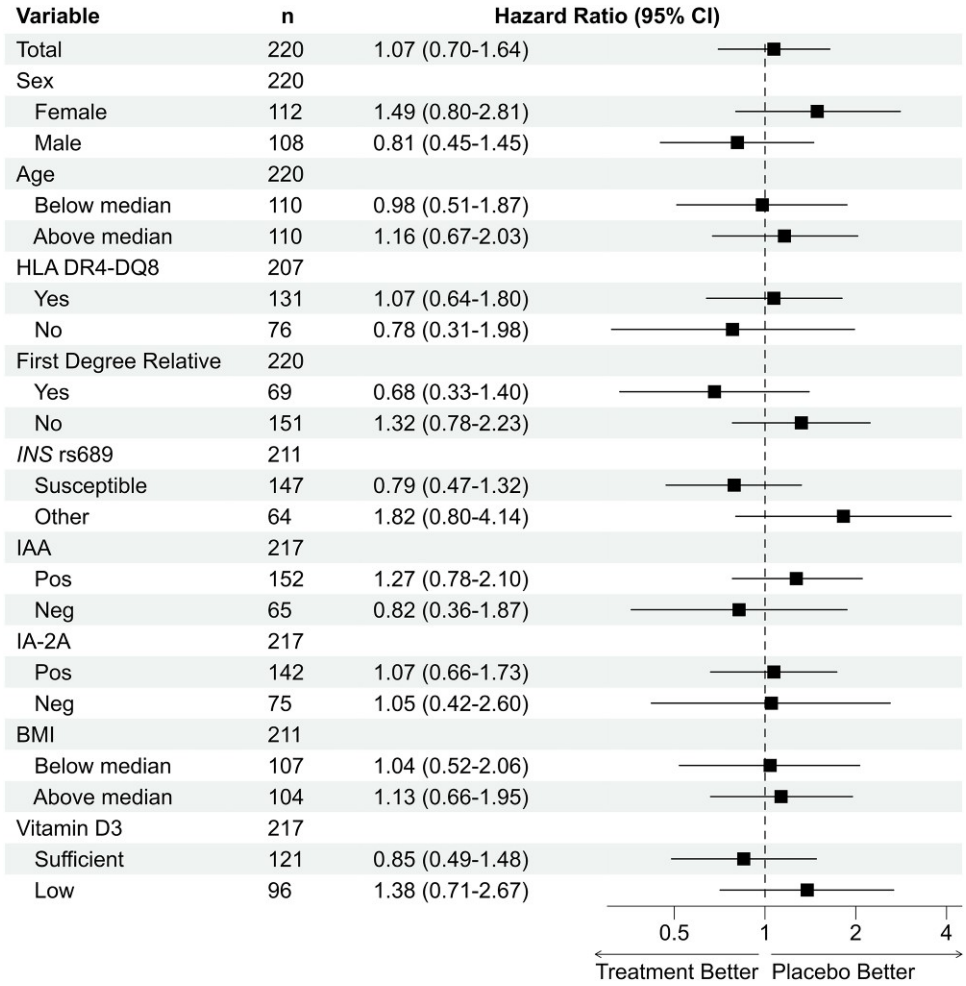

Figure 3—Subgroup analyses of the effect of oral insulin versus placebo treatment on the risk of developing the coprimary outcome of dysglycemia or clinical diabetes in participants with stage 1 T1D. Results of a univariate Cox proportional hazards model of covariates associated with the effect of treatment on the development of dysglycemia or clinical diabetes. Subgroups are defined by status at baseline.

placebo group (17.9 μU/mL [10.6–31.1]; $P = 0.02$) and an increased ratio of insulin to C-peptide in the blood 30 min after administration compared with those receiving placebo (median [IQR] 0.20 [0.16, 0.26] vs. 0.18 [0.13–0.23]; $P = 0.007$) (Supplementary Table 2). Otherwise, no differences were observed between the treatment groups in values for blood glucose, insulin, C-peptide, and ratio of insulin to C-peptide after study drug intake (Supplementary Table 2). No treatment-related effects were observed for height, weight, or BMI (Supplementary Table 3). In addition, no differences in peripheral blood mononuclear cell subpopulations were observed between groups (Supplementary Table 4).

A total of 905 adverse events were reported during the at-risk period for the study participants, including 496 events in the insulin group and 409 events in the placebo group. The most common adverse event was infection ($n = 454$), with 252 events in the insulin group and 202 in the placebo group, affecting 85 (77%) and 78 (71%) participants, respectively. Eleven events were classified as serious adverse events. Nine serious adverse events were observed in four participants in the insulin group, and two occurred in a single participant in the placebo group. None of the adverse events were classified as related to the study drug.

Conclusions

This first interventional clinical trial conducted in children with stage 1 T1D identified by screening for islet autoantibodies in the general population showed that daily oral insulin administration at a dose of up to 67.5 mg per day over a period of 12 months did not delay disease progression to dysglycemia or clinical diabetes and did not alter immune response to insulin, as measured by serum insulin autoantibodies, salivary IgA antibodies to insulin, or blood $CD4^+$ T-cell response.

The dosing strategy was based on previous smaller studies performed in young islet autoantibody–negative children with an elevated genetic risk of T1D (21, 24). We had estimated that an increase in immune response to insulin would be observed in 20% of participants in the placebo group. In this trial, we observed a response in 31% of the participants who received placebo and 25% of those who received insulin. Most of the responses resulted from increases in IAA titer. Few participants had or developed $CD4^+$ T-cell response to insulin in the proliferation assays used. Of potential relevance, an increase in the $CD8^+$ T-cell response to proinsulin was observed in the placebo but not the oral insulin group. However, no overall differences between treatment groups were observed. Two previous studies of oral

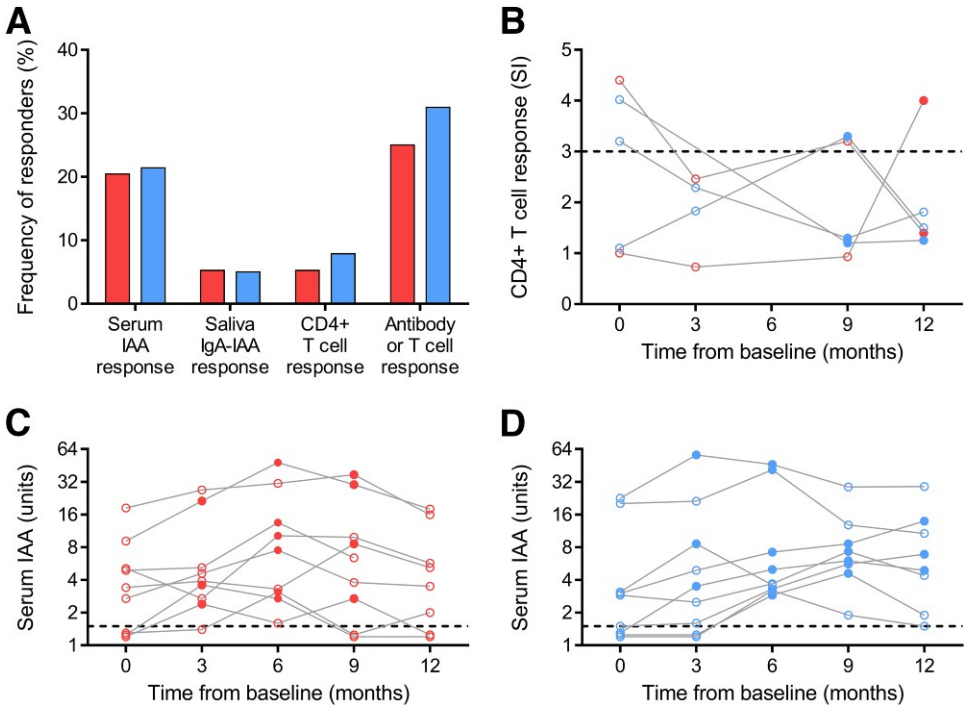

Figure 4—Coprimary outcome of immune response to insulin. *A*: Frequency of a positive immune response to insulin during the treatment phase, as defined by the outcome criteria for serum IAAs, salivary IgA IAAs, $CD4^+$ T cells, or any antibody or T-cell response to insulin, for children receiving oral insulin (red) or placebo (blue). No significant differences in the frequency of responders were observed between the treatment groups. *B–D*: Temporal course of $CD4^+$ T-cell responses to insulin (*B*) and serum IAA levels (*C* and *D*) for participants with a positive immune response to insulin who received either oral insulin (red circles) or placebo (blue circles). Dashed lines indicate the respective thresholds for IAA positivity (1.5 units) or at a stimulation index (SI) of 3 for T-cell responses. Filled circles represent time points at which the outcome criteria for a positive immune response to insulin were met.

insulin used a dose of 7.5 mg (18, 19). No mechanistic studies have been reported with this dose, thus preventing a direct comparison of immune response and efficacy with the results presented here. Previous studies using intranasal insulin showed treatment-related changes in immune response to insulin (30, 31).

Similar to the findings of this study, no effects on progression to clinical T1D were observed in the overall intention-to-treat analysis population in the previous studies using oral or intranasal insulin. However, a protective effect of the 7.5-mg oral insulin dose was observed in a predefined subgroup with impaired first-phase insulin response, as well as in post hoc analyses performed in participants with high IAA titers, HLA DR4, or elevated IA-2A titers (18–20). In comparison, we observed no effects on the coprimary outcome of dysglycemia or clinical diabetes or the secondary outcome of clinical diabetes in subanalyses of HLA DR4 or IA-2A categories, nor for participants with and without a first-degree family history of T1D.

We observed a modest interaction between treatment and *INS* rs689 genotype on the outcome of clinical diabetes, with opposing directional effects of treatment in participants with susceptible and nonsusceptible genotypes. However, no significant protection or acceleration by insulin treatment in the subgroups was observed. Nevertheless, the opposing directional effects were similar to those observed with another *INS* SNP (rs1004446) in the POInT (Primary Oral Insulin Trial) prevention study, where protection against dysglycemia or T1D by treatment in participants with susceptible *INS* genotypes was reported (32). *INS* genotype has been shown to be associated with the risk of IAA development (13, 33) and was associated with higher titers of IAA at baseline in the current trial.

In addition to dose, a difference between the current and previous oral insulin trials was the length of treatment. The current trial treated participants for a maximum of 12 months. The TrialNet TN07 trial had a median treatment duration of 2.7 years (19). It is possible, therefore, that potential treatment effects were lost or required longer or sustained exposure to oral antigen. Finally, it was noticeable that the beneficial effects of oral insulin observed in the substrata and subgroup analyses within the TN07 trial occurred in participants with a higher baseline risk, defined by a low first-phase insulin response to glucose challenge (annualized risk in placebo group 34.1%) (19) or IA-2A positivity (20). No effect of oral insulin was observed in other strata or groups where the annualized risk was <10%. In the POInT trial, participants who experienced progression to stage 1 T1D while receiving oral insulin showed a slower rate of progression to stage 3. The annualized risk in the placebo-treated children in the POInT trial was 29.4% (32). Our current trial was performed in participants who had stage 1 T1D, with an annualized progression rate to T1D of ~8%, which is similar to that observed in placebo-treated participants with stage 1 T1D in the TrialNet abatacept trial (26). It is possible, therefore, that the efficacy of oral insulin and treatments in general may be greatest at more active preclinical disease stages or when administered before the development of autoantibodies.

Limitations

A limitation of the study design was the short treatment duration, which prevented conclusions regarding potential long-term therapeutic effects. Because treatment was administered largely to individuals while they had stage 1 T1D, whether high-dose oral insulin modifies progression to T1D in individuals with stage 2 T1D remains unknown. The dropout rate was 18.6%, which was higher than the 13% predicted rate. Furthermore, the progression rate to the coprimary outcome was lower than predicted. Although the trial duration was longer than originally planned, the overall power of the study to detect differences in the progression rate between treatment groups was <80%.

Conclusion

Our results do not support the use of 12-month treatment with high-dose oral insulin for delaying disease progression in children with stage 1 T1D.

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