Review

MOLECULAR METABOLISM

Cause or effect? A review of clinical data demonstrating beta cell dysfunction prior to the clinical onset of type 1 diabetes

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ABSTRACT

Background: Limited successes of conventional approaches to type 1 diabetes (T1D) prevention and treatment have highlighted the need for improved understanding of risk factors contributing to or hastening progression to clinical diagnosis.
Scope of review: This review summarizes beta cell function metabolic phenotyping data from clinical studies conducted in at-risk individuals before T1D onset and healthy controls. Data are drawn from studies comparing at-risk individuals who progress to T1D to at-risk individuals who do not progress to T1D, as well as from studies comparing at-risk individuals to controls without a T1D family history.
Major conclusions: Rapid loss of beta cell insulin secretion occurs in the months immediately preceding clinical onset. However, evidence of beta cell dysfunction is present even years earlier. Comparisons to controls without a family history suggest that many individuals in families impacted by T1D have evidence of beta cell dysfunction, even individuals who are unlikely to develop clinical disease. These findings may mean that underlying metabolic beta cell dysfunction contributes to T1D development and may explain some of the heterogeneity observed in the disease.

Keywords Beta cell; Type 1 diabetes; Insulin secretion; Risk prediction

1. INTRODUCTION

The incidence of Type 1 Diabetes Mellitus (T1D) is rising at a rate of nearly 3% per year; and the disease constitutes billions of dollars of annual healthcare expenditures [1–4]. Furthermore, despite improved formulations of insulin and improved technologies for glucose monitoring and insulin administration, most persons with T1D do not achieve the glycemic control recommended to avoid acute and chronic diabetes-associated complications [5]. These statistics highlight the need for effective T1D prevention and treatment strategies. Although T1D is classically defined as autoimmune destruction of the pancreatic beta cells, immunomodulatory interventions alone have been unable to produce durable disease remissions [6–11]. There are multiple reasons for the limited successes of these interventions – including heterogeneity of clinical disease, initiation of therapies at later stages of disease, and a need for repeated dosing [11,12]. Additionally, effective T1D prevention or treatment will likely require combination therapies of agents targeting multiple pathologic mechanisms of disease, with personalized regimens targeting differing pathologic features of T1D based on clinical presentation [11,12].
In parallel, emerging preclinical and clinical data from persons at risk of, or with, early T1D have emphasized an expanding role for activation of intrinsic beta cell pathways, such as endoplasmic reticulum and oxidative stress. These pathways may either trigger autoimmunity through neoantigen formation or act independently to accelerate autoimmune-mediated beta cell death [10]. These data suggest that, in combination with immunomodulatory interventions, therapeutics specifically targeting the beta cell may improve disease remission efforts. However, interpreting treatment effects of interventions targeting beta cell health in T1D requires a clear understanding of the natural history and contributions of beta cell dysfunction to T1D development. Recent evidence supporting the idea that beta cells are stressed and dysfunctional in individuals with established T1D has been comprehensively reviewed [13]. Here, we describe what is known about changes in beta cell function before clinical T1D development in humans at increased risk for T1D, and data suggesting that beta cell dysfunction may predate detectable autoimmunity in individuals at genetic risk for T1D.

2. BETA CELL FUNCTION RELATIVE TO T1D PROGRESSION

To date, most cohort studies have ascertained factors associated with progression to T1D in individuals with higher risk human leukocyte antigen (HLA) genotypes or islet autoantibodies (Abs) [14–23]. Predictors of progression to diabetes among these high-risk individuals include high-risk HLA genotypes, age at Ab seroconversion, increasing

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Abbreviations

Ab	Antibody
AUC	Area under the curve
DIPP	Diabetes Prediction and Prevention Study
DPT-1	Diabetes Prevention Trial-Type 1
FDR	First degree relative
FPIR	First phase insulin response
GAD	Glutamic acid decarboxylyase
HLA	Human leukocyte antigen
HOMA-IR	homeostasis model assessment of insulin resistance
IA2	islet tyrosine phosphatase 2
ICA	Islet cell antibody
ICARUS	Islet Cell Antibody Registers Users Study
IVGTT	Intravenous glucose tolerance test
OGTT	Oral glucose tolerance test
PI/C	proinsulin/C-peptide
PTP	Pathway to prevention
T1D	type 1 diabetes
T2D	type 2 diabetes

numbers of positive islet autoAbs, and dysfunctional glucose stimulated insulin secretion [14–16,20–22].

2.1. Early studies describing decreased first phase insulin response before clinical T1D onset

Early metabolic testing studies in nondiabetic persons who eventually developed clinical T1D (progressors) identified first phase insulin response (FPIR) reductions prior to the development of dysglycemia. The Joslin Diabetes Center described IV glucose tolerance testing (IVGTT) of 9 individuals before T1D diagnosis (including first degree relatives (FDRs) of individuals with T1D, as well as individuals who had been incidentally found to have glucosuria) [24,25]. These analyses demonstrated progressively reduced FPIR on serial intravenous glucose tolerance tests (IVGTTs), with responses decreasing dramatically in the year preceding overt T1D [24,25]. In all but one of these individuals islet cell antibody (ICA) or activated T cell positivity predated clinical diabetes onset [24]. Abnormal fasting or stimulated glucose values were only present the year before T1D clinical diagnosis [24]. Follow-up IVGTT data from 35 ICA+ FDRs over ~4 years confirmed that decreases over time to below the 1st percentile were highly predictive of impending diabetes [14].

Subsequent studies confirmed that reduced FPIR is associated with Ab+ status and diabetes progression. In a cross-sectional IVGTT analysis of 66 ICA+ FDRs, 38% of children and 16% of adults showed FPIRs <2 standard deviations below normal [26]. Among children followed longitudinally for diabetes development, very severely decreased FPIR was strongly associated with progression over the next 12 months [26]. Prospective analyses of additional nondiabetic FDRs in the US and Australia have shown that, compared to FDRs with undetectable Abs, FDRs positive for ICA or insulin Abs exhibit reduced FPIR on IVGTT [16,27–30]. However, these studies yielded conflicting results regarding whether adding FPIR to other variables known to be associated with progression to T1D in relatives with multiple Abs improved T1D prediction or whether adjusting FPIR for insulin resistance added benefit [16,27–30]. The Islet Cell Antibody Register Users Study (ICARUS) combined data sets from >20 research groups to explore interactions of Abs, age and FPIR on T1D risk in 456 verified ICA+ FDRs, including 245 who underwent at least one IVGTT [15]. ICARUS confirmed that FPIR loss is highly predictive of short-term risk, but that FPIR's performance as a risk predictor is significantly modified by Ab titers and age [15].

The British Diabetic Twin Study followed 27 monozygotic twins of T1D probands for at least 18 years after diagnosis of the index twin [31]. Over this period, 12/27 twins progressed to T1D (progressors) [31]. Compared to twins that did not progress over this period (non-progressors), progressors exhibited higher fasting insulins, but lower FPIRs on initial IVGTTs (obtained a mean of 3 years before diagnosis) [31]. The Childhood Diabetes in Finland Study followed siblings of affected individuals from the time of proband diagnosis, performing IVGTTs at 6–12 month intervals once the sibling developed ICA or insulin Ab positivity [32]. Among 13 ICA and/or insulin Ab+ siblings in this cohort who progressed to T1D and had repeated pre-diagnosis IVGTT data available, FPIR was reduced in progressors compared to non-progressors at all time points [32]. Intraindividual values fluctuated considerably, and no significant reduction from baseline values was detected in tests performed more proximal to diagnosis, pointing away from a linear progression of beta cell decline before diagnosis [32]. Among these Ab+ siblings, higher ICA titers, higher risk HLA genotypes and HLA identical genotypes to probands were associated with lower FPIR values [33]. These studies hinted that more chronic beta cell dysfunction (before the period immediately preceding T1D diagnosis) may be present in those destined to progress to diabetes.

2.2. Metabolic natural history data arising from prevention studies

Subsequently metabolic analyses performed as part of several large-scale T1D prevention trials became available. These data permitted more systematic prospective analysis of the natural history of metabolic progression of T1D.

The Finnish Type 1 Diabetes Prediction and Prevention Study (DIPP) followed children with high-risk HLA genotypes based on general population newborn screening for development of ICA, insulin Ab, glutamic acid decarboxylase Abs (GADA), or IA2 Abs [34]. Children who had >1 islet Ab were monitored with serial IVGTTs. Most participated in a randomized double-blinded trial of intranasal insulin, which had no effect on diabetes development [34]. For the 218 children with metabolic data, decreasing quartiles of FPIR were associated with higher rates of progression [22]. Here, higher homeostasis model assessment of insulin resistance (HOMA-IR) values were associated with increased risk of progression for individuals with the lowest FPIR [22].

The European Nicotinamide Diabetes Intervention Trial (ENDIT) treated 552 nondiabetic ICA+ FDRs aged 3–40 years with nicotinamide or placebo [35]. All study participants had baseline IVGTTs and serial OGTTs (with fasting and 120-minute timepoints) [36]. A FPIR <10th percentile for age at study entry was associated with a hazard ratio of 2.94 (95% Confidence Interval 2.06–4.20) for clinical diabetes development by the end of the ~5 year follow-up period [36]. However, not everyone who progressed had low baseline FPIR; 36% of individuals who eventually developed T1D had baseline FPIRs above the 10th percentile for age [36]. Similar to the DIPP, study, higher HOMA-IR was only independently associated with progression for individuals with very reduced FPIR [37].

The Belgian Diabetes Registry studied baseline metabolic status of 17 nondiabetic IA-2A+ (16/17 multiple Ab+) FDRs with OGTTs and hyperglycemic clamp studies as part of an ultimately negative prevention study testing the effects of low-dose subcutaneous insulin injections [20,38]. Compared to nonprogressors (followed for~ 94 months) first and second phase C-peptide absolute values were reduced among progressors (obtained 3–62 months before diagnosis) [20]. 5/5 FDRs with low first phase release progressed to T1D (over 3–21 months)

but 2/7 FDRs with normal first phase and abnormal second phase release also developed T1D after 34 and 63 months of follow-up, suggesting that other secretory defects may be present further out from T1D diagnosis [20]. Follow-up data from a larger group classified 81 Ab+ FDRs based on outcomes over 3 years of follow-up [21]. Among the 14 progressors, fasting glucose and OGTT glucose area under the curve (AUC) were increased while both OGTT and clamp-derived (first and second phase) C-peptide measures were decreased. Here, clamp-derived first-phase C-peptide more clearly distinguished progressors from nonprogressors than the OGTT derived parameters tested (fasting and 2 hr glucose, glucose AUC, C-peptide AUC, and peak C-peptide) [20,21].

2.3. The Diabetes Prevention Trial-Type 1 (DPT-1)

The Diabetes Prevention Trial-Type 1 (DPT-1) study group conducted two randomized control trials testing effects of oral and parenteral insulin to prevent T1D. This study group's publications are the source of the most extensive available published pre-diagnosis metabolic analyses.

In DPT-1 non-diabetic 1st and 2nd-degree relatives 3–45 years of age were screened for ICA [39,40]. ICA+ relatives underwent risk assessments including IVGTT and 2-hr OGTT. Individuals with low FPIR or abnormal glucose tolerance were included in the parenteral insulin trial; those with normal FPIR who tested positive for insulin Abs were included in the oral insulin trial. Participants in both trials underwent biyearly OGTTs that included q30 min glucose and C-peptide measurements. The DPT-1 verified reduced FPIR as a risk factor for progression to T1D, and also yielded multiple publications of analyses of the longitudinal OGTTs [41].

2.3.1. Changing OGTT C-peptide responses during DPT-1 monitoring

Longitudinal DPT-1 data analyses suggested that the peri-onset period (defined as 6 months prior to diagnosis), was associated with a rapid metabolic decompensation, including markedly increased fasting and stimulated glucose values, decreases in C-peptide peak and AUC values, and increases in fasting C-peptide [42,43]. Interestingly, longitudinal analysis of glucose and C-peptide OGTT values from 52 progressors over the earlier time period of 30 to 6 months prior to diagnosis demonstrated other abnormalities [17]. Glucose measures increased gradually during this period, without significant changes in fasting, peak, or AUC C-peptide [17]. Further analyses suggested that this was due at least partially to a timing shift in peak insulin secretion [44]. Thirty-six progressors with >=2 years of pre-diagnosis OGTT data were then compared to 80 nonprogressors. The progressors' 30 min minus 0 min (30-0) early C-peptide secretion, which correlates with the FPIR, was already decreased at 2 years before diagnosis and continued to fall until 6 months prior to diagnosis. However, over this same period, they also exhibited delays in the peak C-peptide and an increase in the late C-peptide response [44]. Altogether, 40% of progressors compared to 22% of nonprogressors exhibited a "late" C-peptide response; however, these shifts resulted in similar total AUC or peak C-peptide values [42,44]. Because of this phenomenon, during this period of monitoring, the timing of the C-peptide peak was more predictive of progression than its absolute value [44].

2.3.2. Dysglycemia in DPT-1

During DPT-1 dysglycemic OGTTs were common amongst both progressors and nonprogressors; for 136 progressors and 275 nonprogressors with at least 3 OGTTs, incident dysglycemic OGTTs occurred in 95% of progressors and 55% of nonprogressors [45]. Although dysglycemic OGTTs were associated with increased risk of progression, it is important to note that progressors frequently oscillated between dysglycemic and normal OGTTs [45]. Longitudinal analysis of these oscillating OGTTs showed that the disease was not truly remitting since glucose levels, although sometimes technically in the normal range, exhibited progressive increases over time [18]. Intraindividual OGTT C-peptide values and early C-peptide responses showed that dysglycemic OGTTs or OGTTs transiently in the diabetic range were actually associated with higher C-peptide values [18]. These results suggest that transient dysglycemic or diabetic range OGTTs are associated with changes in insulin sensitivity in progressors.

2.3.3. Reduced beta cell glucose sensitivity is present in progressors

OGTT glucose and C-peptide data from the 328 relatives enrolled in the DPT-1 placebo arms (followed for a median of 2.7 years, 3.2 years for nonprogressors alone) were placed into mathematical models to calculate insulin sensitivity and beta cell glucose sensitivity (the slope of the curve plotting dose–response of insulin secretion relative to plasma glucose on OGTT) [46]. At baseline, despite similar fasting and stimulated insulin levels as nonprogressors, progressors exhibited reduced beta cell glucose sensitivity [46]. In fact, along with young age, decreased baseline glucose sensitivity was the strongest independent predictor of progression to clinical diabetes [46]. Consistent with other reports of rapid metabolic decompensation in the months immediately prior to diagnosis [17,42], for the 208 individuals who had >= 4 OGTTs performed, 70% of progressors exhibited a biphasic pattern to increasing 2-hour glucose values, with rapid increases only occurring 0.78 years before diagnosis [46]. This worsening dysglycemia was accompanied by a rapid decrease in insulin sensitivity. By contrast, dramatic reductions in beta cell glucose sensitivity from baseline began to occur 1.45 years before diagnosis, suggesting that this dysfunction predates hyperglycemia progression [46]. These findings suggested that measures of beta cell function that are interpreted concurrently with changes in glucose and insulin/C-peptide are more physiologically relevant than measures of glycemia or absolute insulin measures alone.

2.3.4. T1D TrialNet pathway to prevention

Given the challenges with feasibility of larger-scale T1D prevention studies, the National Institutes of Health, in cooperation with the American Diabetes Association, and Juvenile Diabetes Research Foundation developed Type 1 Diabetes TrialNet, an international network of clinical sites performing T1D intervention and prevention trials (www.trialnet.org) [47]. TrialNet also conducts the Pathway to Prevention (PTP) study, designed to improve understanding of the natural history of developing T1D. The design of PTP was based on DPT-1, but incorporates screening of first, second, and third degree relatives of individuals with T1D with biochemical islet Abs instead of ICA [47]. Risk assessments to date have involved 2-hour OGTTs performed every 6–12 months [47].

Based on lessons learned from DPT-1 suggesting that better risk assessments can be achieved using measures incorporating timing of C-peptide peak, as well as glucose and insulin levels in concert, metabolic risk scores and indices have been generated and validated using DPT-1 and TrialNet PTP data. These scores/indices can more accurately identify metabolic dysfunction and better predict T1D progression than insulin/C-peptide or glucose measures alone [48–53]. Work from TrialNet has also linked other markers to changes in insulin secretory kinetics in progressors, including circulating cell-free unmethylated insulin DNA (marker of beta cell death) and altered shapes of the OGTT glucose response curve [54,55].

Table 1 — Studies comparing beta cell function in relatives of probands with T1D to controls without a T1D family history.

Study/Year	Country	Methods	Results
Rosenbloom et al. *Metabolism* 1973 [67]	US	OGTTs in 180 siblings of children with diabetes compared to 54 "normal" controls.	58/180 siblings had glucose values that were abnormal by "liberal criteria" (fasting blood glucose >115 mg/dL, 1hr glucose>150, and 2hr glucose >130.) Repeat OGTT testing revealed that half of these children had delayed peak insulin secretion and half were hyperinsulinemic.
Johansen et al. *NEJM* 1975 [71]	US	OGTT, cortisol primed OGTT, and IV tolbutamide testing in 11 monozygotic twins matched by age, sex, and weight to nonrelative controls.	Insulin tended to be increased on OGTT, but only significant on cortisol primed OGTT of twins, while glucose was normal. Growth hormone release increased in twins.
Ginsberg-Fellner et al. *Diabetes* 1982 [74]	US	OGTT in 83 siblings, 67 parents, and 48 nonrelative pediatric controls.	In siblings, HLA identical status (n = 25) but not ICA+ status were associated with higher glucose excursions and lower insulin secretion. ICA+ parents (n = 13) showed higher glucose and higher insulin levels than to ICA- parents.
Hollander et al. *Diabetes* 1982 [73]	US	IV glucose and arginine testing on 14 siblings of T1D probands and nonrelative controls matched for age, sex, weight, and height	Acute insulin response was increased in HLA identical siblings only.
Srikanta et al. *Ann Intern Med* 1983, *Diabetes* 1984 [24,25]	US	IV GTT in 15 ICA- monozygotic twins of T1D probands	Although repeated insulin measurements were variable among twins, compared to a historical control population, no twins had insulin levels < 1st percentile. Only 1/15 twins exhibited progressive reductions in stimulated insulin levels.
Schober et al. *Arch Dis Child* 1983 [72]	Austria	OGTT in 66 siblings of T1D probands stratified by HLA similarity to proband and compared to 33 unrelated controls.	HLA identical siblings (n = 19) mostly showed significantly higher insulin response to glucose (except for 3/19 with very low insulin responses)
Johnston et al. *Diabetes* 1987 [75]	US	IVGTT and arginine stimulation in 12 ICA- adult siblings (>16 years since diagnosis of proband) compared to age, sex, weight matched controls	Insulin sensitivity was reduced in siblings. Absolute maximum acute arginine response was reduced in siblings, but all phases of insulin secretion were reduced after adjustment for insulin sensitivity.
Heaton et al. *Br Med J* 1987 [79]	UK	OGTTS and IVGTTs performed on 10 "low-risk" ICA+ identical twins of probands with longstanding T1D (developed 11—23 years prior), but no personal history of dysglycemia. Compared to age, sex, and BMI matched nonrelative controls.	Compared to controls, low-risk twins had 2-fold increase in fasting proinsulin despite similar C-peptide, glucose, and insulin levels. Twins also showed increased insulin responses to IV glucose and OGTT despite similar glucose excursions.
Heaton et al. *Diabetologia* 1988 [80]	UK	IVGTT and OGTT performed on 11 identical twins with recently diagnosed twin with T1D (<2 years). 5/11 ICA+, 6/11 were ICA-. 2/6 of ICA- group were insulin Ab+. Compared to controls.	Compared to controls, fasting proinsulin increased ~2 fold among ICA- and ICA+ twins, despite similar glucose, insulin, and C-peptide. Stimulated glucose excursions were increased in ICA+ twins.
Vialettes et al. *Diabetologia* 1988 [69]	France	IVGTT in 150 first degree relatives (16/150 were ICA Ab+) compared to 67 controls as well as 31 first degree relatives of individuals with T2D.	12% of T1D relatives had FPIR <5th percentile of controls. No effect of ICA+ on FPIR was detected. Rates of decreased FPIR were similar in T2D relatives (13%).
Hartling et al. *Diabetes* 1989 [76]	Sweden	Fasting proinsulin and insulin were measured in 99 siblings of T1D probands and 41 nonrelative controls matched for age and sex. All siblings were insulin Ab- and only 2/99 were ICA+. Most siblings had been followed for >6 years without T1D development.	Fasting proinsulin was >2 fold increased among siblings, although insulin was slightly reduced. Fasting proinsulin/insulin ratios were significantly increased. Effect was independent of high-risk HLA status.
Lindgren et al. *Diabetic Medicine* 1991 [81]	Sweden	Performed IV glucose infusion tests and somatostatin-insulin-glucose infusions on 93 ICA- and insulin Ab- siblings of T1D probands (same cohort as [76]) compared to 41 nonrelative controls matched for age and sex.	After adjustment for insulin sensitivity, siblings showed reduced total insulin response and increased proinsulin release in response to IV glucose infusion.
Spinas et al. *Diabetes Care* 1992 [82]	Denmark	Fasting glucose, insulin, and proinsulin measured in 85 first degree relatives (siblings, parents, and children; primarily adults) compared to 90 age and weight matched non-relative controls.12/85 relatives were ICA+ and 11/85 were insulin Ab+.	Similar glucose and insulin among relatives and controls but fasting proinsulin was >4 fold increased in relatives. ICA positivity associated with higher proinsulin values. No effect of HLA similarity to proband or insulin antibody positivity was observed.
Carel et al. *JCI* 1993 [70]	France	IVGTT performed on 98 ICA- and Insulin Ab- pediatric siblings of T1D probands compared to 167 nonrelative controls.	Siblings had a 25% reduction in FPIR. Subgroup analysis revealed this effect was only present in siblings >8 years of age.
Hawa et al. *Diabetes Care* 2005 [31]	UK	Follow-up to British Twin Study- At least 2 IVGTTs performed on 27 monozygotic twins of T1D probands followed prospectively for >= 18 years and compared to nonrelative controls with similar age and body habitus.	15 twins who did not develop T1D (mean 24.1 years of age at first test) remained Ab- and showed no differences in FPIR, HOMA-IR, fasting insulin, fasting glucose, or glucose clearance compared to nonrelative controls.
Truyen et al. *Diabetologia* 2005 [62]	Belgium	Random proinsulin/C-peptide ratios obtained in in 561 Ab+ FDRs and 561 Ab- FDRS.	Manuscript notes per personal communication that values for Ab- relatives and nonprogressor Ab+ relatives were similar to a group of 22 nonrelative controls.
Ferrannini et al. *Diabetes Care* 2010 [46]	US	Modeling of insulin sensitivity and beta cell glucose sensitivity using OGTTs in 213 ICA+ nonprogressors (followed median of 3.2 years) in DPT-1.	Discussion notes that at baseline, nonprogressors had reduced beta cell glucose sensitivity and insulin sensitivity compared to historical controls.
Sosenko et al. *Diabetes Care* 2012 [42]	US	Classification of OGTT data from ICA+ DPT-1 nonprogressors into "early" or "late" responders based on timing of OGTT C-peptide peak from historical data from nonrelative controls.	Nonprogressors noted to have significantly higher prevalence of late responders (22%) compared to controls (6%).
Vandemeulebroucke et al. *Diabetologia* 2010 [20] and Balti et al. *JCEM* 2014 [21]	Belgium	1. Hyperglycemic clamp and OGTT in 10 IA2+ (mostly multiple Ab+ FDRs) that did not progress to T1D (7.8 year median follow-up) and 21 nonrelative controls. 2. Hyperglycemic clamps and OGTTs in 67 Ab+ nonprogressors (over 3 years of follow-up), Ab- FDRs (n = 10, n = 20, for first phase and second phase measurements) and transient Ab+ FDRs (n = 7—9) vs. controls.	No differences in glucose or C-peptide measures among Ab+ nonprogressors, Ab- FDRs, transiently Ab+ FDRs, and nonrelative controls.
Siewko et al. *Endokrynol Pol* 2014 [65]	Poland	IVGTT performed in 90 adult FDRs of T1D index cases (only 30% were Ab+) and compared to 60 nonrelative controls.	FPIR was significantly reduced among relatives. Ab positivity was not significantly different between quartiles of first phase insulin secretion.
Campbell—Thompson et al. *Diabetes Care* 2019 [83]	US	Pancreatic MRI performed to calculate pancreas volume in 49 nonrelative controls, 61 Ab- FDRs, 67 Ab+ FDRs, and 52 participants with recent-onset T1D.	Pancreas volume was reduced in Ab- FDRs and Ab+ FDRs compared to controls, although not as severely reduced as in participants with recent-onset T1D.

2.4. Elevations in circulating proinsulin/C-peptide (PI/C), a marker of beta cell stress and dysfunction, are associated with T1D progression

In addition to traditional metabolic measures of beta cell function, circulating biomarkers that reflect events occurring within the beta cell on a molecular level can provide additional insight into beta cell dysfunction [56]. For example, beta cells undergoing intracellular stress accumulate inadequately processed, immature proinsulin molecules [57]. Along these lines, compared to nondiabetic control donors, islets from Ab+ individuals contain normal insulin+ beta cell mass, but increased proinsulin+ area, while islets from individuals recently diagnosed with T1D display reduced insulin+ beta cell mass, and increased proinsulin/insulin positive area [58]. Because accumulating proinsulin molecules are also released into the circulation, elevations in circulating proinsulin levels relative to insulin or C-peptide can be used as a measure of beta cell stress and dysfunction [57,59,60].

Several groups have linked T1D progression to elevations in the ratios of circulating proinsulin to mature insulin or to C-peptide. In the Childhood Diabetes in Finland study, 11 ICA+ and/or insulin Ab+ siblings with severely reduced FPIR on IVGTT were compared to age and sex-matched siblings with normal FPIR [61]. Fasting proinsulin/C-peptide (PI/C) values were 2–3 fold higher in the group with low FPIR and were inversely related to first-phase and stimulated insulin responses [61]. Of 11 siblings with high PI/C and low FPIR, 9/11 progressed to T1D over the next 1–28 months, while none of the siblings with lower PI/C and higher FPIR progressed over this period [61].

Increases in PI/C levels have also been linked to progression among Ab+ relatives of T1D probands. Within the Belgian Diabetes Registry, baseline random PI/C ratios were increased among 338 Ab+ FDRs compared to 561 age and sex matched Ab- FDRs [62]. Longitudinal samples from 32 individuals who progressed to T1D suggested that PI/C values were inversely associated with length of time until diagnosis. PI/C ratios above the 66th percentile were associated with a risk of progression of 50% for multiple Ab+ relatives and 68% for islet tyrosine phosphatase (IA2)+ relatives vs. a risk of 13% for those with lower ratios [62]. Further analyses suggested that fasting PI/C ratios may perform even better for T1D risk prediction when adjusted for insulin sensitivity [63]. Among Ab+ relatives in the TrialNet PTP study in serum samples obtained ~12 months before T1D diagnosis higher PI/C ratios were associated with increased odds of T1D progression, even after adjustment for age, sex, and BMI-Z scores [64]. Interestingly, direct comparisons of progressors to non-progressors divided into young pediatric, adolescent, and adult age groups suggested that in the PTP cohort, significant differences between progressors and nonprogressors were only present in the youngest pediatric group (<=10 years of age) [64].

3. BETA CELL DYSFUNCTION IS PRESENT LONG BEFORE T1D ONSET

These cohorts have provided data to support the critical understanding that, although rapid beta cell losses occur in the years and months immediately preceding T1D onset, abnormalities in insulin secretion are present even years earlier [17–19,23,25]. As an example, 22/52 (42%) of Finnish children followed as part of the DIPP Study with increased-risk HLA genotypes who had recently converted to ICA+ status showed subnormal FPIR by IVGTT [65]. At the time of publication, half of the children with low FPIR had not progressed to T1D, despite ~2.9 years (2–4.4 years) of follow up, suggesting that altered insulin secretion may be present before the period immediately preceding clinical diagnosis [65]. Follow up data from the DIPP cohort compared FPIR among ICA+ progressors and nonprogressors (age of seroconversion 1.6 and 4.6 years, respectively, and age of T1D diagnosis in progressors of 6.6 years) [23]. Here, despite similar glucose values to nonprogressors, progressors had significantly lower FPIR starting around 4–6 years prior to T1D diagnosis [23]. In Ab+ relatives monitored through the TrialNet PTP for at least 5 years before diabetes progression (mean age of diagnosis of 21.6 years), compared to Ab- relatives, abnormalities in fasting and stimulated C-peptide levels were already present at the initial OGTT, a mean of 6.6 ± 1.3 years before diagnosis [66].

These findings not only suggest that chronic abnormalities in beta cell function in Ab+ individuals with genetic risk who progress to T1D, but also raise the possibility that inherited abnormalities in beta cell function may be present and contributing to ultimate T1D risk. Because most cohorts studied only performed metabolic analyses after the development of Ab positivity, these works are unable to make this distinction. However, a significant body of work exists that suggests that family members of individuals with T1D are at increased risk for altered beta cell function compared to nonrelative controls.

4. ARE ABNORMALITIES IN BETA CELL FUNCTION PRESENT IN FAMILY MEMBERS OF INDIVIDUALS WITH T1D COMPARED TO NONRELATIVE CONTROLS?

Because much of the newer available data have focused on the use of large cohorts to better understand factors associated to progression of T1D to clinical disease, these works often compare features of "progressors" to "nonprogressors". However this approach may obscure abnormalities that are present in at-risk individuals who may not progress to disease, but exhibit abnormal beta cell function compared to true unrelated "controls". Although such abnormalities may not exclusively define individuals progressing to clinical disease, they could still provide insights into mechanistic contributors to T1D development. Along these lines, a significant body of cross-sectional data examining family members of individuals with T1D compared to non-relative controls suggests that β cell abnormalities may be present in relatives (Table 1) [25,67–70]. These works describe abnormal insulin release, as well as dysglycemic responses to OGTTs [67–70].

4.1. Early work suggests increased insulin secretion in relatives

Several papers published in the 1970s alluded to this phenomenon. Interestingly, initial descriptions often described increased insulin stimulatory responses in relatives of individuals with T1D. In an analysis of 180 siblings of children with diabetes, 1/3 had increased glucose values on a 2hr OGTT based on "liberal criteria" (fasting glucose > 115 mg/dL, 1hr > 150 mg/dL, and 2 hr > 130 mg/dL) [67]. 55 of these children completed a repeat 4 hr-OGTT; the OGTT was normal in 26 (47%). Compared to nonrelative controls, both groups with normal and abnormal glycemia on repeat OGTTs exhibited differences in plasma insulin in response to glucose. Siblings with abnormal repeat OGTTs exhibited a delayed peak in insulin rise, while those with normoglycemic OGTTs exhibited pronounced hyperinsulinemia in response to glucose [67]. Along these lines, analysis of 11 monozygotic twins showed normal glucose but increased insulin responses to cortisol-primed OGTTs [71]. OGTT analyses of 66 siblings stratified by HLA similarity to probands identified an exaggerated insulin response only in siblings who were HLA-identical to probands [72]. Similarly, IVGTT and arginine stimulation analysis of 14 siblings with known HLA haplotypes suggested that, compared to controls, acute insulin response was exagerated in the 9 individuals who shared HLA haplotypes with affected siblings [73].

4.1.1. Reduced insulin secretion in relatives after adjustment for important confounders

Subsequent to initial work, describing increased insulin excursions in T1D relatives, other groups detected reduced insulin responses in cross sectional analyses of relatives compared to controls. Compared to matched nonrelative controls, IVGTT analysis of 150 FDRs (mean age of 15.8 years, 16/150 ICA+) identified severely reduced first phase insulin responses in 12 participants [69]. This rate was not impacted by ICA positivity and was similar to a 13% rate of reduced FPIR in FDRs of individuals with type 2 diabetes (T2D) (13%) [69]. IVGTT of 98 ICA-, insulin Ab- pediatric siblings of individuals with T1D showed a 25% reduction in insulin response compared to pediatric controls [70]. This reduction was predominantly present in siblings who were >8 years of age [70]. IVGTTs performed on a group of 90 adult FDRs (only 31% biochemical Ab+) revealed normal glucose tolerance, with higher fasting insulin values but reduced FPIR compared to controls [65]. Analysis of insulin response by quartiles did not reveal any differences in prevalence of Ab positivity among quartiles [65]. OGTT analysis of siblings and parents of individuals with T1D suggested that differences in insulin response may be related to the nature of the relationship to the proband with T1D [74]. Here, for siblings (n = 119), identical HLA status was associated with higher (although not frankly abnormal) glucose excursions and lower insulin responses, with no effect of ICA positivity on OGTT measures. By contrast, in parents (n = 128), ICA positivity was associated with increased glucose and increased insulin release on OGTT, with no effect of HLA status [74].

The differences between studies in observed increases vs. decreases in insulin secretion could be related to several factors, including varying metabolic testing techniques, genetic and demographic study group differences, control group matching, and importantly, differences in insulin sensitivity. IV glucose and arginine stimulation of 12 young adult HLA identical siblings (considered low risk based on >= 16 years having elapsed since diagnosis of T1D in the sibling) compared to matched controls suggested that siblings exhibited decreased insulin sensitivity [75]. Accordingly, all phases of insulin release were reduced only after adjustment for insulin sensitivity [75].

An analysis of 99 normoglycemic siblings of individuals with T1D from the Swedish Karolinska Institute examined secretory function using IV glucose infusions adjusted for insulin sensitivity (as measured with somatostatin-insulin-glucose infusions) [76]. Of these siblings, 97/99 were ICA- and insulin Ab- [76]. After adjustment for insulin sensitivity, insulin responses were significantly reduced among siblings compared to age-matched controls. Interestingly, when ICA- siblings were grouped with HLA-similar probands, although considerable variability existed, there were no differences between HLA groups in insulin responses to glucose infusions after insulin sensitivity adjustments [77]. Rather, age was the predominant factor linked to differences in insulin response [77]. Cross sectional OGTT analysis of a large number of nondiabetic FDRs (aged 2–75 years) suggested that, similar to reports in control populations, insulinogenic index increased with age through adolescence and decreased with age thereafter [78]. In this group, multiple Ab+ status (ICA, insulin Ab, GADA, and/or IA-2A) was associated with reduced insulinogenic indexes but not with changes in insulin sensitivity [78]. Reports from DPT-1 have also noted that, despite similar insulin sensitivity, Ab+ relatives that did not progress to T1D had reduced baseline beta cell glucose sensitivity (a measure that accounts for both C-peptide and glucose levels) compared to historical controls [46].

4.1.2. Studies suggesting similar insulin secretion between relatives and non-relative controls

Several studies have also pointed away from altered insulin secretion or insulin sensitivity in relatives of individuals with T1D at lower risk for diabetes progression. Subsequent work from the Joslin twin registry noted that among 15 nondiabetic ICA- twins, although substantial variability in insulin responses to IVGTTs was present, only one twin exhibited progressive decrease in insulin release over 17 years of follow-up, and none had insulin levels < the 1st percentile compared to the group's control population [24,25]. Comparison of IVGTTs from 15 Ab- nondiabetic monozygotic twins of individuals with T1D in the British Diabetic Twin Study to nonrelative controls with similar age and BMIs showed no differences in FPIR, HOMA-IR, fasting insulin, fasting glucose, or glucose clearance [31]. Hyperglycemic clamp and OGTT studies from the Belgian Diabetes Registry did not identify any differences in glucose or C-peptide measures among 10 multiple Ab+ FDRs that did not progress to T1D over a 94 month period compared to a group of nonrelative controls or amongst a group of Ab- FDRs or transiently Ab+ FDRs [20,21]

4.2. Other markers of beta cell dysfunction are abnormal in some relatives of individuals with T1D

Several older studies have detected PI/C ratio elevations in relatives of T1D probands [61,79–82]. When compared to age, sex, and BMI matched nonrelative controls, 10 ICA+ nondiabetic identical twins of individuals with longstanding T1D judged to be low risk for progression (based on long durations ranging from 11 to 23 years since the diagnosis of the twin proband) exhibited normal glycemic excursions on OGTT, but abnormally increased C-peptide responses, as well as abnormally increased fasting and stimulated serum proinsulin levels [79]. Interestingly, 6 ICA- identical twins (2/6 positive for insulin autoantibodies, mean of 17 months since diagnosis) exhibited normal glucose and insulin/C-peptide responses to OGTT but also displayed elevated fasting proinsulin levels [80].

Another analysis of fasting proinsulin levels performed in a cross-section of 85 FDRs suggested that, compared to age and weight-matched non-relative controls, although fasting glucose and insulin values were similar, fasting proinsulin levels were increased in parents, siblings, and children of individuals with T1D [82]. The 12 ICA+ individuals analyzed as part of this study had higher fasting proinsulin than ICA- relatives, but ICA- relatives also had elevated values compared to controls. No effect of insulin Ab positivity or HLA similarity to proband was detected [82]. The Karolinska Institute analysis of 99 predominately ICA- and Insulin Ab- normoglycemic siblings of T1D probands described above also identified increases in fasting proinsulin/insulin ratios compared to nonrelative age-matched controls [76]. HLA status had no effect on ratios [76]. Similar to fasting values, stimulated proinsulin levels in response to glucose infusions were increased in siblings, even after adjustment for insulin sensitivity [81]. By contrast, similar to reports of clamp derived measures, a Belgian Diabetes Registry report noted that random ratios of Ab+ nonprogressor FDRs and Ab- FDRs were similar to values from a group of 22 nonrelative controls [62].

Finally, although not an analysis of beta cell function per se, a recent cross-sectional study described reduced pancreas volumes on MRI in Ab- FDRs compared to nonrelative controls [83]. Volumes were progressively further decreased in Ab+ nondiabetic relatives and in individuals with recent onset T1D [83]. These findings raise the possibility that inherited differences in whole pancreas, including islet, function and/or mass may exist in families with T1D.

5. IMPLICATIONS OF DATA SUGGESTING BETA CELL DYSFUNCTION EXISTS IN SOME RELATIVES WHO DO NOT PROGRESS TO T1D

There are multiple possible interpretations of these data suggesting longstanding beta cell dysfunction in progressors, as well as in Ab+ nonprogressors and Ab- relatives. The observed abnormalities in Ab- FDRs or Ab+ nonprogressors could reflect early signs of an ultimate progression to clinical diabetes over the course of the individual's lifetime that has not become manifest by the end of the period of study observation. Because longitudinal metabolic analyses are not typically performed until after the development of Abs, it is impossible to know the true "baseline" metabolic status of these individuals. In Ab- relatives, abnormal beta cell function could reflect very early ongoing autoimmunity undetected by current Ab measurements or sequelae of a prior autoimmune attack that subsequently resolved. Alternatively, some individuals at risk for T1D may also inherit abnormalities in beta cell function. The presence of this beta cell dysfunction in "nonprogressors" and Ab- FDRs could reflect that these beta cell abnormalities do not increase risk, since most of these relatives will not ultimately get diabetes. Alternatively, beta cell dysfunction may be associated with increased risk, but not sufficient for T1D development. In this way, T1D may develop due to "multiple hits", including inherited predisposition to autoimmunity, environmental exposures that activate autoimmunity, and defects in beta cell mass, function, turnover, or survival.

Although differences in HLA genotype account for the largest known contributor to T1D risk, candidate gene and genome-wide association studies have also identified other genetic variants that have smaller effects [84]. Although these variants are classically linked to differences in immune function and tolerance, at least half are also expressed in islets [85]. Many of these genes regulate proinflammatory and apoptotic pathways in the beta cell, and undergo alternative splicing in the islet, which is substantially impacted by treatment with inflammatory cytokines [85–87]. Inherited differences in beta cell gene expression may interact with the immune system to trigger or exacerbate the autoimmune response. As another example, among subgroups of individuals with T1D, polymorphisms in the *transcription factor 7-like 2 (TCFL2)* gene, which are associated with beta cell dysfunction in Type 2 diabetes, have been linked to increased risk of progression despite a milder immunologic phenotype than that of other progressors [88,89]. This phenotypic difference may reflect to a lower threshold for progression due to inherited beta cell dysfunction.

The body of work comparing nondiabetic relatives of individuals with T1D to nonrelative controls, while intriguing, has some caveats which make interpretation of the work as a whole challenging. Because older studies were performed before newer pancreatic autoAb assays became available, characterization of islet autoimmunity was more limited, and thus, likely underestimated. Nonetheless, beta cell dysfunction was identified at a higher prevalence than would be expected for Ab+ positive status in relatives [90]. Older proinsulin and insulin assays often exhibited significant cross-reactivity, complicating interpretation of circulating proinsulin and insulin values. Additionally, these data were often obtained only cross-sectionally, rather than longitudinally. Longitudinal analyses with multiple time-points would be preferable to exclude individuals who went on to develop diabetes, especially given the now appreciated relapsing-partially remitting nature of T1D onset [91].

Another important limitation in interpretation of this body of work is differences in study designs. Studies have utilized multiple different analyses for beta cell response, which could have led to differing confounding variables, such as the incretin response or differences in glucose absorption during OGTTs. Definitions of abnormal results differed, even when using the same testing modalities. Studies also enrolled different age groups of participants. Given the well-recognized effects of age on beta cell function, insulin sensitivity and diabetes risk, differences between beta cell function in study populations could be related to age differences among groups. Appropriately-matched controls for age and body habitus are not always documented and could also be a contributing factor to differing study results. Lastly, given the heterogenous nature of T1D [91], inherited beta cell dysfunction may only be present in subpopulations of at-risk individuals, and so may not be reflected in small sample sizes or comparison of summary statistics between larger groups.

6. CONCLUSIONS

Notwithstanding these limitations, these important data suggest that contributions of beta cell dysfunction to the pathology of developing T1D need to be further explored as we work toward prevention and cure of this disease. Continued identification of molecular signaling pathways leading to beta cell stress, dysfunction, and death associated with genetic variants linked to increased T1D risk will be key to providing mechanistic insights into these relationships. Robust analyses of beta cell function and stress in Ab- relatives tested with newer Ab assays, as well as individuals harboring high risk polymorphisms potentially contributing to beta cell dysfunction or T2D susceptibility genes will be important to better understand *in vivo* contributions of beta cell dysfunction to T1D development. Identification of the optimal tools or biomarkers to identify beta cell dysfunction in this context will maximize applicability of studies moving forward. Additionally, identification of markers differentiating reversible (ie treatable) vs irreversible beta cell dysfunction will be needed to determine which individuals may benefit from therapies targeting beta cell health. Lastly, studies evaluating impacts of beta-cell targeted therapies will be crucial to determine if these types of reagents may improve outcomes of immunomodulatory therapies in individuals exhibiting indicators of potentially reversible beta cell stress/dysfunction. Ultimately, these efforts may lead to more of a precision-medicine type approach to diabetes prevention and treatment, in which agents targeting beta cell health are combined with immunomodulatory therapies to improve efficacy in groups displaying high levels of reversible beta cell dysfunction.

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CONFLICTS OF INTEREST

The authors have no competing interests to declare.

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