# Revisiting the pattern of loss of β-cell function in preclinical Type 1 Diabetes.

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**Abstract**

Type 1 diabetes (T1D) results from beta cell destruction due to autoimmunity. It has been proposed that beta cell loss is relatively quiescent in the early years after seroconversion to islet antibody positivity (stage 1) with accelerated beta cell loss only developing around 6-18 months prior to clinical diagnosis. This construct implies that immunointervention in this early stage will be of little benefit since there is little disease activity to modulate. Here we argue that the apparent lack of progression in early stage disease may be an artefact of the modality of assessment used. When substantial β-cell function remains, the standard assessment - the oral glucose tolerance test - represents a submaximal stimulus and underestimates the residual function. By contrast, around the time of diagnosis, glucotoxicity exerts a deleterious effect on insulin secretion giving the impression of disease acceleration. Once glucotoxicity is relieved by insulin therapy, β-cell function partially recovers ("the honeymoon effect"). However, evidence from recent trials suggests that glucose control has little effect on the underlying disease process. We therefore hypothesise that the autoimmune destruction of β-cells actually progresses at a more or less constant rate through all phases of T1D and that early stage immunointervention will be both beneficial and desirable.

ARTICLE HIGHLIGHTS

- The autoimmune process in T1D begins long before the onset of clinical symptoms
- Most studies in preclinical T1D have used an OGTT and concluded that beta cell loss occurs late; however the OGTT underestimates beta cell function in the earliest preclinical stage (stage 1)
- Loss of beta cell function is said to accelerate around the time of clinical diagnosis, but this is likely attributable to glucotoxicity rather than an acceleration of the autoimmune process
- We conclude that autoimmune mediated beta cell loss is likely progressive throughout the preclinical period and immunointervention efforts should focus earlier in the process

## Introduction

Current estimates of C-peptide secretion after stimulation in the oral glucose tolerance test (OGTT) in type 1 diabetes (T1D) suggest an impairment in β-cell function is already present 6 years before diagnosis but remains largely stable until a rapid fall that occurs 6-18 months before clinical diagnosis (1-2).

Some weeks after starting insulin therapy there is usually a recovery in function signalled by a drop in insulin requirement (referred to as the 'honeymoon' or a remission phase) but this period is transient, and in a few months the decline resumes again (3).

The recent approval of teplizumab in the USA in November 2022 to slow the autoimmune disease process and delay the onset of clinical disease in T1D, opens up the possibility of using more drugs or drug combinations early in the disease process to delay the onset of disease further (4).

Teplizumab is only licensed for use in stage 2 T1D, a late preclinical phase in which in addition to autoimmunity as evidenced by two or more islet autoantibodies, there is also dysglycaemia (similar to impaired glucose tolerance in type 2 diabetes) (5). Intervening earlier in the disease process – namely stage 1 (multiple autoantibody positive but normoglycaemic) – has the potential to preserve more β-cell function as well as allowing time for other interventions to be added before clinical diagnosis. Additional interventions could include switching non-responders to alternative immunointerventions or escalation to combinations of therapies. However, the current paradigm as described above suggesting that little progressive β-cell loss occurs in stage 1, argues against this approach.

Here we aim to revisit this paradigm, which has largely been developed using the oral glucose tolerance test (OGTT) as the challenge test for assessing β-cell function. However, the OGTT was designed for metabolic staging rather than to evaluate β-cell function. Identifying more precisely the changes in β-cell function at the time of autoantibody development (seroconversion) and the subsequent early phases of preclinical T1D will allow us to better understand when to intervene with new therapies aimed at preserving β-cell mass and how to monitor them. This quest for improved

understanding has also led us to revisit the origins of the "honeymoon" phase, its dependence on current delays in the clinical diagnosis of T1D resulting in severe hyperglycaemia, and how this might change once widespread screening and intervention for early-stage disease is introduced.

## 1. The loss of β- cell function after the onset of clinical disease is relatively linear

Multiple studies suggest that in the first few years after clinical disease onset (stage 3), the loss of C-peptide over time is essentially linear (2, 6). Age modifies this trend: younger subjects have lower random serum C-peptide concentrations at diagnosis and progress faster to absolute insulin deficiency (7, 8).

For years it has been controversial whether an intense insulin regimen could slow or prevent this decline. Buckingham et al. in 2013 showed that 72 hours of hybrid closed loop (HCLC) followed by sensor augmented pump therapy (SAP) did not provide benefit in preserving β-cell function compared with standard of care (9). More recently two early intervention hybrid closed loop studies (CLOuD (10) and CLVer (11)) demonstrated how also a longer and more intensive insulin regimen can lead to glycemic improvement but do not impact on the preservation of residual C-peptide. Hence, the transient increase in C-peptide seen in the first few months after diagnosis in studies where monitoring began early (10, 11), seems likely related to the correction of glucotoxicity reversing transient impairment of β-cell function at this time rather than a true increase in β-cell mass or reversal of the autoimmune process.

## 2. The loss of β-cell function prior to disease onset appears not to be linear

Using cohorts of unaffected relatives has provided information on how β-cell dysfunction precedes diabetes diagnosis by more than 5 years in most subjects with positive autoantibodies (1).

This impairment can be split into three different phases that finally lead to overt disease (1). The first phase is characterized by a reduction of C-peptide secretion compared to autoantibody negative subjects (although glucose levels are similar), 5 or more years before disease onset. It remains

unclear whether this is due to the disease itself, or it predates the onset of the disease process and represents a "risk" factor pre-determined by genetic and/or environmental factors (1).

The second phase, from the start of observations up to 1-2 years before diagnosis, is characterized by a pattern of relative stability of C-peptide as determined in the oral glucose tolerance test, or in some studies a paradoxical increase in the area under the curve (AUC) C-peptide released after the glucose stimulus. The late increase in C-peptide levels may represent a response to compensate for rising glucose levels (1, 12) (Figure 1) – see further discussion later in this review. Finally, there is a third phase, characterized by a large glycemic increase and a decline in C-peptide secretion (corresponding to late stage 2), most evident in the 6 months before diagnosis, and that culminates in the clinical onset (1) (Figure 1). This has previously been interpreted as representing an acceleration in the disease process occurring just prior to diagnosis (13).

However, alternative measures of β- cell function appear to reflect a different picture. Findings from the DIPP study, which followed Finnish children with high-risk HLA showed an early reduction in first-phase insulin responses (FPIR) after seroconversion to autoantibody positivity (14). Intravenous GTTs (IVGTTs) performed at the time of seroconversion in children under 5 years did indeed show that 42% already had a first-phase insulin responses (FPIR) below the fifth percentile as described in the current paradigm. The FPIR inversely correlated with the number of autoantibodies (14). However, following this there was a progressive decline in FPIR and a rise in 60 minute glucose after the IVGTT beginning around 4-6 years prior to diagnosis (Figure 2) (15). Similarly, analysis of FPIR responses in the DPT-1 cohort showed a significant decline of FPIR between 4.4 and 2.5 years prior to diagnosis with an apparent acceleration between 1.5 and 0.5 years prior to diagnosis (16).

The temporal relationship between blood glucose alterations and islet autoantibody seroconversion further fuels the discussion on the pivotal role of the beta cell damage in the initiation of the disease, reducing the role of autoantibodies and seeing their onset more as an epiphenomenon.

**How could the failure to demonstrate progressive β-cell failure in early stage 1 be explained? – the limitations of the OGTT**

Historically the majority of studies have used the OGTT to stage T1D before diagnosis (17) and the Mixed Meal Tolerance Test (MMTT) to test for C-peptide secretion after diagnosis to exploit the secretagogue stimulus of a complete meal (18) with less glucose load. The reported metric is the area under the curve of C-peptide (AUC C-peptide) across a 2 - 4 hour period following the oral challenge. The AUC C-peptide is reported to show high correlation between MMTT and Oral Glucose Tolerance Test (OGTT) after diagnosis (2), but this has not been tested in early stage T1D. Note that the MMTT contains amino-acids which represent a stimulus for C-peptide secretion independent of the glucose rise.

Although the OGTT following 1.75 g/kg (75 g maximum) oral glucose administration (17) is the standard of care to stage the disease (19) it was not developed to detect β- cell functionality, especially in early stage 1. In this preclinical period, there are multiple reasons why the C-peptide AUC in the OGTT may misrepresent the actual beta cell function, underrepresenting it in the early preclinical period and overrepresenting it the late preclinical period.

Firstly, AUC C-peptide in the OGTT does not separate the first and second phases of C-peptide secretion following stimulation. Specifically, time to peak C-peptide >60 min in the OGTT appears to indicate loss of the FPIR and a change to a monophasic instead of a physiological biphasic insulin secretion curve – a pattern that is more frequent in progressors than nonprogressors (20). However, the delayed peak is followed by an initial compensatory second phase and relatively preserved overall AUC C-peptide across the 2 hours. Indeed, in the DPT-1 cohort where both IVGTTs and OGTTs were performed, it was observed that although the total AUC C-peptide in sequential OGTTs remained stable for some time, the time to peak c-peptide response became progressively delayed starting 2 years or more before diagnosis (12,13). This is similar to what is observed in the prediabetic phase of type 2 diabetes (21) . It represents an adaptive period of pancreatic β-cell function with relative preservation of glycemia, at least until β-cell function declines further.

Secondly, AUC C-peptide reported alone does not take into consideration either the timing or the overall magnitude of the glycemic rise that occurs during the test (Figure 1). Glucose levels are both

the stimulus to insulin secretion and the end point of the action of the released insulin. With relatively good β-cell reserve, as is the case in stage 1 and to a lesser extent stage 2 pre-diabetes, the rise in glucose is small and therefore the OGTT represents a submaximal stimulus. By contrast, in late stage 2 (dysglycemic stage) and stage 3 (clinical diagnosis), there is a substantial glucose rise in the absence of compensatory insulin dosing, with no FPIR. This represents a maximal or near maximal stimulus to the β- cells, resulting in comparability between the OGTT and MMTT AUC C-peptide results (2). Note that this attenuation of the stimulus by good β-cell function in early stage T1D is less of a confounder in the IVGTT where the glucose rise is more rapid and the sampling time for C-peptide is limited to the first 10 minutes.

An improved estimate of β-cell function in pre-clinical disease can be obtained by taking the level of stimulus – the glucose excursion – into account in evaluating the C-peptide secretion. Different approaches have been used to make this adjustment. The Diabetes Prevention Trial-Type 1 Risk Score (DPTRS), DPTRS60, Index60 and M120, and C peptide index include the change in glucose with the change in C-peptide using different formulae, in some cases taking into consideration intermediate timepoints. These have been shown to be superior to using only standard dysglycemic criteria or AUC C-peptide to evaluate risk of progression (22). It is noteworthy that DPTRS60 (using only 60 minute values) is as predictive as DPTRS (using 120 minute values) and this may be because it also indirectly reflects time to peak C-peptide, itself reflective of the loss of FPIR (see above) (23). The comparison of these different indices in pre-diabetes has recently been systematically explored by Baidal et al using data from the DPT-1 (oral insulin) and TN07 (oral insulin in insulin antibody positive subjects) studies (22, Table 1). Although AUC C-peptide over 2 hours correlated with all the indices, it correlated less well with FPIR and was substantially less predictive of progression to clinical diabetes (AUC in ROC curve, 0.538 – 0.611, Table 1) than those indices which took the glucose rise in the OGTT into account. C-peptide index, evaluating the change in C-peptide and glucose from 0 to 30 minutes, correlated best with FPIR while Index60 and C-peptide index had the highest predictive accuracy for T1D and were comparable (22). Consistent with this, Ismail et al. recently demonstrated

in two different cohorts (DPT-1 and TrialNet Pathway to Prevention Study, TNPTP) an inflection point in the Glucose C peptide Response Curves (GCRC) 1.5 years prior to clinical symptoms, with a decline in C-peptide in the face of rising glucose (13).

Thirdly, OGTT measured AUC C-peptide (and many of the derived indices) does not include adjustment for insulin sensitivity, which varies between individuals and within individuals over time and modifies the relationship between glucose and insulin. Measures of insulin sensitivity generally require measurement of insulin as well as C-peptide which is not always recorded. Ideally, the interaction between glucose and insulin should take into account insulin secretion as well as insulin action. This can be quantified by estimating the Disposition Index (DI) (24). The DI evaluates the ability of the β-cell to compensate for transient or persistent insulin resistance. DI is traditionally calculated by measuring each component using intravenous glucose clamps. An oral disposition index for the first 30 minutes of the OGTT was described by Utzschneider et al in pre-clinical type 2 diabetes (25). This correlated less well with FPIR than other indices in the study of Baidal et al but its ability to predict progression in T1D was not reported. Recently it has been shown that a 2-compartment mathematical model using C-peptide and glucose to estimate insulin secretion, and insulin levels and glucose to estimate insulin sensitivity can quantify DI using data from an OGTT with an accuracy approaching that of clamp techniques. One version of this is the Oral Minimal Model (OMM), a mathematical model widely applied as a sensitivity-adjusted measure of insulin secretion (26). This calculation only applies in the absence of exogenous insulin – i.e. it is only relevant to the preclinical phases of T1D. Several studies in type 2 diabetes in the absence of insulin therapy have demonstrated that lower DI is a strong predictor of future diabetes (27), and genetic studies have identified predictive variants related to DI (28).

Although the role of insulin resistance has been considered to be relevant mostly in type 2 diabetes, it represents also an important component of T1D (29). The assessment of β-cell responsiveness and insulin resistance evaluated through a mathematical model from OGTT data in the DPT-1 cohort demonstrated a lower ß-cell glucose responsiveness at baseline in autoantibody positive relatives who

progressed to clinical diabetes, but the control group was clinically similar (30) and ß-cell glucose sensitivity and insulin sensitivity may have been less than normal even in the nonprogressors of DPT-1 at the time of staging. In a different cohort, a lower DI due both to a reduced insulin sensitivity and a reduced β-cell secretion has been described in stage 1 when compared to their healthy peers (31). These results confirm the hypothesis that insulin resistance is present in T1D and becomes important to evaluate when quantifying the sufficiency of β-cell function. The physiologic changes of insulin sensitivity through the pediatric ages may represent an additional confounder while evaluating the trajectory of insulin secretion in disease progressors – as well as during intervention trials – that involve pediatric population. Glucose-stimulated insulin secretion in fact is normally increased during puberty, a response that may compensate for puberty-induced defects in insulin sensitivity (32).

Performing clamps in a pediatric population is very challenging and DI calculated from the OMM allows for an accurate assessment of β-cell function on OGTT derived data. Recently a 2h-7 point OGTT (instead of the classical 9 points used in a research setting) was validated in a paediatric cohort to evaluate ß cell function through DI (33). This should increase the compliance in young children and the incorporation of the analysis of DI will permit to more accurately quantify risk of progression (33). The major limitation of the OMM is the need for qualified personnel for the analysis and the requirement of multiple samples during the test: 7 points (the dynamic component relies on early sampling) glucose, C peptide and insulin.

Fourthly, AUC C-peptide in the OGTT does not take into account the negative effects on beta cell function of chronic hyperglycaemia ("glucotoxicity") that occur in the later phases (stage 2 and early stage 3). This is discussed in more details in the review of the "honeymoon" effect below.

However it is noted that recently, continuous glucose monitoring (CGM) has been used to stage children in pre-clinical T1D. Although neither insulin secretion nor insulin resistance is measured, the variability in CGM especially in the post-prandial period may represent an indirect reflection of both with the advantage of being less invasive. Consistent with this, it has been reported that spending ≥5% of time ≥140 mg/dL (7.8 mmol/L) is associated with an increased risk of progression

to T1D in the following 2 years, reaching around 40% (34). Furthermore, it has been reported that small rises (0.2-0.3 mmol/l) in glucose can be detected even prior to seroconversion in large population studies (34-36). However, the reliability of CGM values in predicting progression as compared to OGTT indices has recently been challenged (22, 37), and we await further studies in larger cohorts at different stages of pre-T1D to evaluate the contribution of CGM to measuring disease progression and to define more precisely which metrics are the most sensitive.

Finally, (in contrast to the MMTT) the OGTT only measures the beta cell response to glucose, not to amino-acids, further underestimating beta cell function on a protein containing diet. In fact the β-cell response to arginine is preserved after it is lost to glucose. The MMTT peak C-peptide is highly associated with the acute C-peptide response to glucose potentiated arginine test (GPA) in established T1D (38).

One argument against substantial disease progression in early stage T1D, aside from the results of metabolic assessments, has been the report of low levels of insulitis in early stage disease (39). However, insulitis is certainly present in cases with multiple antibodies (40) but its quantification is challenging and recent 3d imaging suggests the degree of insulitis may be underestimated in 2d tissue sections (41). Unfortunately, reliable methods to measure the activity of the autoimmune process with a high degree of accuracy using blood samples do not exist currently. It has been suggested that the immune response may occur in waves and with some evidence of increased proinflammatory activity in individuals who progress to stage 3 diabetes (42) but more discriminatory techniques are required.

### 3. The loss of β-cell function around the time of clinical diagnosis: pre-diagnosis acceleration and "the honeymoon" period.

At the time of clinical diagnosis there is a breakdown of residual functionality, sometimes combined with an infectious/inflammatory trigger that further increases the glycemic rise.

The combination of insulin deficiency and high counterregulatory hormone concentrations increases glycogenolysis, gluconeogenesis, lipolysis and ketogenesis, which result in hyperglycemia, ketonemia and metabolic acidosis. Hyperglycemia exceeding the usual renal threshold of approximately 10 mmol/L (180 mg/dl) together with hyperketonemia cause osmotic diuresis leading to dehydration, often aggravated by vomiting associated with severe ketosis (43). From 15% to 70% of children in Europe and North America experience Diabetic Ketoacidosis at presentation (43). This is a life-threating condition and the resolution depends on exogenous insulin therapy together with fluid and electrolyte administration (43).

The "honeymoon period" or remission phase is a spontaneous and transient period of recovery of β-cell function that patients with new onset T1D usually experience a few weeks after insulin therapy has been started (44). It is characterized by a reduction (sometimes even withdrawal) of the exogenous insulin requirement associated with good metabolic control and can last from a few months to a year post-diagnosis (45).

The incidence of the honeymoon phase varies from 35 to 80% between studies with the highest rate reported in Sweden (45, 46). The variation in the rate partly reflects the use of different definitions for remission and the ages of the patients included in the studies. In the past authors identified this phase by a Total Daily Insulin (TDI) ≤0.5 UI/Kg but a more recent and complete definition considers insulin dose–adjusted HbA1c (IDAA1C ≤9 indicates a remission phase) (47).

The underlying mechanisms remain still unclear (see below), but the correction of hyperglycemia represents a key factor. A younger age, DKA or recent infection prior presentation, female sex, adolescence, absence of HLA DR3 e DR4 are poor prognostic factors for remission (48, 49). Initial HbA1c was negatively associated with remission phase occurrence and length (45).

## 4. The role of glucotoxicity in the honeymoon effect

The honeymoon period appears to reflect a temporary slowing or even reversal of β-cell loss. We have already noted that an apparent acceleration of β-cell loss starts 1 year before diagnosis and is

more evident in the last 6 months. This raises the question of whether this is a true acceleration in the underlying disease process (i.e. autoimmunity) or rather the apparent rapid decline of β-cell function is the result of the metabolic decompensation that occurs at this time (50).

It is known that hyperglycemia exerts a deleterious effect on insulin secretion and sensitivity which is reversible when normoglycemia is restored. Normalization of the plasma glucose profile by phlorizin treatment in diabetic rats completely corrected β-cell abnormalities (51, 52). Glucotoxicity leads to progressive but still reversible changes in β-cell gene expression (53) that in turn lead to dysfunction in insulin secretion (54). In addition, the DiViD study revealed with pancreatic biopsies from recently diagnosed subjects that a restored biphasic insulin release was obtained from isolated islet cells after some days in a nondiabetogenic environment in vitro (55). The suggestion that any effect of glucotoxicity around the time of diagnosis is reversible and does not result in long-term β-cell compromise, is consistent with the finding as discussed above that intensive glucose management after diagnosis (with closed loop therapy) has no long-term advantage in terms of β-cell function at 1 year (9-11).

Consistent with this, restoration to essentially normal FPIR can be seen in people with T2D who have a full remission after bariatric surgery (56) or with effective glucose lowering treatments (57). In addition, being diagnosed early prior to major glucotoxicity appears to reduce the remission/honeymoon phase. Children followed to clinical diagnosis from pre-diabetes in the TEDDY study had a mean HbA1c of 6.8% at diagnosis compared to 10.5% in matched community controls. Honeymoon rates were not reported, but a fall in HbA1c and IDAA1c between 3 and 6 months was only seen in the community cohort (6) (Figure 3).

Note that it is possible than severe or prolonged exposures to high glucose levels and or ketoacidosis results in a degree of long lasting β-cell impairment (58), as suggested by improved HbA1c up to 10 years later in individuals diagnosis without DKA as opposed to with DKA (59).

## 6. What is the real picture?

As discussed above despite working well when dysglycemia is evident (stage 3), the static and dynamic tests used so far appear not to return a complete view of the trajectory of β-cell function and evaluate AUC C-peptide in the OGTT is misleading, inadequately quantifying beta cell function in early stage T1D (Table 2).

The data on FPIR appear to show a gradual decline in β-cell function starting in stage 1 and progressing into stage 2 (Figure 2) in contrast to the steady rise in AUC C-peptide, and the description of C-peptide stability from the fitted mixed model prior to diagnosis (Figure 4). Indeed, data from FPIR and GCRC data suggests an inflection and decline at – 1.5 years, earlier than the OGTT/MMTT data.

We propose that once the disease has started, the decline in general is continuous and occurs at a similar rate across all three metabolic phases of T1D. Towards the end of stage 1 when a substantial number of β-cells are no longer functional and/or have been lost, the glucose levels begin to rise. This increases the static and dynamic stimulus to the β-cells. Conceivably this may also increase the level of β-cell stress leading to greater self-antigen presentation and autoimmune activity, but this has yet to be demonstrated (41). As the glucose levels rise further functional impairment via glucotoxicity begins resulting in a self-perpetuating cycle of falling insulin secretion and further increases in glucose. The result is an apparent accelerated decline in β-cell function at the time of entry into stage 3 and prior to beginning insulin therapy when glucose levels are at their highest, without necessarily any acceleration in the autoimmune process (Figure 5). Importantly, this decline is reversible as the β-cells are impaired but not lost. Once insulin therapy is started and glucose levels fall, there is substantial recovery of functionality due to the relief of glucotoxicity. This underlies the honeymoon phenomenon. In reality, the disease process has continued at a steady pace throughout despite the glucotoxic process giving the appearance of relapse and subsequent remission. We await further data from more integrative approaches to measuring β-cell function, such as the oral minimal model enabling calculation of the disposition index, to provide further clarification on this (26).

## 7. Why is this important?

The diagnosis and the management of early stage T1D is undergoing a major change. Following the recent licensing of teplizumab for stage 2 in November 2022 screening programs based on autoantibody detection amongst relatives as well as the general population are expected to expand and indeed in Sept 2023 Italy passed a law offering universal screening for T1D and coeliac disease (60, 61). Teplizumab's regulatory approval has opened a new era and it is now expected that other immunotherapies will become available: at least nine therapies have already shown some efficacy in beta cell preservation in clinical trials (62, 63). The majority of screened individuals will be in stage 1, and there will be an increasing opportunity to intervene at this stage rather than waiting until stage 2. Note that it is possible that different immune mechanisms predominate at different stages of the disease and hence that drugs that are effective in stage 2 (e.g. teplizumab) may not be effective in stage 1. Currently this can only be addressed by well designed and well powered trials in early stage disease.

Until recently, there has been a widespread view – fuelled in part by the data form AUC C-peptide measurements - that there is little disease activity until the onset of stage 2 and hence immune interventions in stage 1 are likely to serve little purpose. By contrast, if the disease process is continuous across all stages as we propose (Figure 5), then intervention in stage 1 offers the opportunity of saving the greatest amount of β-cell function buying time to switch to alternative therapies in non-responders and delaying/reducing downstream complications and hypoglycaemic events. Using a drug at such an early stage requires us to be able to precisely predict the progression of the disease in that individual and OGTT derived metrics such as Index-60, Disposition Index and CGM (but not AUC C-peptide alone) could be useful tools.

In addition, we anticipate that as a result of screening, the diagnosis of stage 3 will occur as soon as glucose levels begin to rise rather than several months later, reducing DKA rates, which currently remain high. The earlier introduction of insulin will obviate major glucotoxicity which in turn will, as a "side effect", result in the disappearance of the honeymoon phase as illustrated in Figure 6.

Regarding parental anxiety related to islet autoantibody positivity, the Fr1da study showed how maternal distress exists but is low or moderate and dissipates over time. Importantly, parental distress at diagnosis was lower than that reported from parents of children diagnosed before introduction of the screening program (64).

In closing, there is an urgent need to develop more accurate measures of β-cell function that integrate glucose levels, insulin secretion and insulin sensitivity, such as the disposition index, if possible without requiring more invasive assessment. These measures will allow us to confirm whether the disease is indeed steadily progressive across all disease stages and permit the development of early stage (stage 1) interventions that can delay the onset of the need for insulin longer.

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| | TN-07 | | DPT-1 | |
|---|---|---|---|---|
| | AUC | (95% CI) | AUC | (95% CI) |
| FPIR | 0.707 | (0.602-0.812) | 0.628 | (0.537-0.719) |
| C-Peptide AUC | 0.611 | (0.479-0.742) | 0.538 | (0.432-0.643) |
| C-Peptide Index | 0.717 | (0.621-0.813) | 0.721 | (0.638-0.803) |
| C-Peptide 30-0 min | 0.648 | (0.546-0.751) | 0.626 | (0.532-0.721) |
| Index60 | 0.778 | (0.698-0.858) | 0.763 | (0.677-0.848) |

Table 1: *Prognostic accuracy of FPIR- and OGTT-derived measures for T1D development*

AUC and 95% CIs for 2-y ROC curves for FPIR and OGTT derived measures across two different studies (TN-7 and DPT-1). AUC C-Peptide has the lowest ROC curve AUC (approaching 0.5 in DPT-1); FPIR/change in C-peptide over the first 30 mins and OGTT derived variables that take glucose into account perform better.

Adapted from (22).

| Factor not taken into account | Impact | Alternative |
|---|---|---|
| Failure to distinguish first and second phase insulin secretion | Underestimate loss of beta cell function/ability to control blood glucose as first phase declines and insulin secretion is delayed into the second phase | Use IVGTT to measure FPIR or use OGTT metrics that reflect the balance of first and second phases (e.g. C-peptide index in first 30 mins, timing of peak C-peptide or Oral Minimal Model) |
| Glucose change during the test | Does not account for a lesser glucose stimulus due to less glucose rise, underestimating beta cell function in early stage T1D | Composite indices including glucose change in first 60 mins (e.g. Index 60, DPTRS 60, C-peptide index, Disposition index) |
| Insulin sensitivity | Insulin sensitivity affects relationship between glucose and insulin/C-peptide and may change over time in the same individual (e.g. puberty) | Use of modelled indices including insulin measurements to estimate insulin sensitivity |
| Glucotoxicity | Rate of disease progression and loss of beta cell function may overestimated in stage 2 | Take care with interpretation of C-peptide measures in late stage 2/stage 3 (prior to insulin correction of hyperglycaemia) |
| Insulin secretory effect of non-glucose components in diet (e.g. amino-acids) | Ability of beta cell function to control blood sugars in normal diet underestimated | Consider measurements such as HbA1c and CGM that reflect glucose control outside the OGTT |

Table 2: *Reasons why AUC C-peptide in the OGTT inadequately quantifies beta cell function in early stage T1D*

Figure 1*: Longitudinal patterns of metabolic decline in Progressors ≥5 years from seroconversion to diagnosis*

Shown are the mean values for the fasting C-peptide (A), early C-peptide response (B), C-peptide AUC (C), fasting glucose (D), 2-hour glucose (E), glucose AUC, (F) and Index60 (G) at baseline and then each year. Red dotted line shows mean values of the aAb– individuals at baseline.

From (1) with permission.

Figure 2 (A-B): *FPIR is decreased several years before the diagnosis*

Figure 2A shows the decline in FPIR in progressors (continuous line) compared with non-progressors (dotted line) during an intravenous glucose tolerance test (IVGTT). Figure 2B shows the 60 minute glucose levels in progressors (continuous line) compared with non-progressors (dotted line) during IVGTT . Point 0 indicates the time of the diagnosis or the last IVGTT.



Figure 3: *No 3 month-HbA1c reduction in cases from TEDDY study, diagnosed with a lower HbA1c at the beginning*

Hemoglobin A1c (HbA1c) in The Environmental Determinants of Diabetes in the Young cases and community controls during the first year follow up after diagnosis of diabetes. Box plots with minimum, first quartile, median, third quartile, and maximum values. The line in the box plots indicates the median value, while the mean is denoted by " ◯" for TEDDY cases and "+" for community cases.

In red the pattern of HbA1c in TEDDY cases and in blu in community ones.



Figure 4: *C-peptide before and after type 1 diabetes diagnosis*

The mean rate of C-peptide decline before and after type 1 diabetes diagnosis from a fitted mixed model in which age is included as a continuous variable.



Figure 5: *The disappearance of the honeymoon*

In panel A how glycemic profile will change with screening programs and early detection of the disease (dotted line): the sustained glycemic rise, the glucotoxicity and the honeymoon (continuous line) will disappear. In the green area normal glycemic range. In panel B how AUC C-peptide will change in consequence of the absence of a sustained glycemic elevation with early diagnosis (dotted line) compared with late diagnosis (continuous line).

Figure 6: *Paradigm shift*

Adding an immunopreventive/ß-cell preserving therapy (alone or combined) to the early detection of the disease, the beta cell function will be preserved for a longer time.

## Figures

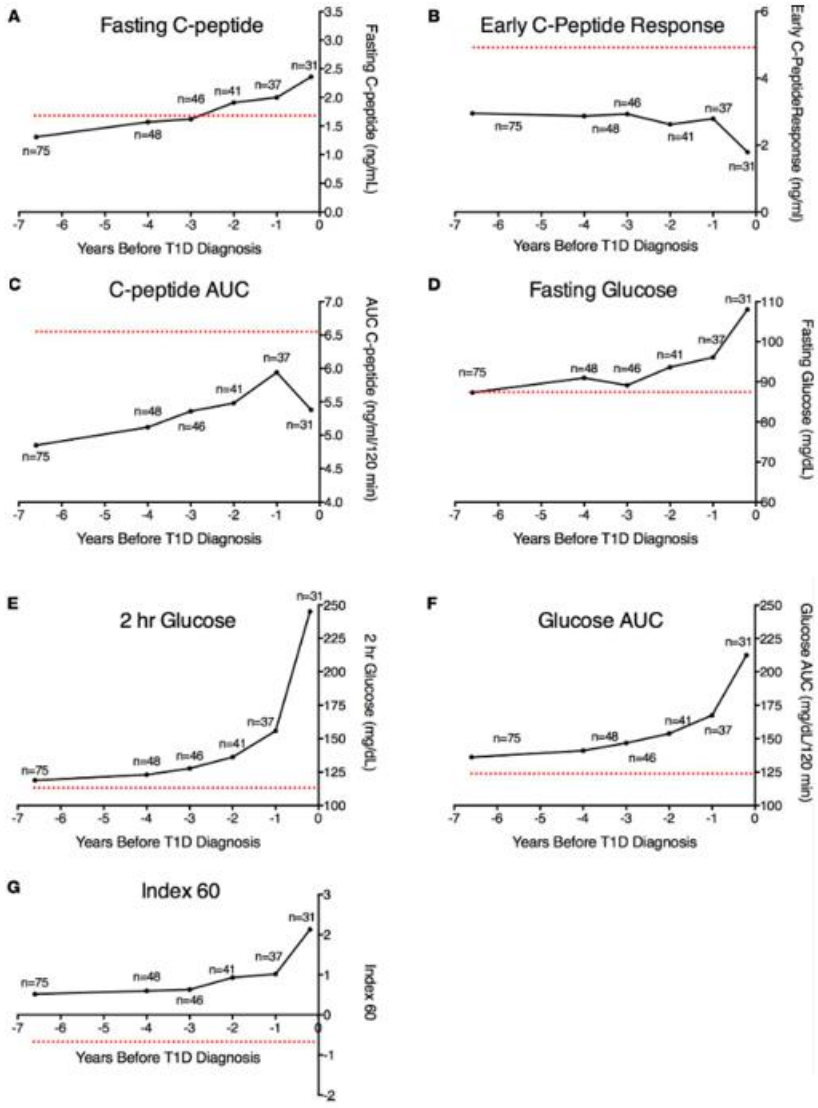


Figure 1

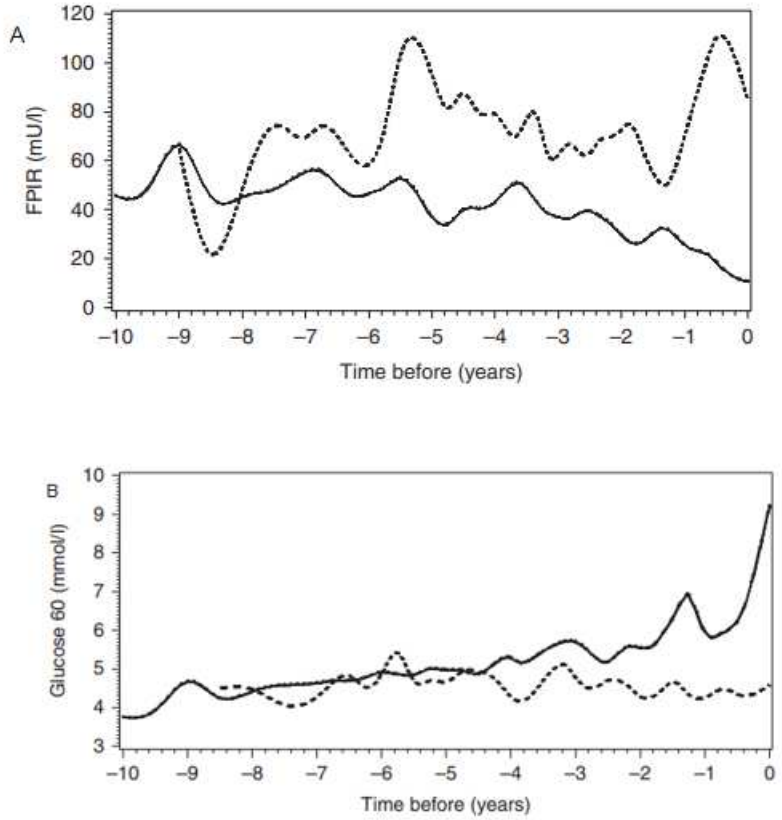
A
FPIR (mU/l)
120
100
80
60
40
20
0
−10 −9 −8 −7 −6 −5 −4 −3 −2 −1 0
Time before (years)
B
Glucose 60 (mmol/l)
10
9
8
7
6
5
4
3
−10 −9 −8 −7 −6 −5 −4 −3 −2 −1 0
Time before (years)

Figure 2 (A-B)

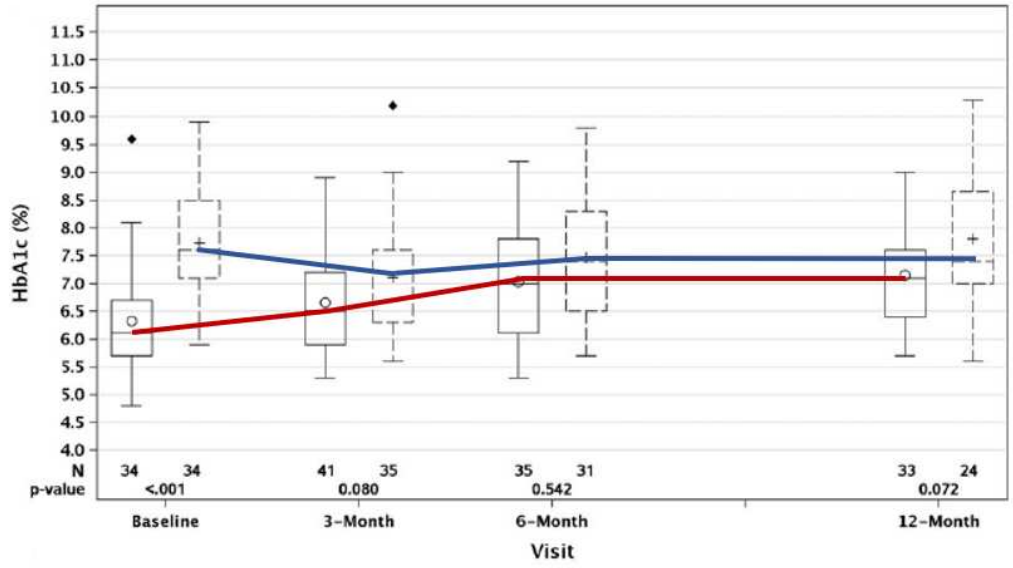


Figure 3

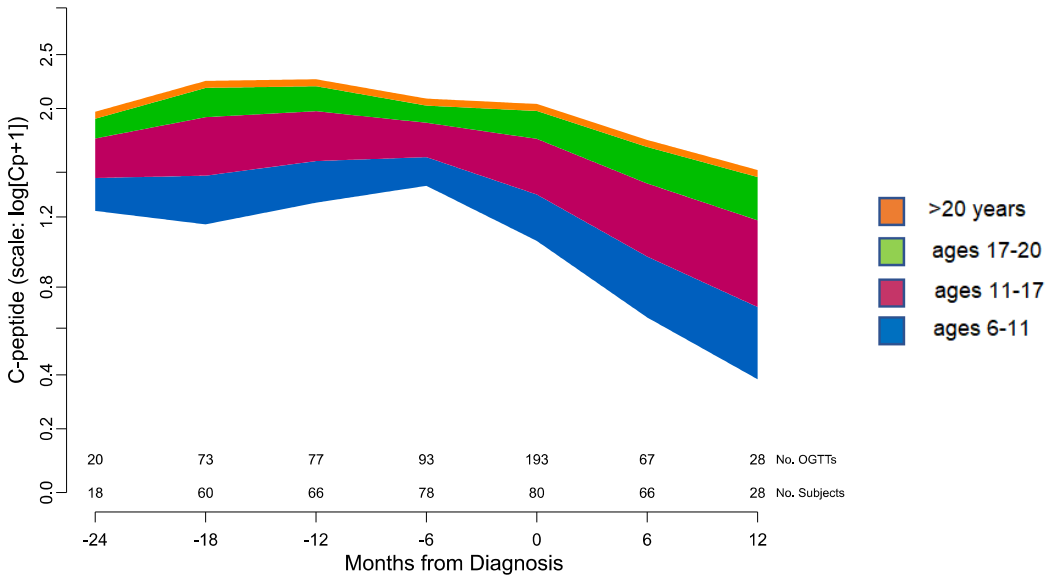


Figure 4

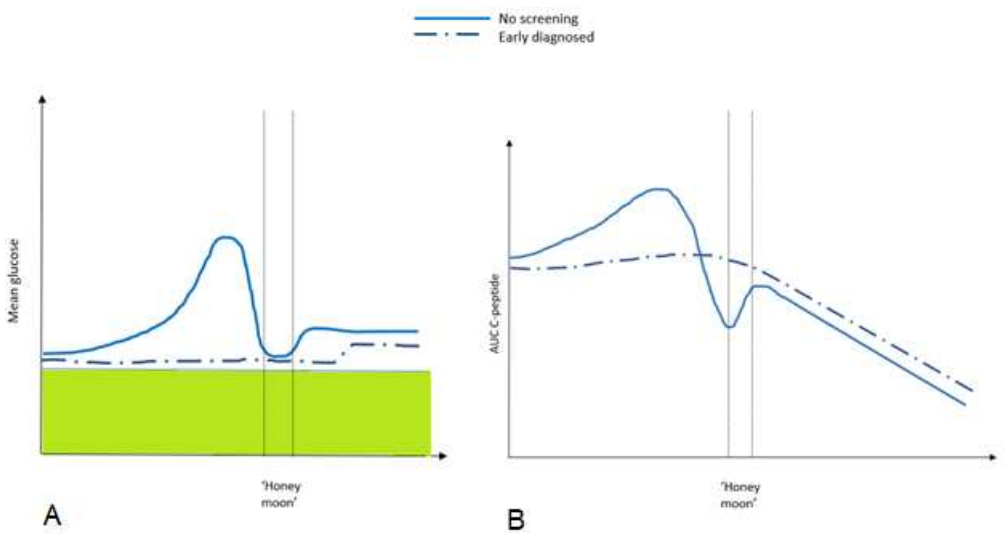


Figure 5

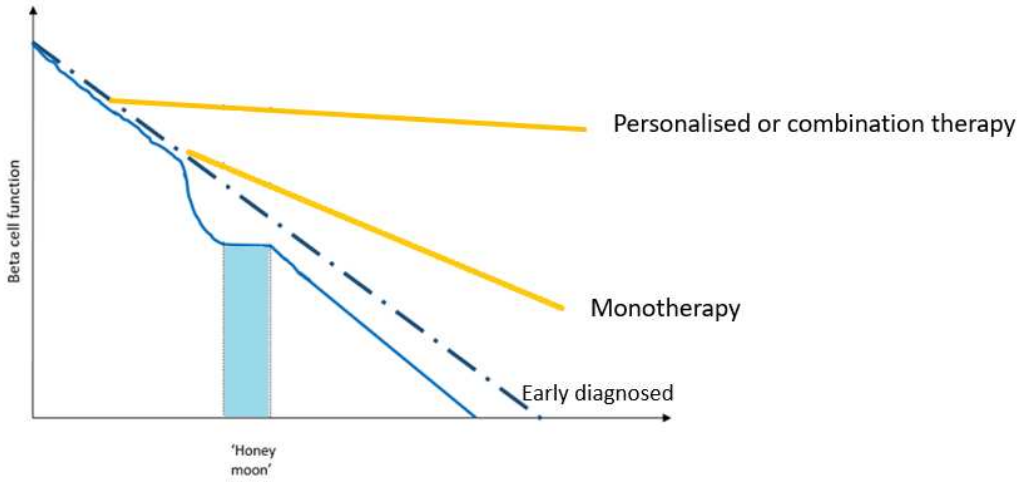


Figure 6