C Peptide and Type 1 Diabetes: Concise Review of Fundamental Concepts

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Abstract

Analyzing the loss of insulin secretion has strengthened our understanding of type 1 diabetes mellitus (T1D) pathogenesis. Pancreatic beta cell injury is fundamental to this disease process. It is generally accepted that we can evaluate B cell function by measuring C peptide levels. We will discuss the various ways to measure C peptide that are used in clinical and research settings and emphasize the reasons for assessing insulin secretion, C peptide levels and shapes of oral glucose curves in response to mixed meal or oral glucose tolerance tests (OGTT) in investigative trials. This review will highlight the patterns and variations that occur metabolically before the clinical presentation of T1D and their significance, specifically emphasizing data that shows the decline in beta cell function is most rapid during the last 6 months before T1D diagnosis. Preserving Beta cell function has essential long term advantages and consequences for patients with T1D. We will draw attention to data that comes from several landmark trials including Diabetes Control and Complications Trial (DCCT), Diabetes Prevention Trial-Type 1 (DPT-1) and Trial Net. It is extremely valuable to be able to quantify and monitor risk of future T1D. A main message from this review is to have an understanding and appreciation of temporal C peptide changes that occur before T1D diagnosis as most future research questions and trials investigating therapies continue to direct efforts to intervene earlier and target this peri-diagnosis period before accelerated C peptide decline is observed.

Introduction

Type 1 diabetes (T1D) results from damage to and destruction of pancreatic βeta-cells. The pathogenesis of T1D begins long before the onset of the usual recognized features of T1D. The clinical presentation of T1D is heterogeneous and ranges from minimal symptomatology to fulminant diabetic ketoacidosis (DKA). In this review, when we reference T1D diagnosis, we will be referring to either surveillance of subjects at increased risk of T1D or presentation with symptoms and testing confirms diagnosis by ADA standards (also reviewed below).

TID develops in individuals with a genetic predisposition. The earliest indication of the T1D disease process is the detection of diabetes related autoantibodies. Most common antibodies are glutamic acid decarboxylase antibodies (GAD), islet cell (ICA), insulin (IAA), tyrosine phosphatase (IA-2) and to a zinc transporter (ZnT8). In most individuals, changes in insulin secretion and glucose tolerance occur months to years after these islet autoantibodies are detected and we will review this progression.[3,4] While the exact genetic, immune and environmental mechanisms remain unclear, there is a 'inflection point' where a critical amount of beta cell failure leads to the onset of hyperglycemia." Overt diabetes presents with the glucose concentrations meeting current ADA diagnostic criteria, either through fasting plasma glucose concentration of >126 mg/dL, 2-hour plasma glucose concentration of >200 mg/dL or HbA1c value ≥6.5%. At this point, there is still some residual beta cell function, albeit less than prior to diagnosis but over time, this usually continues to decrease.[3,4,,] This review will offer a concise examination of the fundamental advancements and knowledge we have gathered from T1D and C peptide research trials and summarize key take home points.

C Peptide: Assessing Beta Cell Function

The best assessment of β-cell function and secretion available is through measurement of C peptide levels.[2,4,7-,10,11] Proinsulin is cleaved and secreted as insulin and C-peptide by the pancreatic beta cells. The half-life of C peptide is 20-30 minutes, approximately 5 times longer than insulin. C-peptide and insulin are secreted into the portal circulation in equimolar ratios and C peptide avoids the first pass clearance by the liver. [9] In addition, C-peptide that is secreted by βeta cells is not contained in insulin medications thus making it an optimal measurement of β-cell function even in patients on insulin therapy. [9,] Two general points about C peptide are important to recognize. First, C-peptide levels must be interpreted cautiously in the setting of renal failure. Approximately half of C-peptide produced is renally cleared., Therefore, levels of C-peptide can be falsely elevated when there is renal impairment and this should be taken into consideration. Next, more recent C peptide assays can detect very low C peptide levels, as little as 0.0015-0.0025 nmol/l and cross reactivity with proinsulin is rarely seen. [4,7,8]

C peptide: Methods of Testing in Clinical Setting

C peptide can be measured in the urine and blood. One can obtain urine C peptide as a random sample or 24 hour collection but these are less often used as there are conflicting studies regarding correlation of urine and serum C peptide.[17] Also urine collections would have limitations and difficulties that are recognizable, especially in children. [17] Therefore, serum c-peptide levels are the principal way of measuring C peptide concentrations. [18] This can be measured at random, fasting, or after stimulation. Random or fasting C peptide measurements may be more convenient to obtain with patients in the clinical setting and can offer valuable information, especially if a patient has undetectable or low C peptide level.

C peptide: Methods of Testing in Research Setting with Stimulated C peptide

In contrast, in the research setting, stimulated C peptide after a meal has been most commonly measured in clinical trials and has provided significant data and insights into beta cell function and insulin secretion in T1D. [2-4, 8,] There are various ways to assess stimulated C peptide levels. First, glucagon can be administered intravenously (glucagon stimulation test or GST) with C peptide levels obtained immediately before and 6 min after glucagon injection. This test has been mainly used in Europe. Second, 2 hour oral glucose tolerance tests (OGTTs) are obtained where, after fasting, 75 grams of oral glucose is given and blood glucoses with corresponding C peptide levels are tested 10 minutes prior, at start and then, 30, 60, 90, and 120 minutes later. [18,19] The peak or highest C peptide level can be variable but is usually seen at 90 minutes with a decrease and return to baseline levels at 120 minutes in non-diabetic patients.[18-] In patients with impaired beta cell function, this C peptide response at different time points is frequently altered. Thus, the overall area under the C peptide curve (AUC) is often utilized as an indicator of overall beta cell secretion. [18-22] The third method of stimulated testing is intravenous glucose tolerance tests (IVGTT). This protocol involves after 12 hours of fasting, administering IV glucose bolus intravenously within 60 seconds and serum is sampled at 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60 and 75 min for glucose, insulin, and C-peptide values. [18,19] Lastly, there is the Mixed meal tolerance test (MMTT). MMTT involves having a patient consume a weight-based liquid meal, such as Sustacal or Boost, over 5 min and glucose and C peptide samples are measured 10 minutes prior, start and then at 15, 30, 60, 90, and 120 min. The AUC during a mixed meal can also be calculated. The 90-min– stimulated C Peptide level is recognized as highly sensitive and specific measure for peak insulin secretion [2,4,18,]. Generally, OGTT's are repeated and used for surveillance of C peptide and glucose changes prior to T1D diagnosis while MMTT is used after diagnosis.

With so many methods of stimulated C peptide testing available, various workshops and organizations including The Diabetes Prevention Trial of Type 1

Diabetes (DPT), Type 1 Diabetes TrialNet (TrialNet) and European C-peptide Trial (ECPT) Study Group have evaluated each of the stimulated C peptide tests to determine if there is an optimal one that should be most commonly used. Greenbaum et al reported the peak and AUC C-peptide values obtained during MMTT and OGTT correlated but the C-peptide value were higher after OGTT compared to after MMTT stimulation. One explanation for this is the lower glucose concentrations after the mixed meal. They cautioned about making direct comparisons with trials that use OGTT vs. MMTT. Trial Net Research Group and ECPT Study Groups compared MMTT vs GST.[22-] The MMTT was concluded to be more sensitive, providing higher post-stimulus C-peptide levels and subjects reported less side effects during MMTT testing. With this information, to bring standardization among clinical trials and limit variations in study designs, it was decided that based on sensitivity and reproducibility, MMTT was the preferred method for testing beta cell function in subjects with T1D. MMTT became the recommended stimulated C peptide testing endorsed by the ADA Workshop and Immunology of Diabetes Society. [18,]

C Peptide Measurements: Why perform stimulated C peptide testing in diabetes investigative trials?

Fasting C- peptide correlates with MMTT and GST stimulated C peptide. ' So what is the purpose of testing stimulated C peptide tests in research trials? Our knowledge and insights of T1D disease progression, especially before, during and right after new onset T1D has come from studying the relationship of glucose and C peptide variations after stimulated testing. We will review these distinctive stages that have been identified and their clinical significance.

Metabolic Progression of T1D: Time course and C peptide Changes Before and During Clinical Diagnosis

Observations made for several years before and at T1D diagnosis have reformed our understanding of the disease course [27-29]. There are 3 major stages that have been seen and classified: Stage 1 is Autoimmunity+/Normoglycemia/Presymptomatic Type 1 Diabetes, Stage 2 is Autoimmunity+/Dysglycemia/Presymptomatic Type 1 Diabetes and lastly Stage 3 is Autoimmunity+/ Dysglycemia/Symptomatic Type 1 Diabetes and these occur as a progressive continuum. The first period is characterized by development of diabetes related autoantibodies. After pancreatic autoantibody detection, the next change we see is in the first-phase insulin response (FPIR). FPIR is the pulsatile release of insulin in response to an intravenous glucose load. [4,18,21,,29] FPIR was shown to have distinctive pattern of deterioration in many trials including in the Diabetes Prevention Trial–Type 1 study (DPT-1). DPT-1 followed IVGTTS of patients who were termed as progressors and non-progressors towards T1D using the OGTT criteria by the ADA. Non-progressors were not diagnosed with T1D during the study data collection. The study showed that during pre-clinical phase of T1D, there is a modest decrease in FPIR after IVGTT with the slope (FPIR vs

time in months) showing a slight decline. Approximately 1.5-0.5 years prior to T1D diagnosis, a much steeper decline in slope occurred [7,8,27-30]. This is schematically depicted in figure 1 with bell cell function over time before and after T1D diagnosis.

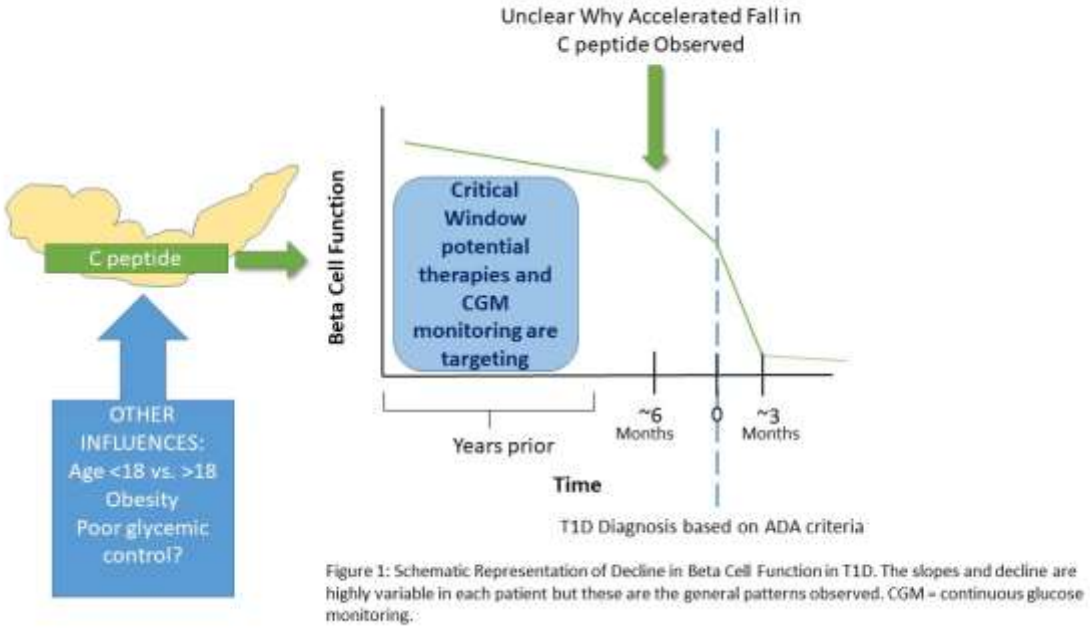

Figure 1: Schematic Representation of Decline in Beta Cell Function in T1D. The slopes and decline are highly variable in each patient but these are the general patterns observed. CGM = continuous glucose monitoring.

After the loss of first-phase insulin response, the next component in the metabolic progression that occurs is abnormal post meal glucose with preservation of fasting glucose levels. Glucose levels gradually increase and we start seeing evidence of glucose intolerance, or dysglycemia frequently, at least 2 years before diagnosis and this dysglycemia continues until 6 months prior. Following this, there is a much steeper increase in glucose levels that is observed before T1D diagnosis. [27-28] This is evidenced by studying and analyzing the OGTT curves. During the OGTT, the 30–0-min C-peptide level, also referred to as the early peak C peptide response declines in progressors to T1D.[29-30] In addition, C-peptide levels increase at later time points in the OGTT curve, especially during 60-90 minute interval. This increase in C peptide later in the OGTT curve could be a compensation from the decreasing early C peptide response. However, the later C-peptide response unfortunately does not prevent elevated blood glucoses. With the changes in early and later peak C peptide levels, the overall AUC remains fairly unchanged until 6 months prior to T1D diagnosis. The timeframe from approximately 6 months prior to diagnosis and 3 months after diagnosis has been identified as the peri-onset period. During this peri-onset phase, there is a sharp increase in the glucose levels as well as a much more rapid fall in AUC C-peptide and the peak C-peptide.

Furthermore, the peak C peptide falls even more dramatically in the following 3 months after diagnosis time.

We don't know why there is this accelerated decline in C peptide and AUC during this time. Speculations have included β-cell apoptosis, insulin resistance or some form of environmental trigger that triggers this event. " But, these changes in C peptide responses during oral glucose testing offer insights to the damage and destruction of β-cells that occurs much before the clinic presentation of T1D. Furthermore, the key findings are that Beta cell secretion is impaired early during the disease process with a much more sudden fall just approximately 6 months prior to diagnosis has implications for future treatment targets to intervene during this critical window in the disease process and attempt to delay or prevent this sharp fall in C peptide.[29-32]

Vital Role of Residual Endogenous Insulin secretion for Long Term Microvascular Complications and Hypoglycemia

With this information, it is clear that by the time the OGTT reveals impaired fasting or impaired glucose tolerance, a considerable amount of C peptide is absent. Why is it imperative to try to maintain even small amount of C peptide? Our understanding of the clinical benefits and long term outcomes of maintaining the C peptide levels and beta cell function comes largely from the DCCT. First, it was found that patients with a stimulated C peptide levels >0.2 pmol/mL had a significantly lower mean fasting plasma glucose level, and lower hemoglobin A1c. Thus, this C peptide cut off has been used as the critical point in clinical research trials. Next and most important, the concept that maintaining beta cell function to minimize end organ complications emerged from this trial. The DCCT had 1,441 T1D patients with 1-15 years of disease. Of these, 855 had T1D for 1-5 years. They were further classified as either C peptide responder or non-responder based on stimulated levels with C peptide responders having C peptide levels of 0.2-0.50 pmol/mL after mixed meal testing. C peptide non responder had <0.2 pmol/mL cutoff. The patients were randomly assigned to conventional treatment or intensive insulin management. The intensively treated arm that had stimulated C peptide > 0.20 nmol/l past one year had fewer complications, including a significant risk reduction in progression of retinopathy and micro-albuminemia.[34,35]

In a subsequent analysis, the DCCT subjects were divided into cohorts based on stimulated C-peptide responses: Non-responders with C peptide<or=0.03, Minimal or C peptide of 0.04-0.20, Baseline Responder or 0.21-0.50 nmol/l at entry, and Sustained responder who had C peptide of 0.21-0.50 nmol/l at entry and at least 1 year later. Patients with higher and sustained levels of stimulated C-peptide were again associated with lower rates of retinopathy and nephropathy. Despite lower A1c among the C peptide responders in the intensively treated arm, the risk for hypoglycemia was found to be 65% less than the intensively treated C peptide Non responder group. Also, intensive therapy reduced the risk of losing C peptide response by 57% over the

6.5 year study period. Thus, intensive insulin treatment helps sustain Beta Cell function with C peptide secretion and allows reduction in diabetes complications while minimizing hypoglycemia. [32,34,35] Finally, follow up DCCT analyses also added that while this C peptide of 0.2pmol/mL has clinical advantages, any small increase in C peptide levels, even at lower levels is also beneficial.[35]

Effects of Age and BMI on C Peptide Levels

What influences these C peptide levels? Age has been identified as one factor. For example, Davis et al reported that 78% of participants diagnosed at >18 years of age compared to only 46% of those diagnosed at ≤18 had residual C-peptide defined in this study as ≥0.017 nmol/L, 3–5 years from diagnosis. Most of those diagnosed as age <18 years of age who had detectable C-peptide long after diagnosis had markedly lower non-fasting C-peptide values than those with similar disease duration who were diagnosed as adults or >18 years of age. Greenbaum et al found the rate of decline of C-peptide also varied based on age with the youngest subjects between ages of 7-12 years of age starting with lower C peptide concentrations at their first MMTT testing. The Joslin 50-Year Medalist Study followed a large group of diabetic patients for more than 50 years. They found 64.4% with minimal (C peptide of 0.03-0.2 nmol/l) and 2.6% with sustained (C peptide ≥0.2 nmol/l) random C peptide levels after 50 years of insulin treated diabetes. They also concluded that alater age of onset of T1D patients was associated with higher C-peptide levels. Multiple studies have confirmed C peptide can be maintained for long periods of time. The two important take-away points are the presence of C-peptide does not rule out a T1D diagnosis and T1D patients will gradually lose their C peptide secretion.[8,18]

Obesity also affects beta cell function. Higher body mass index (BMI) typically results in more insulin resistance. Recent studies have postulated that higher weights for individuals may accelerate the T1D disease process but nothing has been affirmed. Yu et al reviewed retrospectively data of 135 children aged 2.1-16.5 years with autoimmune T1D and classified them using the 2007 Korean Growth Chart. They found that younger age of onset was linked with lower C-peptide levels, but neither overweight nor obese patients exhibited more rapid onset of T1D. Also, in subjects of older age of onset without DKA on presentation, those that were classified as overweight or obese status were associated with preservation of C-peptide levels at the time of T1DM diagnosis compared to the underweight BMI group. These conclusions were drawn from random C peptide levels. However, similar findings were reported recently by Sosenko et al when they reviewed Diabetes Prevention Trial-Type 1 (DPT-1) patients and used stimulated C peptide levels. The C-peptide values were higher for the highest BMI cohort than for the lowest BMI group at all OGTT time points. Poor glycemic control and human leukocyte antigen (HLA) types may also affect beta cell function, but studies have not been conclusive. Currently age and BMI are recognized as influencing C peptide levels (figure 1) and we still do need more studies

to elucidate whether BMI influences the progression of beta-cell dysfunction.

Using C peptide levels in T1D Risk Scores to Identity and Stage High Risk Individuals

Factoring C peptide levels, age and other influences to the risk of progressing to T1D has been of growing interest, especially since such markers can be used not only in research trials but perhaps clinically. Identifying patients earlier in the disease course could have benefits in terms of attempting to minimize long term complications as well as possibly intervene with medications or therapies much sooner. [8,18,21,27] Both DPT-1 and Trialnet have shown that children who developed multiple autoantibodies were at a very high 10-year risk of progression to T1D. As reviewed, C-peptide levels, especially post stimulation, have also been shown to be predictive of T1D risk. There have been other markers used to assess this T1D risk, including the DPT-1 risk score (DPTRS) which factors several predictors of T1D including age, BMI, fasting C peptide, C peptide and glucose levels during OGTT into one score. A DPTRS score >9.00 almost guaranteed a patient would progress to T1D. This DPTRS score is primarily for subjects with positive pancreatic antibodies.[41,] Another diagnostic score available is the Type 1 Diagnostic Index 60 (Index 60). To apply this, a patient has to also be autoantibody-positive.[21,] It is another valuable score which incorporates both glucose and C-peptide measurements fasting and during the OGTT. An Index 60 score ≥2.00 was diagnostic of T1D. It does not include age or BMI as compared to DPTRS. Validation studies have shown that using the Index 60 with the first OGTT that met the criteria of ≥2.00 occurred approximately one year before a diagnosis would be made using standard OGTT glucose criteria.[44]

Finally, recent studies have also shown that the actual shape of the OGTT may offer more insight in antibody positive relatives of T1D patients and their risk to disease progression. The phasic glucose response curves have been described as 'monophasic' or 'biphasic.' Monophasic refers to glucose that increases after an oral glucose load to the maximum at 30, 60 or 90 min and then decreases until 120 min. Those with glucose curves that decreased after an initial increase and then increased again at a later time point were classified as 'biphasic'. Ismail et al recently commented that compared with the biphasic group, the monophasic group were associated with higher risk to develop T1D, probably related to the lower early C peptide response also seen. Therefore, the glucose curve shapes with varying C-peptide levels could be also valuable to identify progression risk to type 1 diabetes.

Future Directions

On this basis, it's evident that assessing risk of future T1D and maintaining any residual beta cell mass are a major goal as patients with higher levels of endogenous insulin secretion have better long-term outcomes. Over the past few decades, a variety of immune interventions have been studied before new-onset T1D, including nonspecific immunosuppression, antigen-

specific therapies, and cellular therapies. Furthermore, initial trials are underway exploring continuous glucose monitoring (CGM) as a new approach to monitoring and diagnosing type 1 diabetes in children and adults with positive islet autoantibodies (Ab+). Many research efforts are continuing to find ideal interventions that could conserve any beta cell function and even potentially make the disease milder for these patients.

Conclusions

Findings from large clinical trials over the last few decades have helped our understanding of the T1D disease process tremendously. They have offered insights to using C peptide as a measure of residual Beta cell Function and helped us appreciate the different stages with first autoantibody detection, then dysglycemia with a gradual decline in C peptide followed by an abrupt onset of accelerated decline in insulin response and shortly thereafter, T1D diagnosis. Studying the time to peak and shape of C peptide curves has further detailed the disease process in T1D. We have also recognized the need to preserve this remaining C peptide function as higher C peptide levels are associated with improved glycemic control, lower daily insulin dose, less severe hypoglycemia, and less microvascular complications. Given this, various markers to calculate diagnostic risk scores are being utilized and validated so that we can stratify and identify high risk individuals earlier in the disease course when they still have C peptide levels detectable. Hopefully we can continue to build on this momentum and continue efforts to explore interventions, especially in the pre-diagnosis stage and decrease the disease burden for T1D.

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