

# **The Amylin Circuit-Breaker: Restoring Glucagon Counterregulation in T1D**

*Ironically, the key to increasing glucagon secretion during hypoglycemia  
may require the use of an alpha-cell inhibitor.*

## **Executive Summary**

We propose that a new dosing regimen for an amylin analog, pramlintide, is probably the key to restoring most of the glucagon counterregulatory response to hypoglycemia in Type 1 Diabetes. We think the clinical need and the possibility of success warrant clinical research aimed at testing our hypothesis.

Automated insulin delivery systems promise to reduce the therapy burden on T1D patients and improve time in range. But studies have not shown convincing evidence that AID can dramatically shrink the gap between healthy blood glucose levels and the chronic hyperglycemia characteristic of T1D. So long as that gap exists, the comorbidities and early mortality associated with T1D can be expected to continue.

The barrier to closing the gap with more intensive insulin therapy is the risk of hypoglycemia caused by a defect in glucagon counterregulation in T1D: islet alpha-cells fail to respond to the onset of hypoglycemia by increasing glucagon secretion to amplify hepatic glucose production. After decades of clinical research, a pharmaceutical strategy for correcting this defect remains elusive.

To restore normal alpha-cell secretory patterns in T1D, a new drug concept is needed that would suppress glucagon secretion in response to rising blood glucose, and that would stimulate glucagon secretion at the onset of hypoglycemia. Defining that new drug target will require a new model of alpha-cell homeostasis, a paradigm that is novel, yet plausible, and theoretically restores both appropriate glucagon suppression during hyperglycemia and stimulation during hypoglycemia. The paradigm should propose a new perspective on alpha-cell control mechanisms directly caused by the beta-cell deficit, and it should suggest a hypothetical drug target which can form the basis for clinical research. Ideally the paradigm should be testable immediately without requiring new drug discovery or delivery technology.

To this end we propose a new “circuit-breaker” model of counterregulation: During euglycemia alpha-cells are in a state of tonic inhibition by the neuroendocrine hormone amylin, which delivers its suppressing effect via the CNS. When beta-cells detect the onset of hyperglycemia, they increase this inhibition by secreting more amylin. When the brain detects the onset of hypoglycemia, it interrupts the amylin suppression signal, resulting in a rebound of glucagon secretion. Ergo, the circuit-breaker effect.

FDA-approved dosing of the amylin analog pramlintide results in overdosing and nausea at mealtimes and does not provide the tonic inhibition needed between meals and overnight. We propose that a dual hormone AID system with an algorithm that provides different insulin/amylin ratios for basal and bolus infusion rates could be the solution to restoring glucagon counterregulation in T1D, as well as to smoothing postprandial blood glucose.

# The Amylin Circuit-Breaker: Restoring Glucagon Counterregulation in T1D

## Introduction

The purpose of this presentation is to propose a novel hypothesis for a therapeutic strategy that may restore the glucagon counterregulatory response to hypoglycemia in Type 1 Diabetes (T1D). In short, we believe that a neuroendocrine hormone, amylin, acts during euglycemia as a tonic inhibitor of alpha-cell secretion, and that hypoglycemia activates a neural circuit-breaker to interrupt this suppression signal, thereby releasing a glucagon counterregulatory rebound. This model proposes that beta-cells are the primary sensors of hyperglycemia, that the brain is the primary sensor of hypoglycemia, and that amylin is central to the regulation of alpha-cells in glucose homeostasis.

The roadmap for this hypothesis aims to correct a shortcoming of previous alpha-cell model building efforts: amylin is a missing link that has not been considered in models of glucagon counterregulation. As recently as December 2018, a review of the role of the alpha-cell in diabetes contained no mention of amylin's role in regulating glucagon secretion.<sup>1</sup> We have been unable to find a single literature reference to the idea that amylin is a key player in the alpha-cell response to hypoglycemia.

Our discussion is organized into three parts and three appendices:

- **Part 1: A New Model for Glucagon Counterregulation.** Technology advances focused on insulin therapy of T1D have stalled with respect to normalizing HbA1c, and the biggest barrier to achieving euglycemia is the risk of iatrogenic hypoglycemia. None of the available adjunctive pharmaceutical therapies appear to offer promise for solving this problem, and so there is a pressing need for a new model of alpha-cell regulation. We propose a new simple systems model of the alpha- and beta-cell regulatory network whereby (1) the beta-cells are the primary sensory mechanism for preventing hyperglycemia while (2) the brain is the primary sensory mechanism for preventing hypoglycemia. We demonstrate how amylin can be expected to play the pivotal role in this model, and we cite clinical data that is indirectly supportive of our hypothesis.
- **Part 2: Getting the Amylin Dosing Right.** We propose a teleological rationale for beta-cell secretion of two glucoregulatory hormones, and we show how that rationale is supported by the physiology of these peptides and the resulting diurnal peripheral concentrations. We then demonstrate that FDA-approved dosing of pramlintide does NOT mimic the natural profile, but rather causes overdosing at mealtimes and underdosing between meals and overnight. We believe this incorrect dosing is the cause of the poor efficacy and tolerability that has limited the use of amylin replacement therapy and is the reason pramlintide has failed to demonstrate restoration of glucagon counterregulation. We propose the use of an automated Dual Ratio Amylin/Insulin system to correct this problem, and we discuss the merits of other amylin agonist formulation and delivery strategies.
- **Part 3: Where Do We Go From Here.** We begin the process of defining the clinical research needed to confirm our circuit-breaker hypothesis by raising a series of questions that need

answering. We consider this a work-in-process that will evolve over time as we receive feedback on the hypotheses presented in Parts 1 and 2.

- **Appendix A: Alpha-Cell Response to Hyperglycemia.** We take a deep dive into recent data from diurnal studies of glucagon, and we show that (1) mealtime influx of blood glucose explains only about half of the normal daily variations in circulating glucagon concentrations, and that (2) alpha-cell response to hyperglycemia is eliminated by T1D. This data is consistent with the idea that beta-cell secretion of amylin is the glucoregulatory signal which suppresses alpha-cell secretion in response to rising blood glucose.
- **Appendix B: Dual Ratio Amylin/Insulin Dosing.** We present details of the calculations that resulted in recommending 6µg/U basal and 2µg/U bolus as baseline infusion rates for personalizing dual hormone replacement therapy.
- **Appendix C: US Patent 9,656,017 INFUSION DELIVERY DEVICES AND METHODS.** We include a copy of the issued patent with claims covering the dual amylin/insulin ratio AID infusion algorithm.

The goal of this presentation is to stimulate interest in starting clinical research aimed at testing our hypothesis that appropriate amylin replacement dosing can restore the glucagon counterregulatory response in T1D. During the past half-century, much data about alpha-cell function has been accumulated and interpreted based on several physiological models. Many contradictions have been observed, and these models remain controversial. There is good reason to expect that some data aimed at validating these prior models will appear to contradict our hypothesis: glucose homeostasis is a complex system of multiple organs, signals, and redundancies which is difficult to decipher – especially if the physiologic models and confirmatory experiments have not considered a key component of the alpha-cell sensing system, the neuroendocrine hormone amylin.

For readers wishing more detail about amylin, the most comprehensive analysis of the hormone and its role in glucose homeostasis can be found in the book [Amylin: Physiology and Pharmacology](#) by Andrew Young, who is the world's leading expert on amylin.<sup>2</sup>

A note on the use of the royal “we” in this work: The author wishes to acknowledge the collaborative help from a wide range of sources far more knowledgeable about glucose homeostasis and pathology, including both the authors of referenced works and colleagues who encouraged and supported the development of the amylin circuit-breaker hypothesis.

**Howard E (Ted) Greene, Jr.**

Founding CEO of Amylin Pharmaceuticals

April 2020

## Endnotes

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<sup>1</sup> The alpha cell in diabetes mellitus; Nature Reviews Endocrinology; 14:694-704 2018.

<sup>2</sup> Amylin: Physiology and Pharmacology; Advances in Pharmacology Volume 52, Elsevier Academic Press 2005.

# The Amylin Circuit-Breaker – Part 1

## A New Model for Glucagon Counterregulation

In Part 1 we lay out the logic and data supporting a novel hypothesis about the physiology regulating the counterregulatory response to hypoglycemia. This chapter is organized around the following issues:

- **Why did we undertake this project?** Progress toward normalizing HbA1c in T1D has reached a point of diminishing returns well above healthy levels in spite of the advent of automated insulin delivery systems. Inherent limitations in subcutaneous insulin delivery point to the need for adjunctive drug therapy to resume progress toward euglycemia.
- **Are there any promising candidates to fill this adjunctive role?** To date the obvious adjunctive drug candidates have proved disappointing. What's needed is a new target for drug development.
- **Where should the search for a solution begin?** We believe the most promising target is the lack of response by alpha-cells to both rising and falling glucose in T1D. Normalizing glucagon secretion would relax the most important constraint on insulin therapy: the risk of iatrogenic hypoglycemia.
- **What about existing strategies for restoring the glucagon counterregulatory response?** None of the approaches taken to date have successfully restored normal alpha-cell functions in T1D, and we can find no new ideas for doing so in the literature.
- **How should a new hypothesis be developed?** We believe the solution starts with constructing a new model of alpha-cell sensing of hypoglycemia that builds on data in the literature beyond the classical endocrinology.
- **What is the central concept of our new model?** Systems analysis supports the idea that alpha-cells require tonic inhibition during euglycemia, and that the counterregulatory response is a rebound triggered by the onset of hypoglycemia.
- **What is the likely suppression signal to alpha-cells?** We believe the neurohormone amylin is the alpha-cell inhibitor because (1) it is the most potent known suppressor of glucagon secretion, (2) hypoglycemia activates a “circuit-breaker” for this CNS mediated suppression, and (3) healthy amylin secretion is eliminated by T1D.
- **What data supports the amylin circuit-breaker hypothesis?** There is clinical evidence consistent with the idea, but no studies of counterregulation have considered the role of amylin.
- **Is the efficacy of islet transplants consistent with the hypothesis?** We demonstrate how restoration of counterregulation by denervated, transplanted alpha-cells is consistent with the CNS circuit-breaker concept.

In Part 2 we address the issue of why a decade and a half of amylin replacement therapy has not already validated our hypothesis.

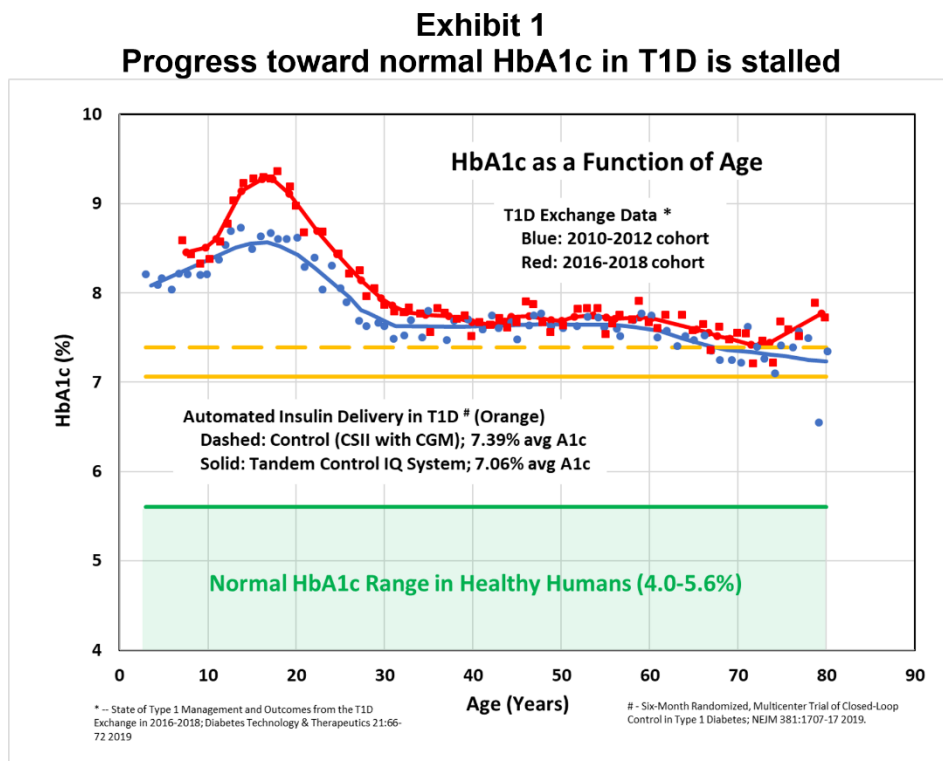


## A BREAKTHROUGH IS NEEDED FOR TREATING T1D

The landmark Diabetes Control and Complication Trial established prospectively a connection between HbA1c and long-term complications.<sup>1</sup> As a result, the goal of T1D therapy is to achieve HbA1c levels as close to the normal, healthy range of 4.0% to 5.6% as possible.

Unfortunately, data published in early 2019 from the T1D Exchange Registry indicates that progress toward this goal is stalled in the broad population of T1D patients. The T1D Exchange Clinic Network includes 81 U.S.-based endocrinology practices in 35 states, and their February 2019 report is based on data from 22,697 participants.<sup>2</sup> As shown in Exhibit 1, during the six years ending 2018 average HbA1c results for T1D patients did not improve (and may have deteriorated in teenagers), and average HbA1c levels were above the ADA guideline of 7%.

This disappointing situation has occurred despite continuing investments in new technologies for treating T1D, as reflected by the patients in the T1D Exchange Clinic Network. More patients are using continuous subcutaneous insulin infusion (CSII), with participation rising from 57% to 63%. And patients reported an even more striking increase in the use of continuous glucose monitoring (CGM), from 7% to 30%. Since patients participating in the Registry can be expected to be more motivated and informed than nonparticipants, the HbA1c averages in the total 1.5 million American T1D population are probably higher.<sup>3</sup>



This failure to achieve euglycemia in T1D has serious consequences: a 2015 study estimated that the loss of life expectancy from age 20 for a T1D patient is 11-13 years.<sup>4</sup> Clearly the number one goal in treating T1D is to permit patients to achieve HbA1c levels in the normal, healthy range.

To this end, Automated Insulin Delivery (AID or the “artificial pancreas”) is a primary focus of research and development aimed at improving therapy of T1D. (For 2018 review of progress toward AID, see reference 5.) AID has been shown to reduce the burden of therapy for T1D patients by automating portions of the therapy regimen; for example, a recent study in children demonstrated the AID system

was associated with less time thinking about diabetes, decreased worry about blood sugars, and decreased burden in managing diabetes.<sup>6</sup> The system improved Time-in-Range (TIR: percentage of a 24-hour period during which CGM readings are in the range 70-180 mg/dl) from 53% without AID to 71% with AID, and mean glycemic control improved without increasing hypoglycemia.

While TIR has been correlated with the risks of retinopathy and microalbuminuria, it remains that “A1c is the only prospectively evaluated tool for assessing the risk for diabetes complications.”<sup>7</sup> So, how well do the AID systems perform with respect to normalizing average blood glucose levels?

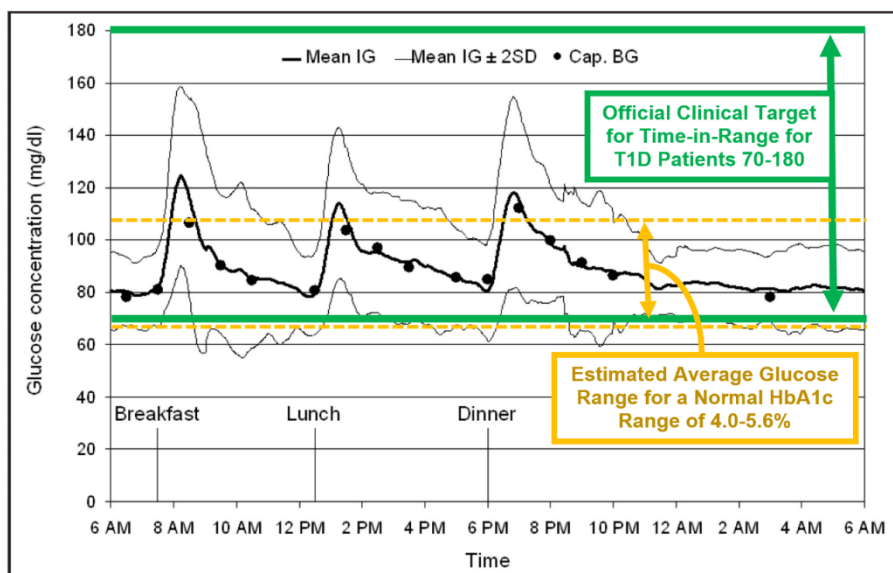
To establish context for this question, Exhibit 2 compares the official TIR goal to the normal glucose excursions in healthy subjects.<sup>8</sup> Also shown in Exhibit 2 is the estimated Average Glucose (eAG) range needed to achieve the normal range for HbA1c of 4.0-5.6%.<sup>9</sup>

Exhibit 2 demonstrates that the official consensus for TIR has been set higher and more broadly than the typical healthy glucose excursion range. This was done presumably to reflect the need for a hyperglycemic “buffer,” given the risk of iatrogenic hypoglycemia. This official TIR is viewed as challenging but doable with present technology, but it does not reflect a healthy, normal range of glucose excursions.

Returning to Exhibit 1, the orange lines demonstrate the improvement in HbA1c achieved with the most advanced AID system to achieve FDA clearance in 2019, the Control-IQ by Tandem Diabetes: “This closed-loop system uses an algorithm with a dedicated hypoglycemia safety module, automated correction boluses, and overnight intensification of basal insulin delivery designed to consistently target near-normal glycemia each morning, which was compared to control subjects using their insulin pumps augmented by continuous glucose monitoring (CGM).”<sup>10</sup>

In this example the AID system lowered HbA1c from 7.39% to 7.06%, a reduction of 0.33%. This is about the same improvement in HbA1c shown with pramlintide treatment, which was not enough to make SYMLIN a marketing success.<sup>11</sup>

**Exhibit 2**  
**Time-in-Range for T1D patients is elevated relative to normal glucose excursions in healthy subjects**



Mean continuous IG trace obtained in healthy volunteers under everyday life conditions by means of the microdialysis technique. Mean interstitial glucose trace  $\pm$  2 SD (corresponding to the 95% range), n = 21; Cap. BG: capillary blood glucose values, used for calibration of the continuous glucose monitoring device. Source: Continuous glucose profiles in healthy subjects under everyday life conditions and after different meals; Journal of Diabetes Science and Technology 1(5):695-703 2007.

For patients in a children's AID study, daily ambulatory glucose values were reduced, especially in the morning; however, most of the reported glucose values were above the healthy range of euglycemia (Exhibit 3).<sup>6</sup>

These disappointing results are widely attributed to two problems with the most convenient and least invasive route of insulin delivery, subcutaneous (SC) infusions:

- **Slow delivery into circulation:**

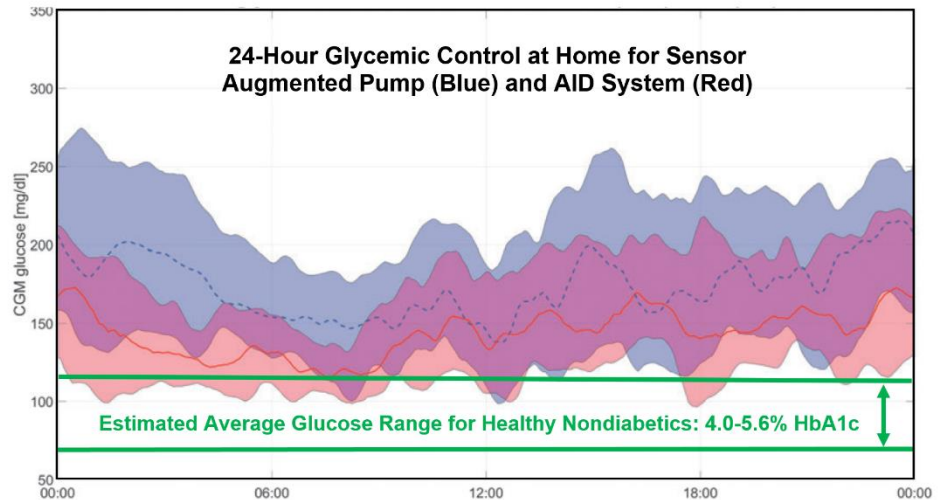
Diffusion through

the subcutaneous tissues introduces delays to insulin action and clearance that make tight control difficult.<sup>12</sup> The hurdles to tight glycemic control caused by these delays are so significant that many AID studies have added meal announcements with full or partial boluses triggered by the patient to improve postprandial glucose control, thereby trading-off autonomy for performance. *“(A) limitation remains on the effectiveness of AP caused by the speed of insulin action, with automated basal rate changes taking time – sometimes several hours – to show full clinical effect.”*<sup>13</sup> Unfortunately, new insulin formulations have offered only modest improvements: A new, more rapid onset insulin resulted in an HbA1c improvement of only 0.15% in one 6-month study.<sup>14</sup>

- **Low hepatic concentrations:** Because about 50% of beta-cell-secreted insulin is taken up by the liver, SC infusions cannot approach normal hepatic concentrations without causing severe hyperinsulinemia in peripheral circulation. To partially compensate for inadequate hepatic insulin, dosing practice in T1D results in patients being about 65% hyperinsulinemic on average during the diurnal cycle, which in conjunction with hyperglycemia may contribute to their relative insulin resistance (see Appendix A). Perhaps as a result of inadequate hepatic insulin, T1D patients are deficient in liver glycogen,<sup>15</sup> which may contribute to their glucose counterregulatory failure. Thus, intraperitoneal (IP) insulin delivery has been studied as an improvement over the SC route. In one study, changing from SC to IP delivery improved HbA1c from 8.8% to 7.2% in ten patients after 24 months.<sup>16</sup> But this more invasive technology is unlikely to replace SC infusions in the near term.

While AID systems are now considered the future of T1D therapy, achieving true euglycemia in T1D will require new pharmaceutical strategies beyond insulin. Our hypothesis is that appropriate therapy adjunctive to insulin may be able to compensate for the limitations of SC insulin infusions. Are there any candidates?

**Exhibit 3**  
The most advanced AID system does not bring T1D blood glucose values into the healthy range



Full-day glycemic control during home use. The red line and shaded area represent Automated Insulin Delivery use, and the blue line and shaded area represent Sensor Augmented Pump use. The shaded area represents the 25th to 75th percentile for glucose values in each group. The center lines (plain and dotted) represent the mean.  
Source: Successful at-home use of the Tandem Control-IQ artificial pancreas system in young children during a randomized controlled trial: Diabetes Technology & Therapeutics 21:1-11 2019.

## NO ADJUNCTIVE DRUG CANDIDATES PROMISE TO OVERCOME INSULIN INFUSION DEFECTS

Conventional treatment of T1D orbits around insulin replacement. The textbook etiology of T1D is straightforward: autoimmune destruction of beta-cells leads to an absolute deficit of insulin, which results in glucose starvation and diabetic ketoacidosis. The obvious solution is to infuse exogenous insulin in a manner that closely mimics endogenous secretions.

Thus, innovations since the discovery of insulin have focused on improving insulin therapy, including new insulin formulations, better delivery technologies, continuous glucose monitoring, and most recently AID. In all cases, insulin remains the center of the T1D universe. *“(T)he existing dogma – that the clinical features of the disease were entirely due to lack of insulin – was not easily abandoned.”* (2017) <sup>17</sup>

As we’ve pointed out, subcutaneous delivery of insulin faces pharmacokinetic and physiological barriers to closely mimicking beta-cell secretions. With insulin development facing diminishing returns, non-insulin adjunctive drug therapies have been under consideration.

To date only two drugs have FDA approval as adjuncts to insulin for treating T1D:

- **Glucagon:** Lilly introduced glucagon in 1960 as a remedy for insulin induced hypoglycemia. More recently several companies have been working on stable liquid formulations that could be used in dual hormone AID systems. To quote a 2017 review: *“In direct comparison, dual-hormone systems have been shown to be superior to single-hormone systems in preventing hypoglycaemia and achieving target glucose concentrations in response to meals and exercise.”* <sup>18</sup> However, as we discuss in a subsequent section, glucagon is indicated for use after the onset of hypoglycemia and does not directly address the pharmaceutical limitations of insulin infusions that raise the risk of hypoglycemia. In effect, glucagon is a band-aid to cover up the wound caused by insulin.
- **An amylin agonist:** Pramlintide has been shown to sharply reduce postprandial spikes in blood glucose. <sup>19</sup> However, the benefit of this for HbA1c is modest at about 0.33% HbA1c. <sup>20</sup> Nausea and additional injections have discouraged use, with the result that, after fourteen years of marketing, pramlintide has not become a major diabetes drug.

Other FDA-approved diabetes drugs have been proposed as candidates for T1D indications:

- **Metformin:** A survey of 197 studies showed insulin-dose reductions, but no benefits for cardiovascular and other key clinical outcomes, including glycemic control. <sup>21 22</sup>
- **GLP-1 agonists:** A survey of 9 clinical trials showed only weight benefits, while gastrointestinal adverse events were common. <sup>23 22</sup> A GLP-1 agonist was also tested for improving hypoglycemia unawareness in T1D; results were negative. <sup>24</sup> From a March 2020 report: <sup>25</sup> *“Short-acting exenatide does not seem to have a future as a standard add-on treatment to insulin therapy in type 1 diabetes.”*

- **DPP4 inhibitors:** Studies demonstrated no increase nor decrease the risk of hypoglycemia, nor was there a decrease in HbA1c levels.<sup>22 26</sup>
- **SGLT inhibitors:** Studies have showed modest HbA1c improvements with increased risk of diabetic ketoacidosis.<sup>27</sup> A recent study showed a reduction of time in hyperglycemia, but no change of time in hypoglycemia.<sup>28</sup> In March 2019 Sanofi's SGLTi indication for T1D received a Complete Response Letter (turn-down) by the FDA.<sup>29</sup> In March 2020 the candidate sponsored by Lilly and Boehringer Ingelheim was also rejected by the FDA.<sup>30</sup> The European Medicines Agency approved an SGLTi only in overweight, obese T1D patients.<sup>31</sup>
- **Triple therapy:** Investigators are testing whether the addition of dapagliflozin and semaglutide could improve HbA1c in T1D.<sup>32</sup>

It's important to note that these candidates target glucose control mechanisms which are not directly linked to beta-cell failure. Thus, they do not address the core defect of T1D.

To summarize, the situation for T1D patients is frustrating. Progress toward normalizing HbA1c has stalled, further breakthroughs in insulin therapy are unlikely short of IP delivery, and other anti-diabetic drugs appear to be of limited value. What's needed now is a new disease model that reconsiders the pathophysiology of beta-cell destruction and looks beyond the obvious insulin deficiency.

As a first step in pursuing this hypothesis, we step back and reassess the basic insulincentric therapy model, and we ask might there be a basic flaw in the popular understanding of T1D pathophysiology?

## **ALPHA-CELL DYSFUNCTION SHOULD BE THE FOCUS OF THE NEW T1D MODEL \***

Let's start the search for a new model by shifting the focus from the beta-cells to the alpha-cells. Some experts have proposed that alpha-cell dysfunction in T1D outweighs the failure to correctly mimic endogenous insulin secretions.

Alpha-cells play a central role in maintaining euglycemia, because glucagon is the primary control mechanism for regulating endogenous glucose influx from the liver to the blood stream. Liver output of glucose is directly proportional to plasma glucagon levels. See reference 33 for an overview of current views (2017) of the roles of insulin and glucagon in glucose homeostasis.

In addition to the canonical role of glucagon in glucose homeostasis, there is long established evidence that glucagon plays a role in energy homeostasis by enhancing satiety, increasing energy expenditures, and inducing thermogenesis.<sup>34</sup>

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\* -- For the remainder of this discussion of alpha-cell regulation we will be referring to analyses of diurnal and postprandial glucagon profiles applied to data generated by Andy Basu's team at the Mayo Clinic as presented in Appendix A:

- Diurnal pattern to insulin secretion and insulin action in healthy individuals; Diabetes 61:2691-700 2012.
- Diurnal pattern of insulin action in type 1 diabetes; Diabetes 62:2223-9 2013.
- A novel natural tracer method to measure complex carbohydrate metabolism: Am J Physiol Endocrinol Metab 317:E483-93 2019.

Alpha-cells are known to respond to nonglycemic plasma signals, e.g. arginine: <sup>35</sup>

*“Amino acid-stimulated glucagon secretion during meals has a different purpose (from protecting against hypoglycemia): amino acids stimulate insulin secretion, which mobilizes amino acid transporters and effects their storage in peripheral tissues. At the same time, insulin obligatorily recruits GLUT-4 glucose transporters in muscle and fat. The hypoglycemic potential of such GLUT4 mobilization is averted only by the simultaneous liberation of endogenous glucose in response to feedforward (anticipatory) glucagon secretion.” <sup>36</sup>*

In healthy, nondiabetic subjects, alpha-cell secretion of glucagon is responsive to the need to restore euglycemia in response to rising or falling blood glucose, and also to respond to nonglycemic signals:

<b>Regulatory Control</b>	<b>Alpha-Cell Phase (from meal start)</b>	<b>Healthy Response</b>
Glucose Responsive Alpha-Cell Secretion	Postprandial Suppression (10-60 minutes)	Prandial glucose flows into the bloodstream from the gut, so liver output of glucose must be suppressed; this suppression requires restraining glucagon secretion by the alpha-cells.
	Counterregulation Stimulation (onset of hypoglycemia)	When blood glucose starts to fall below the levels required by the brain, a hierarchy of protective mechanisms kicks in to raise blood glucose. The second-tier response is for the alpha-cells to increase plasma glucagon levels to stimulate liver glucose production.
Nonglycemic Responsive Alpha-Cell Secretion	Prandial Stimulation (0-10 minutes)	As nutrient flows into the bloodstream, alpha-cells briefly increase secretion of glucagon to protect against hypoglycemia caused by a surge in insulin secretion.
	Postabsorption Stimulation (60-180 minutes)	Between meals, nonglycemic stimulation of alpha-cell secretion to suppress appetite and maintain appropriate energy balance during exercise may be the predominant secretory control factor.
	Sleeping Suppression (180 minutes after dinner to breakfast)	Starting about 3 hours after dinner, circulating glucagon drops below levels correlated with glycemic control, presumably to compensate for a decline in systemic glucose utilization during sleep. <sup>37</sup>
With the exception of counterregulation, these phases are based on Appendix A analyses of published diurnal glucagon profiles in <i>Diurnal Pattern to Insulin Secretion and Insulin Action in Healthy Individuals</i> ; Diabetes 61:2691–2700, 2012.		

Two not mutually exclusive observations have promoted the idea that normalizing glucagon regulation could be the key to effective T1D therapy: excessive postprandial glucagon secretion encourages glucose influx from the liver thereby increasing the need for mealtime insulin boluses, and glucagon counterregulatory failure is the principal barrier to intensive insulin therapy aimed at normalizing blood glucose.

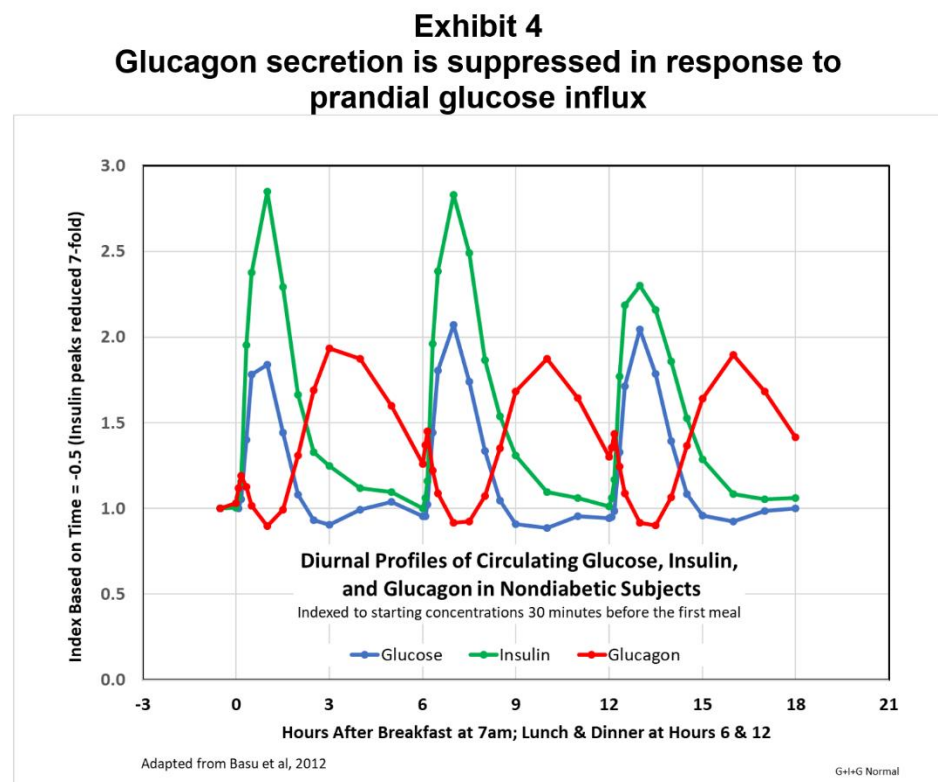


## Suppressing postprandial glucagon mitigates hyperglycemia

Unger et al have pointed out that T1D hyperglycemia is aggravated by excessive postprandial glucagon secretion: <sup>38</sup> “The present studies demonstrate that failure to suppress glucagon following glucose ingestion exacerbates postprandial hyperglycemia in T1D subjects. These data indicate that therapy for T1D subjects is unlikely to result in completely normal carbohydrate tolerance unless both the concentration and pattern of change of glucagon following glucose ingestion is also restored to normal.” <sup>39</sup>

Concern about this “Prandial Problem” of hyperglycemia has led to the suggestion that diabetes drug R&D shift from basal to prandial therapy: “The prandial problem, including postprandial hyperglycemia, weight gain, and hypoglycemia caused by overreliance on injected insulin, is an endocrine and neurologic puzzle that calls for further basic and clinical research. (2017)” <sup>40</sup>

In nondiabetics, following a momentary, arginine-induced spike, prandial increases in circulating glucose cause immediate reductions in circulating glucagon. Exhibit 4 demonstrates this by showing for healthy, nondiabetic subjects glucose, insulin, and glucagon diurnal profiles indexed to preprandial basal levels, with the insulin index divided by seven to allow visual comparisons; in this study, following the amino-acid induced surge, postprandial circulating glucagon was suppressed to a level about 10% below basal levels.



In healthy, nondiabetic subjects, circulating insulin levels are tightly correlated to circulating glucose levels, because beta-cell secretion is mostly responsive to glucose. Linear regression analysis of three different studies confirms that 90%+ of insulin diurnal profiles are explained by glucose profiles: <sup>†</sup>

<sup>†</sup> -- See Appendix A for the data and analyses underlying references to insulin and glucagon diurnal profiles.

<b>Regression Analyses of Circulating Insulin Levels as a Function of Circulating Glucose Levels</b>	
<b>Study Design in Healthy Nondiabetic Subjects</b>	<b>R-Squared</b>
18-hour diurnal profiles of three equal meals of 50 grams <u>simple</u> carbohydrate 6-hours apart	0.8902 (n=20)
6-hour postprandial profiles of single meals of 50 grams <u>simple</u> carbohydrate	0.9487 (n=8)
6-hour postprandial profiles of single meals of 50 grams <u>complex</u> carbohydrate	0.9394 (n=8)

In contrast, circulating glucagon is much less closely correlated with circulating glucose following ingestion of simple carbohydrate, and not at all correlated to glucose following complex carbohydrate and in T1D:

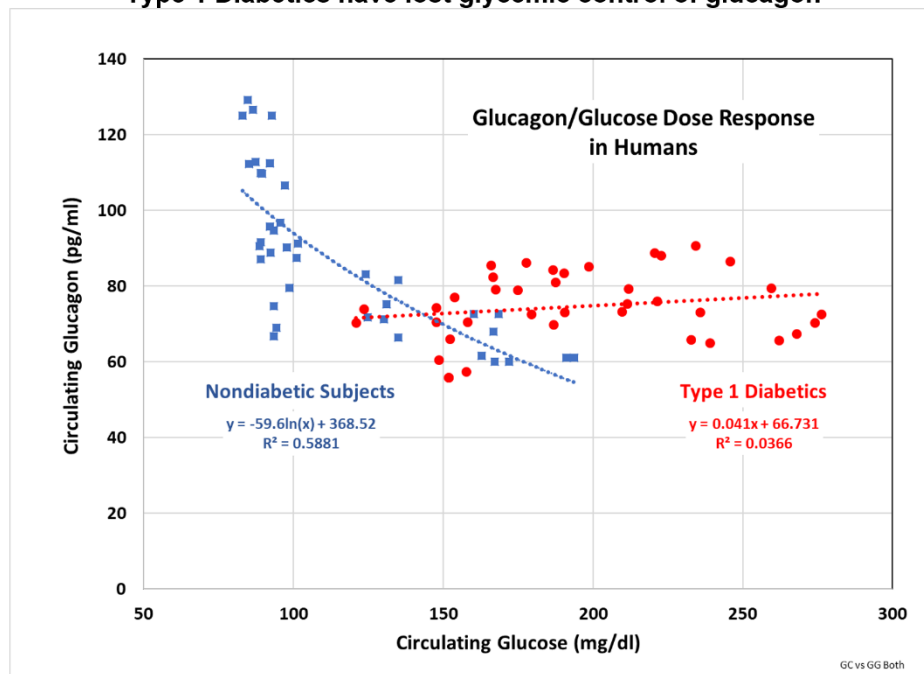
<b>Regression Analyses of Circulating Glucagon Levels as a Function of Circulating Glucose Levels</b>		
<b>Subjects</b>	<b>Study Design</b>	<b>R-Squared</b>
Healthy nondiabetics	18-hour diurnal profiles of three equal meals of 50 grams <u>simple</u> carbohydrate 6-hours apart	0.5881 (n=20)
	6-hour postprandial profiles of single meals of 50 grams <u>simple</u> carbohydrate	0.4882 (n=8)
	6-hour postprandial profiles of single meals of 50 grams <u>complex</u> carbohydrate	0.0033 (n=8)
Type 1 diabetics	18-hour diurnal profiles of three equal meals of 50 grams <u>simple</u> carbohydrate 6-hours apart	0.0366 (n=19) *
* -- The slope of this linear regression was slightly positive, which is contrary to the healthy glucose dose-response and suggests there is no correlation at all.		

Exhibit 5 compares the glucagon dose-response to changes in glucose from a simple carbohydrate meal for nondiabetic and T1D subjects in the diurnal studies.



It's clear from Exhibit 5 that T1D subjects have circulating glucagon levels mostly well above the levels predicted by their chronic hyperglycemia, based on the nondiabetic dose-response. If the T1D diurnal glucose profiles are used in the nondiabetic correlation equation to predict circulating glucagon, the resulting area under the curve compared to actual T1D levels indicates that T1D subjects are exposed to 35% more circulating glucagon than they should be, i.e. they are relatively hyperglucagonemic.

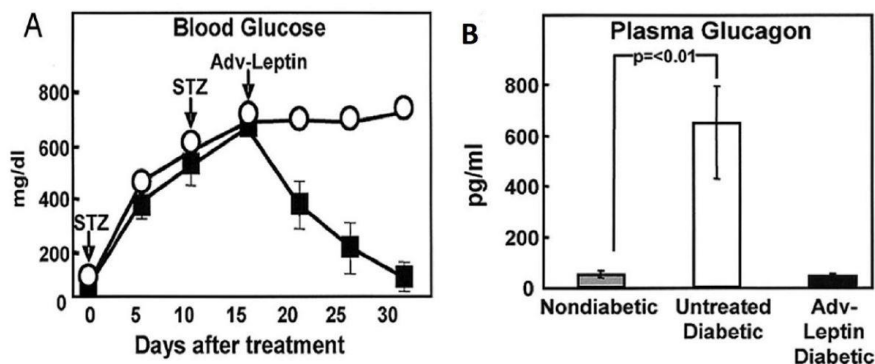
**Exhibit 5**  
Type 1 Diabetics have lost glycemic control of glucagon



Since glucagon stimulates hepatic glucose production, an excess of glucagon can be expected to contribute to the chronic hyperglycemia of T1D. This is supported by a T1D animal experiment in which correcting the excessive glucagon secretion virtually eliminated the need for insulin.<sup>41</sup> In this experiment plasma glucose was normalized in diabetic rats given leptin to suppress their secretion of glucagon; results are shown in Exhibit 6.

It seems clear that dysregulated alpha-cell secretion is a key player in postprandial hyperglycemia.

**Exhibit 6**  
Leptin suppression of glucagon eliminates the need for insulin in T1D rats



Hyperleptinemia reverses abnormalities of uncontrolled diabetes induced by a double dose of STZ.

(A) Comparison of mean ( $\pm$ SEM) blood glucose levels treated with Adv-leptin ( $\blacksquare$ ) ( $n = 5$ ) or untreated double-dose STZ-diabetic rats ( $\circ$ ) ( $n = 5$ ).

(B) Plasma glucagon levels 30 days after treatment (\*,  $P < 0.01$ ).

Source: Making insulin-deficient type 1 diabetic rodents thrive without insulin; PNAS 105:14070-5 2008.

## **Counterregulatory failure is a treatment barrier**

Cryer et al point out that the risk of hypoglycemia is the biggest barrier to normalizing glucose in T1D, as described in a June 2018 review: <sup>42</sup>

*“Iatrogenic hypoglycemia is a fact of life for most people with T1D who must, of course, be treated with insulin. Most have untold numbers of episodes of asymptomatic hypoglycemia which are not benign since they impair defenses against subsequent hypoglycemia. They suffer an average of two episodes of symptomatic hypoglycemia per week – thousands of such episodes over a lifetime of diabetes – and of about one episode of severe, at least temporarily disabling, hypoglycemia per year. Hypoglycemia causes brain fuel deprivation that, if unchecked, results in functional brain failure that is typically corrected after the plasma glucose concentration is raised. Rarely, if it is profound and prolonged, can result in brain death. Hypoglycemia may lead to cardiac arrhythmias, especially in patients with preexisting cardiac abnormalities. Additionally, hypoglycemia has been demonstrated to be pro-coagulant and pro atherothrombotic. Furthermore, severe hypoglycemia has been associated with increased risk of death extending many months after the sentinel episode. Early reports suggested that 2 to 4% of deaths of people with diabetes, largely T1D, were the result of hypoglycemia. More recent reports suggest that 6 to 10% of deaths of people with T1D are the result of hypoglycemia. Regardless of the actual rate, the fact that there is an iatrogenic hypoglycemia mortality rate is alarming.”*

The risk of serious hypoglycemia is inversely correlated with the intensity of insulin therapy aimed at lowering HbA1c. <sup>43</sup> As insulin therapy is intensified to achieve lower average blood glucose, the risk of serious hypoglycemia increases, with the result that patients are inclined to live with a hyperglycemia “buffer.”

Hypoglycemia is a risk because glucagon counterregulation is defective in T1D, i.e. the alpha-cells in T1D do not respond to hypoglycemia by increasing glucagon secretion. <sup>44</sup> *“This is a signaling defect; glucagon secretory responses to stimuli other than hypoglycemia are largely, if not entirely, intact. The mechanism of the absent glucagon response to hypoglycemia that characterizes established type 1 diabetes is not known, but it is linked tightly to, and is possibly the result of, endogenous insulin deficiency.”* <sup>45</sup>

\* \* \* \* \*

The unifying conclusion between the Unger and Cryer camps is that T1D therapy would be more effective if drug therapy could restore glycemic regulation of alpha-cell secretion:

- Appropriate postprandial glucagon suppression would reduce the glycemic burden of hepatic glucose production so that less insulin would be needed at mealtimes, thus lowering the risk of iatrogenic hypoglycemia.

- Restoration of the glucagon counterregulatory response would mitigate the risk of hypoglycemia and permit relatively more aggressive insulin therapy.

We agree with those who believe targeting alpha-cell secretion is also a key to reducing blood glucose variability, which is increasingly viewed as a primary cause of diabetic complications.<sup>46</sup>

## **THERE ARE NO STRATEGIES FOR NORMALIZING ALPHA-CELL RESPONSE**

Various ideas for correcting alpha-cell dysfunction in T1D have been tried and/or proposed:

- **Exogenous glucagon** infusions quickly correct hypoglycemia in T1D. Lilly introduced Glucagon for Injection as an antidote to hypoglycemia in the 1950s. More recently, glucagon has been tested in dual insulin/glucagon infusion pumps to be used in AID systems. However, recent clinical studies showed no compelling benefit,<sup>47 48 49</sup> although there may be some advantage during exercise.<sup>50</sup> (Skeptics have described this idea as one foot pressing the gas while the other foot is pressing the brake.) Mini-dose glucagon packaged in convenient pens may be helpful for mild exercise-induced hypoglycemia.<sup>51</sup> But exogenous glucagon delivery is a treatment for a T1D symptom rather than a correction of the underlying pathophysiology.
- **A leptin agonist** appears to suppress glucagon. Leptin is believed to control glucose homeostasis via a CNS mechanism.<sup>52</sup> Preclinical results were encouraging.<sup>53</sup> However, a human pilot study with metreleptin was disappointing,<sup>54</sup> perhaps because of immunogenicity.<sup>55</sup>
- **GLP-1 agonists** may suppress glucagon secretion, but the interaction of GLP-1 with alpha-cells is controversial.<sup>56</sup> And, in the clinic liraglutide failed to suppress a meal-stimulated glucagon response.<sup>57</sup> As reported above, exenatide failed to improve therapy of T1D.
- **Somatostatin inhibitors** increase the release of glucagon during hypoglycemia. Somatostatin is generally thought to play a minor role in inhibiting alpha-cells in non-diabetic animals and humans.<sup>58</sup> In T1D, elevated somatostatin is thought to suppress alpha-cell response to hypoglycemia, and exercise caused hypoglycemia in rats can be ameliorated by a somatostatin antagonist<sup>59</sup> (a patent has been issued to cover this idea<sup>60</sup>). Before human trials, the safety of non-specific effects would need to be established, because somatostatin targets many tissues, so nonspecific effects are a concern. Moreover, inhibiting suppression of postprandial and fasting plasma glucagon might aggravate hyperglycemia.<sup>61</sup>
- **Glucagon antagonists** have been shown to block the effects of hyperglucagonemia.<sup>62</sup> Glucagon blockade has been studied for almost 40 years.<sup>63</sup> The focus of this research has been on type 2 diabetes, presumably because of the risk of hypoglycemia in T1D. Studies show a potential for improved glycemic control and decreased insulin doses.<sup>64</sup> However, observed side effects include weight gain, increased cholesterol, and alpha-cell hyperplasia. Lilly and Merck have pursued development of glucagon antagonists, but neither reported candidates in their pipelines as of March 2019. The long history of research without a late stage drug candidate is not encouraging.

- **Amylin agonists** have been shown to suppress glucagon secretion.<sup>57</sup> Pramlintide has been on the market since 2006 but has failed to achieve widespread use in T1D because of an unfavorable tradeoff for patients between its clinical benefits and the burdens of extra injections and nausea. Importantly, there is currently no published model that implicates amylin deficiency in the failure of counterregulation.

Several other compounds have been suggested to augment the counterregulatory response to hypoglycemia in T1D; for example: (1) glucose-dependent insulinotropic polypeptide (GIP) increases glucagon responses in humans<sup>65</sup>, and (2) partial blockade of nicotinic acetylcholine receptors can improve the counterregulatory response in rats.<sup>66</sup> However, these are early stage ideas that don't target the underlying etiology of T1D.

In summary: *“Non-insulin adjunct therapies in type 1 diabetes have been proposed as a means of improving glycaemic control and reducing risk of hypoglycaemia. Evidence to support this approach is, however, scant and few pharmacological agents have proved effective enough to become part of routine clinical care.”* (2018)<sup>67</sup>

To restore normal alpha-cell secretory patterns in T1D, a new drug concept is needed that would suppress glucagon secretion in response to rising blood glucose, and that would stimulate glucagon secretion at the onset of hypoglycemia. Defining that new drug target will require a new model of alpha-cell homeostasis, a paradigm that is novel, yet plausible, and theoretically restores both appropriate glucagon suppression during hyperglycemia and stimulation during hypoglycemia. The paradigm should propose a new perspective on alpha-cell control mechanisms directly caused by the beta-cell deficit and suggest a hypothetical drug target which can form the basis for clinical research. Ideally the paradigm should be testable immediately without requiring new drug discovery or delivery technology.

We propose in subsequent sections exactly that new paradigm: the proper dosing of the amylin agonist pramlintide to suppress glucagon during hyperglycemia and restore glucagon counterregulation during hypoglycemia.

## **THE NEW MODEL FOCUSES ON ALPHA-CELL SENSING OF HYPOGLYCEMIA**

Let's start with a controversial puzzle: what disrupts the glucagon counterregulatory response in T1D. *“While the molecular mechanisms involved in the regulation of insulin secretion are well understood, knowledge of those that mediate the inhibition of glucagon release remains fragmentary.”* (December 2018 alpha-cell review.)<sup>68</sup>

Glucagon secretory dysfunction in T1D is probably not due to a global defect in alpha-cell function. They remain normal in number and histological appearance in T1D, and the alpha-cell response to ingested arginine is normal or increased in T1D.<sup>69</sup>

As shown in Exhibit 7, while the amplitude of the T1D diurnal glucagon profile is muted compared to the nondiabetic profile, the T1D pattern is similar.

This similarity of diurnal profiles suggests that the nonglycemic regulation of alpha-cells remains intact, a finding confirmed in studies dating back to 1973.  
44

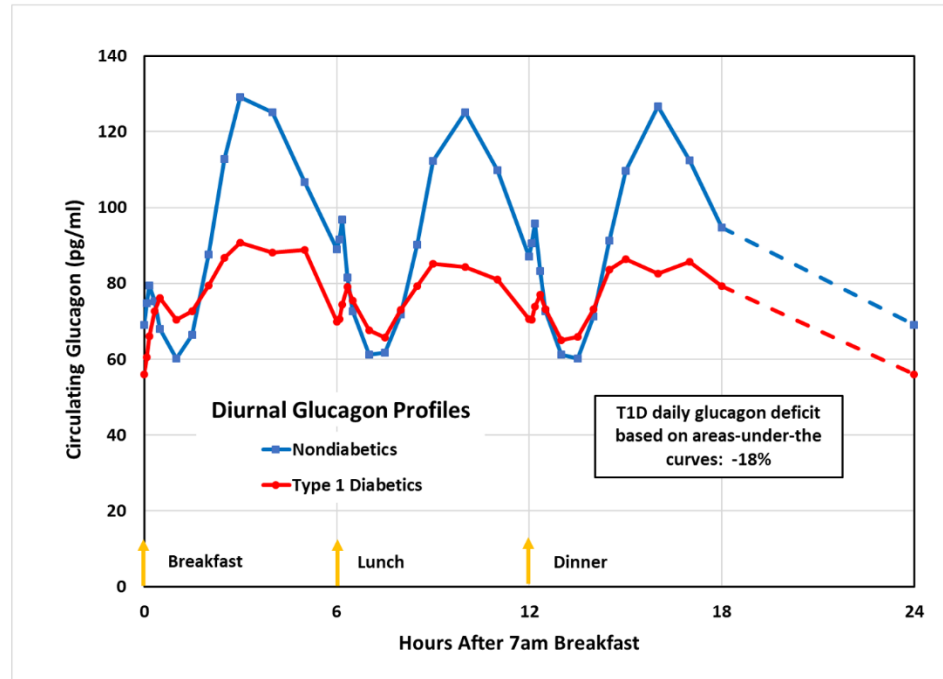
Hypoglycemia sensing appears to lie at the root of defective alpha-cell response, and existing theories of alpha-cell hypoglycemia sensing can be classified into three not mutually exclusive mechanisms:

- **DIRECT** signaling of plasma glucose on alpha-cells: Isolated alpha-cells have been shown in

some experiments to respond to blood glucose levels, but the direct response to hypoglycemia appears to be relatively weak.<sup>70</sup> At the RNA level, glucose regulates proinsulin and prosomatostatin, but not proglucagon.<sup>71</sup> If this were the only, or even most important, glucose sensing mechanism, grossly defective glucagon counterregulation would not be expected to characterize T1D, because the alpha-cells remain sensitive to non-glycemic signals in T1D.

- **PARACRINE** signaling of beta-cell secretions to alpha-cells: Some data (Cryer et al) suggest alpha-cells respond to beta-cell secretions. Over time, T1D patients' decline in C-peptide is mirrored by increasing postprandial glucagon secretions.<sup>69</sup> Insulin, or perhaps zinc, is assumed to be the paracrine messenger.<sup>72 73</sup> This paracrine hypothesis is the basis for the "insulin switch-off model:" insulin secretion downregulates glucagon secretion, especially at mealtime, and hypoglycemia suppresses insulin secretion, causing a glucagon secretion rebound. However, some data (Unger et al 1983) dispute this idea: *"These results indicate that the glucagon response to insulin-induced hypoglycemia is independent of the level of both endogenous intraislet and exogenous arterial insulin concentration in normal man, and that this response may be normal in the absence of endogenous insulin secretion, in contrast to earlier reports. Thus, loss of beta cell function is not responsible for alpha cell failure during insulin-induced hypoglycemia in IDDM."*<sup>74</sup> Suffice it to say that the data with respect to intra-islet regulation of glucagon secretion is contradictory and confusing, probably because it does not consider the possible role of a potent alpha-cell suppressor, the neuroendocrine hormone amylin.
- **CENTRAL NERVOUS SYSTEM** signaling of hypoglycemia to alpha-cells: Autonomic activation is an important player in glucose counterregulation,<sup>75</sup> and the concept of brain control of glucose homeostasis dates back over 150 years to work by Claude Bernard.<sup>76</sup> But a focus on

**Exhibit 7**  
**T1D diurnal glucagon profile is similar to the healthy profile, but muted**



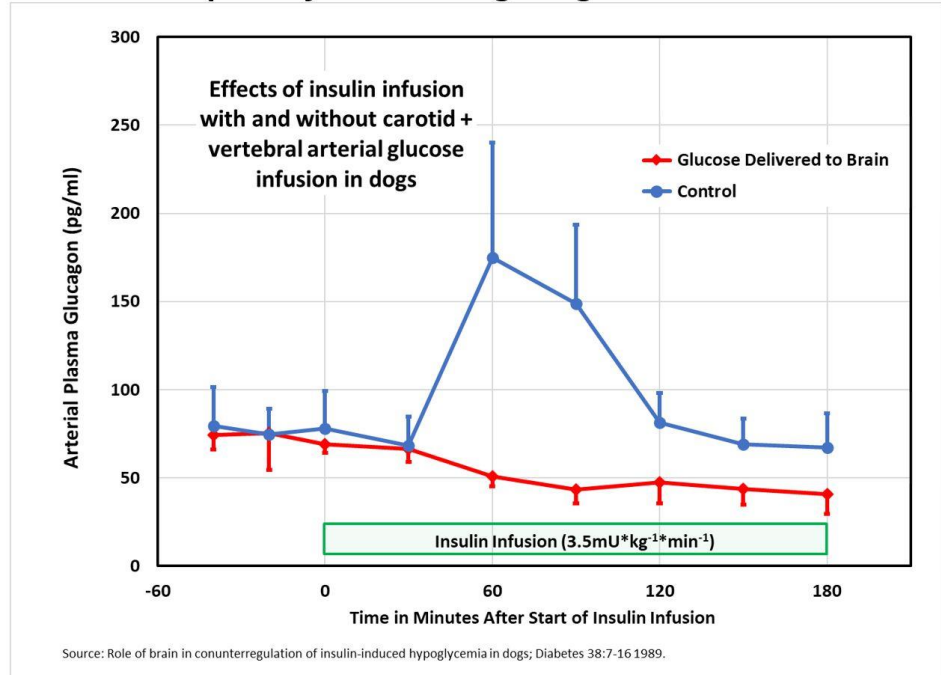
pancreatic regulation by endocrine hormones has overshadowed research on the CNS glucosensory network.<sup>77</sup> The first response to a dangerous fall in glucose is detection by hypoglycemia sensors, including neurons in the hypothalamus and other regions.<sup>78 79 80</sup> *“This indicated that the brainstem may be the most physiologically relevant site of hypoglycemia detection and counterregulation.”*<sup>81</sup>

We favor the view that the CNS is the main mediator of alpha-cell response to hypoglycemia. Substantial evidence exists that autonomic nerves are of major importance for the glucagon response to hypoglycemia in healthy humans and experimental animals.<sup>82</sup> Ganglionic blockade eliminates 75-90% of glucagon counterregulation without affecting glucagon response to arginine administration.<sup>83</sup> Data indicate that the magnitude of the glucagon response to iatrogenic insulin is dependent on the recognition of hypoglycemia by the brain, not the islet.<sup>84</sup>

An experiment in dogs demonstrated that counterregulation is primarily triggered in the brain, not in the pancreas. When dogs were made hypoglycemic, the alpha-cells responded strongly; but, when glucose was infused directly into the brain to maintain local euglycemia, counterregulation was blocked (Exhibit 8).

*“These results suggest that under marked hyperinsulinemic conditions the brain is the primary director of glucagon release and that it is responsible for ~75% of the life-sustaining glucose production.”*<sup>85</sup>

**Exhibit 8**  
**Under marked hyperinsulinemic conditions the brain is the primary director of glucagon release**



CNS control makes teleological sense. Although the adult human brain constitutes only ~2% of body weight, it accounts for ~20% of whole-body glucose utilization.<sup>86</sup> The brain uses 60-80% of blood glucose in a resting state, and the brain cannot store more than a 20-minute supply of glycogen, with the result that low plasma glucose concentrations quickly cause functional brain failure.<sup>87</sup> Thus, the tissue most vulnerable to hypoglycemia is logically the tissue where hypoglycemia is detected.

In summary, we believe that the beta-cells are the primary regulator of alpha-cell response to hyperglycemia, while the brain is the primary regulator of alpha-cell response to hypoglycemia. And we

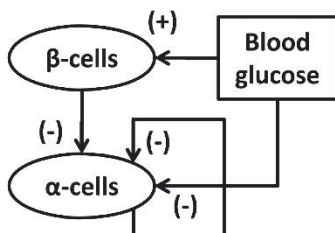
propose that amylin is the signaling mechanism which activates both functions. We now show how a simple systems analysis can support this hypothesis.

## SYSTEMS ANALYSIS SUPPORTS THE IDEA OF AN ALPHA-CELL INHIBITOR

It's been proposed that distributed glucose sensors provide critical inputs to an integrative network, allowing for more finely tuned responses to glycemic challenges.<sup>76</sup> Glucagon, like insulin, is secreted in a basal and pulsatile manner, and studies show pulsatile delivery of glucagon is more potent than continuous delivery, probably because of receptor dynamics. Continuous delivery may suppress receptor expression, or receptors may be more responsive to rate of change in concentration; the latter is supported by the observation that there is a glucagon dose-response to simple carbohydrates that rapidly increase circulating glucose, but there is no dose-response to complex carbohydrates which enter circulation at about half that rate (Appendix A). Alpha-cell pulses are produced in apparently linked antiphase manner to those of insulin and somatostatin, which appear to be highly regulated through a series of controlling influences from within and without the pancreas.

These observations are consistent with the idea that alpha-cells are under constant tonic inhibition by the beta-cells as postulated by the insulin switch-off model. Tonic inhibition of glucagon in T1D rodents reduces glycemic volatility consistent with the insulin switch-off model.<sup>88</sup> However, in a network system, looking at individual interactions doesn't provide a good picture of what's going on, as demonstrated by the apparently contradictory data about alpha-cell sensing. What's needed is a system-level approach combining *in vivo* and *in silico* studies.

To this end, an *in-silico* systems analysis is a new, more comprehensive way of analyzing the switch-off model of glucagon mediated counterregulation. Farhy et al have demonstrated how counterregulation abnormalities can be simulated with a minimal systems network.<sup>89</sup>



Their model builds upon the premise that the specific trigger is probably from the beta-cell. They consider the counterregulatory defect as the failure of a dynamic minimal control network to respond adequately to a hypoglycemia stimulus, and they interpret glucagon counterregulation as a rebound in response to switch-off of the putative alpha-cell inhibitor signal.

Their counterregulatory model simulates glucagon control at this simple systems level:

- In healthy euglycemia, beta-cells generate an alpha-cell inhibitor signal which restrains glucagon secretion. When hypoglycemia occurs, beta-cells shut down secretion of the alpha-cell inhibitor, and alpha-cells rebound with robust glucagon secretion.

- T1D removes the alpha-cell inhibitor signal from the beta-cells, thereby eliminating hyperglycemic control of glucagon secretion, as well as eliminating the tonic inhibition of glucagon secretion required for the shut-off rebound response to hypoglycemia.

As an intervention in T1D, the modelers used somatostatin in animals to validate their *in silico* model.<sup>90</sup> But somatostatin is not a practical solution given the potential side effects of somatostatin and the requirement to quickly lower plasma levels at the onset of hypoglycemia.

As discussed above leptin has also been proposed as an alpha-cell inhibitor in T1D, because leptin's glucose lowering effects are accompanied by normalization of plasma glucagon levels.<sup>91</sup> Preclinical results were encouraging;<sup>54</sup> however, a human pilot study was disappointing.<sup>55</sup>

Thus, we believe the search for a pharmaceutical solution to restoring counterregulation in T1D should focus on finding a drug candidate which satisfies the following criteria:

1. Addresses the core etiology of T1D, beta-cell destruction
2. Is a potent alpha-cell inhibitor
3. Acts at the systemic level, not just the intraislet level, to permit SC delivery
4. Is switched off quickly when blood glucose falls into the hypoglycemic range
5. Is safe to deliver at a continuous basal level
6. Is immediately available for clinical studies
7. Can be scaled up to pharmaceutical production

We propose that an amylin agonist is probably that inhibitor.

## **AMYLIN IS THE IDEAL ALPHA-CELL INHIBITOR**

In 1987 scientists discovered that beta-cells produce a companion hormone to insulin: amylin.<sup>‡</sup> (For a 2015 review of amylin's physiology and pathology, see reference 92.) It seems reasonable to hypothesize that this second secretion is also a key player in glucose homeostasis: teleologically a beta-cell secreted, amidated peptide is an energy hill that Mother Nature wouldn't climb without good reason.<sup>93</sup>

In fact, three decades of research have shown that amylin plays a complementary role to insulin in regulating glucose homeostasis:<sup>94</sup>

- **Insulin** controls glucose efflux (disappearance) from plasma by increasing glucose transport into liver, muscle, and fat storage.

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<sup>‡</sup> Sometimes known as islet amyloid polypeptide (IAPP).



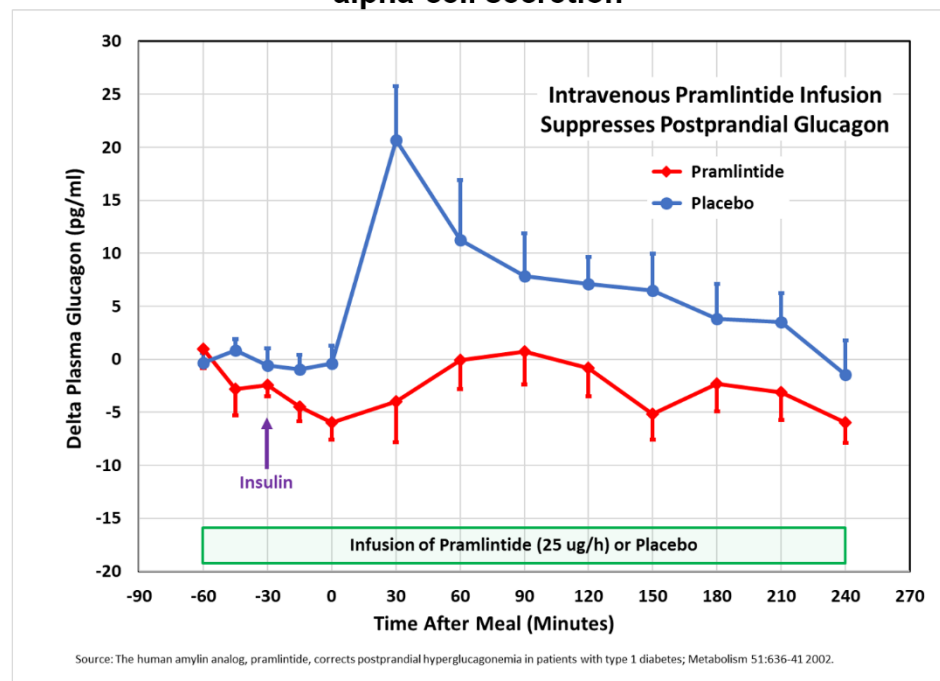
- **Amylin** controls glucose influx (appearance) into plasma by:
  - **Slowing gastric emptying**, which is the primary regulator of the rate of caloric influx from the stomach to systemic circulation.<sup>95</sup> Gastric emptying rate has been estimated to account for about 34% of the variance in peak plasma glucose after a 75-g oral glucose load,<sup>96</sup> and amylin is the most potent among the hormones known to regulate gastric emptying.<sup>97</sup>
  - **Inducing satiety**, which controls the amount of food intake and thereby exogenous glucose absorption.<sup>92</sup>
  - **Suppressing glucagon secretion**, which controls the rate of endogenous glucose production from the liver. Amylin has a potent ( $EC_{50} = 18 \text{ pM}$ ) and profound (~70% inhibition) effect to inhibit amino-acid stimulated glucagon secretion.<sup>98</sup> This suppression is a direct signal to alpha-cells that is not secondary to the slowing of gastric emptying.<sup>107</sup>

As shown in Exhibit 9, amylin is a potent suppressor of postprandial alpha-cell secretion.<sup>99</sup>

Importantly, amylin exerts its glucoregulatory effects through the central nervous system:

- Amylin's primary receptor binding sites are in the brain, and the receptors with access to plasma peptides are in the area postrema.<sup>100</sup> The area postrema provides direct access to neurons of brain areas with vital roles in autonomic control of systems critical to regulating feeding and metabolism, and, the area postrema has been the source of several anti-diabetic and anti-obesity targets.<sup>100</sup>
- Amylin's glucagon suppression effects have been shown to be extrinsic to the pancreas.<sup>101</sup>
- Amylin's modulation of gastric emptying requires an intact vagus nerve.<sup>102</sup>

**Exhibit 9**  
**Amylin is a potent suppressor of postprandial alpha-cell secretion**



Amylin is a neuroendocrine hormone which participates in glucose homeostasis via the central nervous system.

Today it is widely recognized that insulin and amylin balance glucose fluxes at mealtime to prevent hyperglycemia, and an amylin agonist – pramlintide or SYMLIN – is FDA approved as an adjunct to insulin therapy. Pramlintide is indicated for modest HbA1c reductions in T1D: FDA approved labeling says 0.33% HbA1c, which is helpful but not exciting.<sup>20</sup> Moreover, studies have shown pramlintide provides ancillary benefits, including postprandial glucose smoothing<sup>103</sup> and weight loss.<sup>104</sup>

In March 2020, a dual hormone, insulin-amylin AID system was reported that used separate pumps programmed to deliver a fixed dose ratio of pramlintide to insulin for 24-hours.<sup>105</sup> TIR improved from 74% to 84% in the rapid insulin with pramlintide arm compared to the insulin-only arm, and the eAG pointed to an improvement in HbA1c from 6.6% to 6.3%, about the same as with pramlintide delivered with injection pens. These results stimulated an editorial in the same issue titled Rediscovery of the Second Beta-Cell Hormone which concluded, “*The good news for now is that we are rediscovering that diabetes is a two hormone deficiency disorder and beginning to test the potential of co-replacement by continuous infusion systems to overcome the limitations of replacing insulin alone.*”<sup>106</sup>

Encouraging news because the hassle of mealtime injections is the biggest barrier to amylin replacement therapy. However, we are unaware that any of the studies completed, underway, or planned are designed to examine amylin’s role in the glucagon counterregulatory defect of T1D.

### **What points to amylin as the key to restoring counterregulation?**

Amylin replacement is now well documented as helpful in controlling postprandial blood glucose. But, how well does amylin’s known physiology match up to the alpha-cell switch-off signal parameters discussed above? To summarize:

- **Central to T1D etiology.** Amylin is secreted by beta-cells, which is the basic defect in T1D. If it were an important player in glucose homeostasis, its absence in T1D could be expected to play a central role in the pathogenesis of this disease. Thus, it makes sense to consider how amylin deficiency could result in alpha-cell disruption.
- **Potent alpha-cell inhibitor.** Amylin’s known actions on alpha-cells are consistent with tonic inhibition of glucagon as proposed by the *in silico* switch-off model. Amylin is a potent alpha-cell inhibitor, which should make it a key player in any glucagon-centric model of T1D.<sup>107 108</sup> Its alpha-cell regulatory role is implied by its plasma increases at mealtime with insulin, which is the time when glucagon suppression is most needed to minimize hepatic glucose output.
- **Hypoglycemia interrupts inhibition.** Because amylin’s glucoregulatory effect is routed through the CNS, amylin’s alpha-cell inhibitor effect is interrupted when the brain detects hypoglycemia. Amylin suppresses glucagon secretion during euglycemia and hyperglycemia, but during hypoglycemia this suppression is cancelled.

- **Basal levels for tonic inhibition.** Whereas insulin’s diurnal profile concentrates most of its daily exposure in mealtime spikes, amylin’s diurnal profile has a greater basal component.

The next two sections elaborate on these latter two observations.

### The amylin “circuit-breaker”

With respect to gastric emptying, several studies have demonstrated that hypoglycemia accelerates gastric emptying in healthy and T1D subjects. For example, in one study of healthy subjects, during hypoglycemia the half-times for emptying half their stomachs were about 60% less than during euglycemia: <sup>109</sup>

<b>Time to Empty Half the Stomach Contents (minutes)</b>	<b>During Euglycemia</b>	<b>During Hypoglycemia</b>
Solid phase	43.0	16.3
Liquid Phase	38.0	15.4

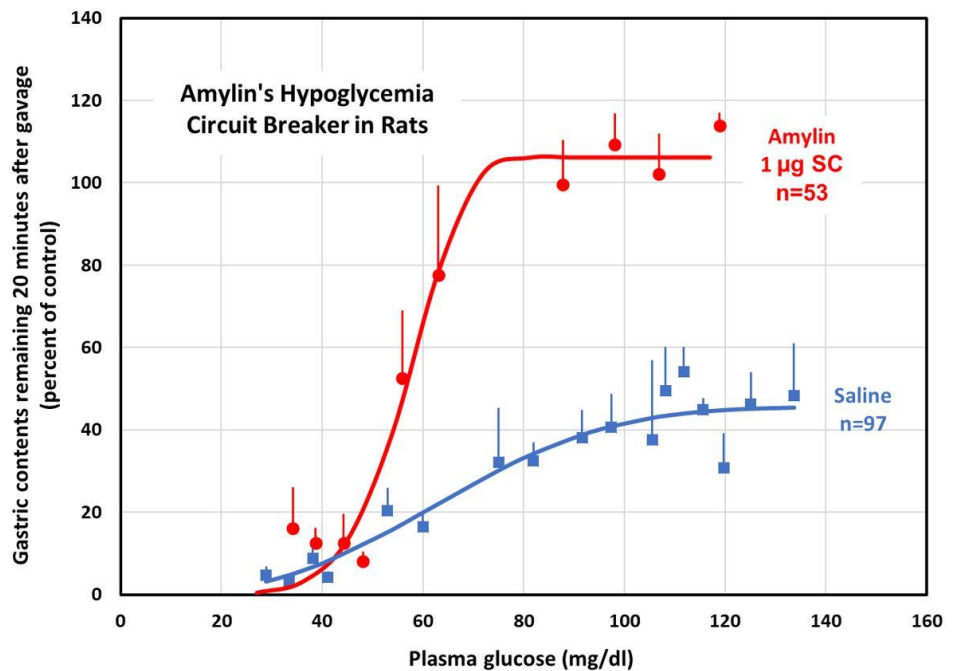
What causes hypoglycemia to accelerate gastric emptying? Answer: in response to hypoglycemia, a CNS-mediated circuit-breaker trips, which cancels amylin’s neural signal to slow gastric emptying. This cancellation occurs independent of circulating amylin concentrations, as shown in Exhibit 10. In this experiment, euglycemic rats were injected with human insulin and either rat amylin or saline immediately before being

gavaged with an acaloric gel containing dye, which produced a wide range of plasma glucose levels. At 20 minutes post-gavage, stomach contents were analyzed for dye retention. <sup>110</sup>

Note that below about 50 mg/dl amylin has no effect on the rate of gastric emptying.

This circuit-breaker effect has also been confirmed for glucagon suppression in humans. Exhibit 11 shows results from a study during which T1D subjects were infused with pramlintide while maintained in either

**Exhibit 10**  
The onset of hypoglycemia cancels the effect of amylin to slow gastric emptying

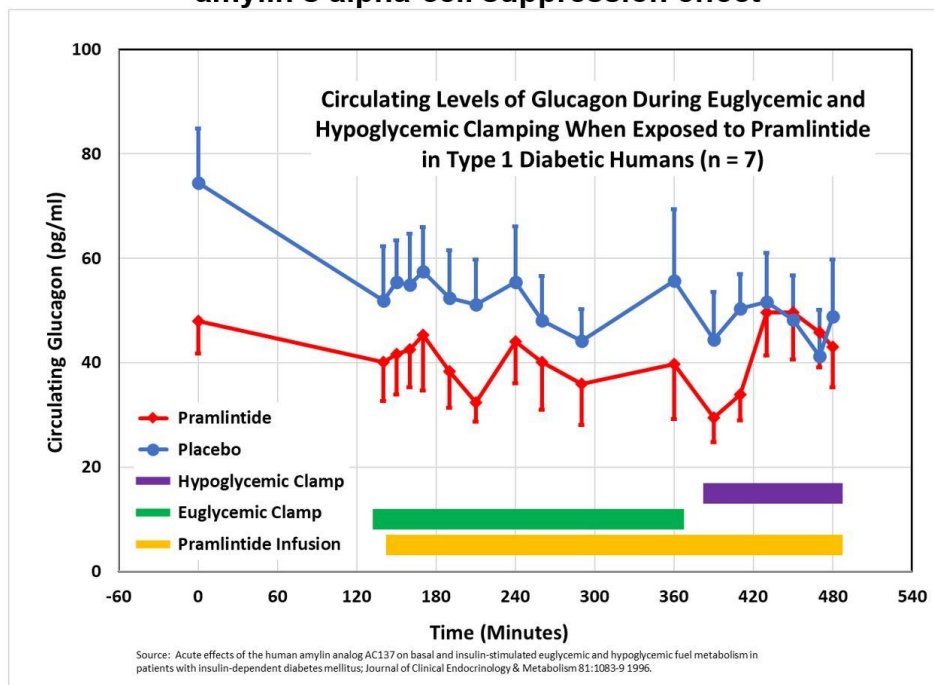


Source: Hypoglycemia overrides amylin-mediated regulation of gastric emptying in rats; Diabetes 47:93-7 1998.

euglycemic or hypoglycemic clamps. During euglycemia alpha-cell secretion was depressed in the pramlintide arm, but this suppression ended with the onset of the hypoglycemic clamp.<sup>111</sup>

These data demonstrate that amylin’s glucose regulatory effects are subject to a CNS-mediated circuit-breaker which kicks open when blood glucose levels in the brain drop into the hypoglycemia range. Even when circulating amylin levels are elevated, the onset of hypoglycemia triggers the circuit-breaker, which immediately shuts down amylin’s restraining effects on glucagon and gastric emptying, thereby amplifying influxes of both hepatic and nutrient glucose.

### Exhibit 11 The onset of hypoglycemia cancels amylin’s alpha-cell suppression effect

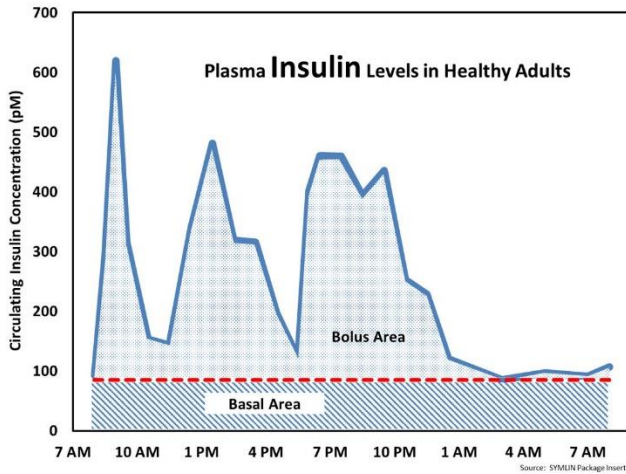


Based on a Google Scholar search, this amylin circuit-breaker does not appear to be widely understood beyond a core group of amylin researchers. In fact, just the opposite impression was created when pramlintide was first approved for clinical use: the drug developed a reputation for *causing* hypoglycemia in T1D patients. During clinical trials the FDA mandated that insulin dosing be held constant, because the studies were designed to demonstrate the independent effects of amylin replacement on HbA1c; as a result patients experienced iatrogenic hypoglycemia from having too much insulin onboard at the same time their glucagon counterregulatory response was defective. To mitigate this risk, the approved labeling for pramlintide recommends reducing premeal short-acting insulin doses by 50%.<sup>11</sup>

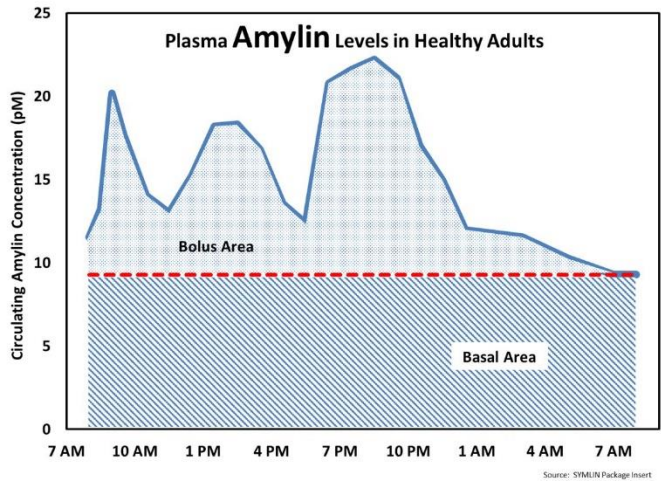
### Amylin provides tonic inhibition

Circulating amylin exhibits a greater basal component than circulating insulin. In one study about 65% of daily insulin exposure in healthy nondiabetics is associated with prandial surges<sup>11</sup>; in another study, the prandial surges accounted for about 74% of daily exposure.<sup>112</sup> Exhibit 12 demonstrates the results from the first study graphically. In contrast, over 60% of daily amylin exposure is from the basal component as shown in Exhibit 13.<sup>11</sup>

**Exhibit 12**  
Circulating insulin is dominated by mealtime boluses



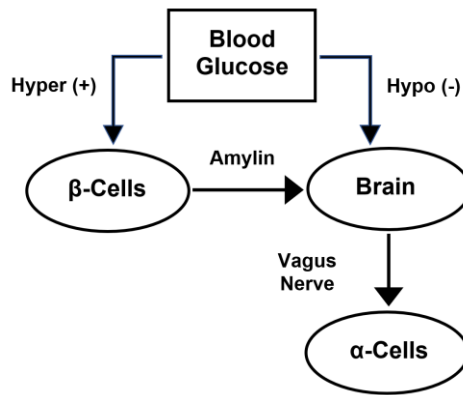
**Exhibit 13**  
Circulating amylin has a large basal component



The amylin basal component of daily exposure is consistent with the need for the tonic inhibition of alpha-cell secretion.

### The amylin circuit-breaker model

We now propose the following revision of the Farhy et al simple system model:



In our model, rising blood glucose is sensed by the beta-cells, which increase secretion of amylin, which activates receptors in the brain, which transmits the amylin signal to the alpha-cells, which respond by decreasing secretion of glucagon. The onset of hypoglycemia is sensed by the brain, which throws the circuit-breaker, which shuts off the amylin signal, causing the alpha-cells to rebound with a surge in glucagon secretion.

If this model is correct, then amylin replacement is an ideal fit with our pharmaceutical criteria for restoring counterregulation in T1D:

Pharma Criteria	Amylin Agonist
Potent alpha-cell inhibitor	Amylin is the most potent peptide inhibitor of alpha-cell secretion reported in the literature.
Acts outside the pancreas	Amylin receptors in the brain respond to circulating levels of amylin
Switches off quickly in response to hypoglycemia	Hypoglycemia has a circuit-breaker effect on alpha-cell suppression
Safe for continuous basal delivery	Pramlintide therapy has shown no safety signals at physiological plasma levels
Addresses the core etiology of T1D	Pramlintide restores amylin deficiency in T1D
Immediately available for clinical studies	Pramlintide is currently marketed by AstraZeneca
Can be scaled pharmaceutically	Pramlintide is already in global distribution for treating T1D

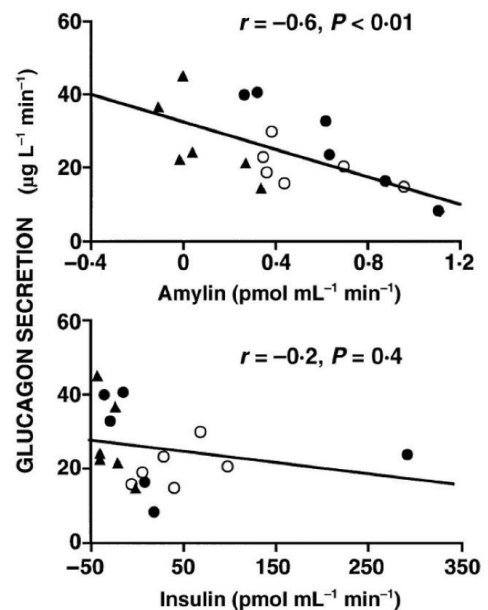
Next, we address the obvious question: is this amylin circuit-breaker model consistent with research results in the field of glucagon counterregulation?

### THERE IS ANECDOTAL SUPPORT FOR THE AMYLIN CIRCUIT-BREAKER HYPOTHESIS

We are proposing a revised model of counterregulation that substitutes amylin for insulin in the alpha-cell switch-off model. Our theory therefore predicts that glucagon secretion should be more closely correlated with amylin plasma levels than with insulin. In fact, this prediction is confirmed by data shown in Exhibit 14.<sup>113</sup>

To quote the source article: “When glucagon secretion was correlated with the insulin and amylin concentrations, both total and supra-basal area-under-the-curve, no significant relationship was detected. However, when glucagon secretion was correlated with the dynamic secretions of amylin and insulin (from C-peptide), i.e. their changes from basal, a significant correlation between the secretion of glucagon and that of amylin ( $R = -0.6$ ,  $P = 0.008$ ), but not with that of insulin ( $R = -0.2$ ,  $P = 0.4$ ) was found. When the subject with very high insulin secretion was excluded, the correlation was even weaker.”

**Exhibit 14**  
Glucagon secretion correlates with amylin secretion, but not insulin



Relationships between glucagon secretion and changes with respect to basal of insulin from C-peptide and amylin. Control (○), obese (●) and diabetic subjects (▲).  
Source: Inverse relation between amylin and glucagon secretion in healthy and diabetic human subjects; European Journal of Clinical Investigations 33:316-22 2003.



If C-peptide secretion is used as a proxy for residual beta-cell function, C-peptide has been shown to correlate negatively with glucose instability in T1D as shown in Exhibit 15. <sup>114</sup>

To quote the authors: “Given that T1DM is the only condition in which such glucose volatility occurs and that T1DM is the only condition in which the islets are devoid of beta-cells, the possibility of a causal relationship between the volatility and the loss of paracrine control of glucagon secretion by insulin (substitute amylin!) seems quite plausible.” <sup>115</sup>

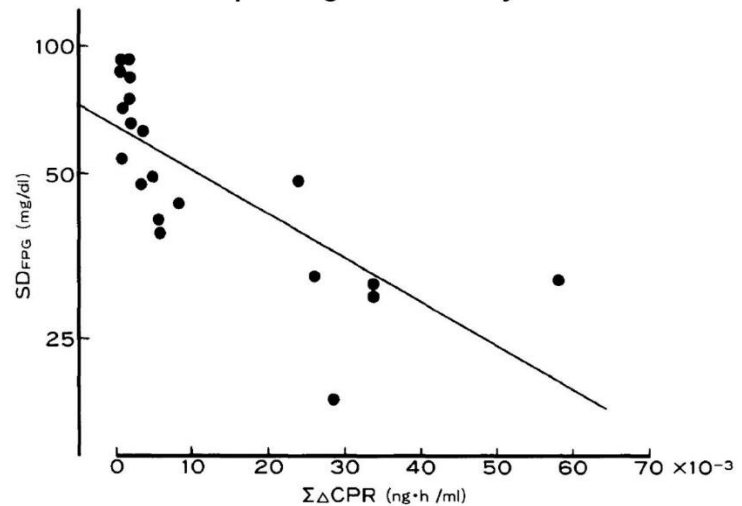
A study published in February 2020 found the following: “(W)e demonstrated significant associations between residual C-peptide secretion and lower glucose variability and low-glucose events in flash glucose monitoring users. These associations were independent of prevailing HbA1c and diabetes duration.” <sup>116</sup>

These findings point to the beta-cells as playing a central role in maintaining blood glucose stability, which is consistent with our theory that amylin is the regulatory link between beta- and alpha-cells.

It is important to note that the effects of amylin have NOT been considered in any interpretation of results aimed at confirming the insulin switch-off model. Recall the 1983 experiment by Unger et al which implied that beta-cells do not regulate alpha-cell secretion; by manipulating only insulin, they clearly missed considering any effects of amylin and the CNS circuit-breaker. <sup>117</sup>

- “(G)lucagon responses were absent during insulin-induced hypoglycemia in diabetic patients who were plasma C-peptide negative but present in patients who were plasma C-peptide positive and suggested that it was the absence of beta-cell function that might be causally related to defective alpha-cell dysfunction during hypoglycemia.”
- “Cryer et al. have championed the hypothesis that defective glucagon secretion during hypoglycemia in diabetic patients might be due to the lack of a switch-off signal from the beta-cell. This hypothesis had earlier been rejected by Bolli et al., who examined glucagon responses during hypoglycemia under conditions of varying exogenous insulin and glucose levels in clamp studies in normal subjects. They found similar glucagon responses under all conditions and concluded that hypoglycemia is the primary signal for glucagon secretion independent of insulin levels.”

**Exhibit 15**  
In T1D, residual beta-cell secretions predict glucose stability



Relationship between incremental C-peptide immunoreactivity areas after intravenous glucagon measured by highly sensitive method (XACPR) and standard deviations of 10 values of fasting plasma glucose (SDFPQ) in 20 patients with IDDM.  $r = -.74$ ,  $P < .01$ .

Source: Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover; The Journal of Clinical Investigation 122:4-12 2012.

In these experiments Bolli et al administered exogenous insulin, but NOT exogenous amylin. So, the results support the conclusion that insulin is not the alpha-cell suppression signal. But, we believe they were infusing the wrong beta-cell hormone to confirm the switch-off model.

Bottom line, experimental designs and interpretations of glucagon counterregulation studies would have been very different had researchers considered the biology of amylin. Reflecting the lack of interest in amylin, at the 2018 American Diabetes Association Scientific Sessions, there were only three presentations that mentioned amylin out of 2,490 oral, poster, and late-breaking submissions.<sup>118</sup>

## **INTRAHEPATIC ISLET TRANSPLANTS DEMONSTRATE CORRECT AMYLIN REPLACEMENT**

The most obvious way to administer amylin replacement therapy in T1D is the use of pramlintide. A less obvious approach has been tested in Phase 3 clinical trials: intrahepatic islet transplants.

*“Special enzymes are used to remove islets from the pancreas of a deceased donor. The islets are purified and counted in a lab. On average, about 400,000 islets are transplanted in each procedure. The islet transplant infusion procedure involves inserting a thin, flexible tube called a catheter through a small cut in the recipient’s upper abdomen. A radiologist uses x-rays and ultrasound to guide the catheter into the portal vein of the liver. The islets are slowly infused through the catheter and into the liver by gravity. Alternatively, a minimally invasive open procedure can be used to directly visualize a vein near the liver to insert the catheter.”<sup>119</sup>*

Islet transplantation is indicated for patients with T1D who suffer from disabling, severe hypoglycemia events despite optimized insulin therapy. It is NOT indicated for patients as a way of lowering HbA1c or of eliminating the need for insulin injections. Because transplants are precisely directed at correcting a potentially catastrophic failure of counterregulation, it is instructive to consider whether observed effects of transplants are predicted by our amylin circuit-breaker model.

With respect to glucose counterregulation, islet transplants have been shown to be remarkably effective.

- **Transplants increase the glycemic threshold for glucagon activation:** In a study of activation thresholds, islet transplants were found to almost restore the level observed in nondiabetic control subjects:<sup>120</sup>

<b>Glycemic Thresholds for Glucagon Activation (mg/dl)</b>	
Nondiabetic Control Subjects (n=8)	74 ± 4
Type 1 Diabetes Subjects (n=6)	48 ± 2
Islet Transplant Recipients (n=7)	70 ± 7

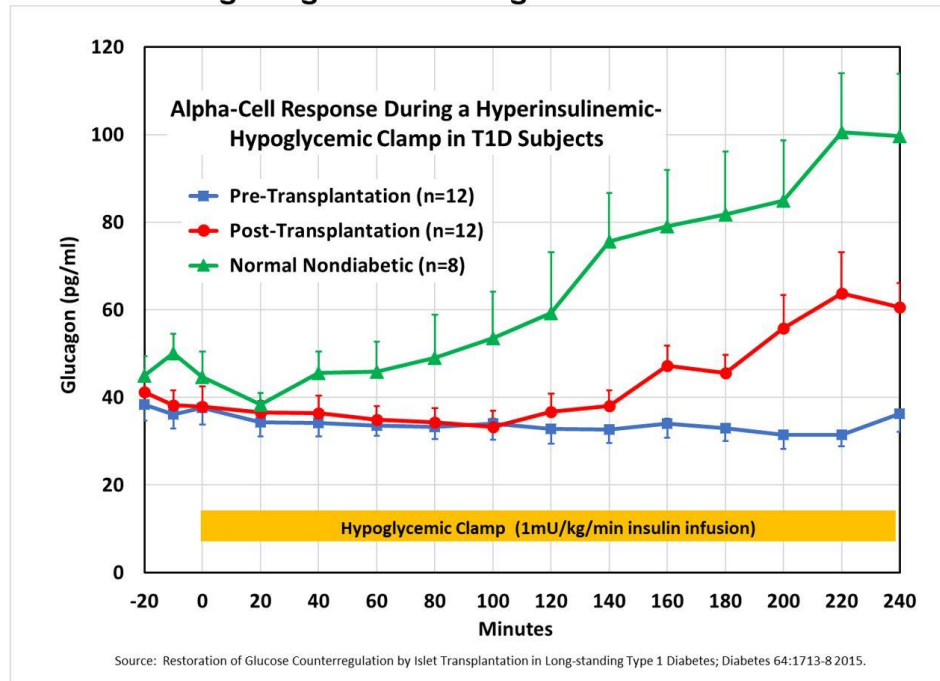
- **Transplants restore partial glucagon counterregulation:** Another study demonstrated a partial restoration of the glucagon response to hypoglycemia, as shown in Exhibit 16.<sup>121</sup>



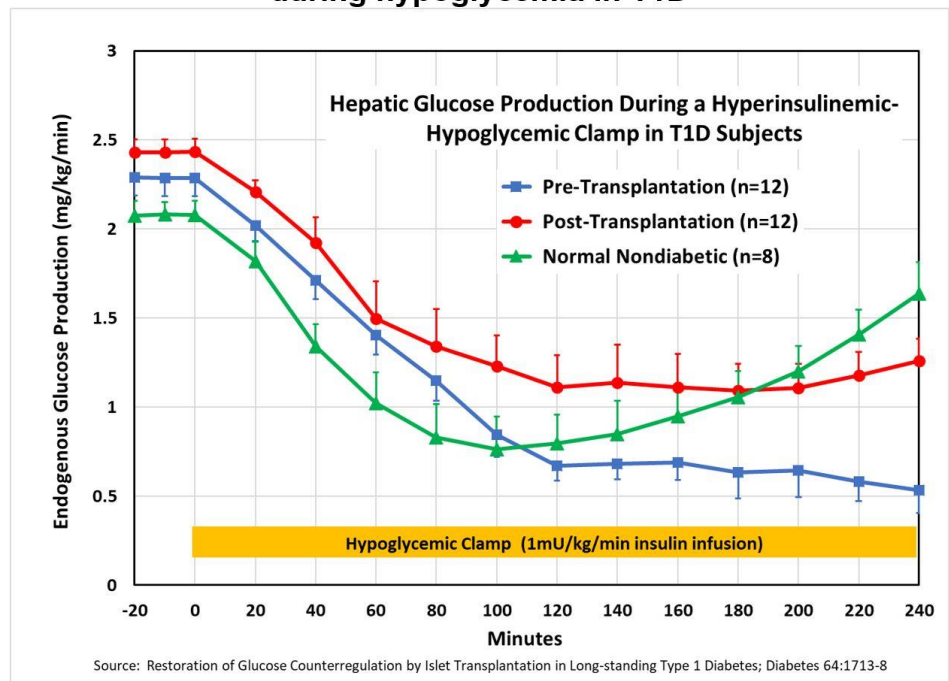
- **Transplants increase hepatic glucose output during hypoglycemia:** The same study demonstrated islet transplants increased the supply of glucose from the liver during hypoglycemia, as shown in Exhibit 17.

- **Transplants improve HbA1c and prevent serious hypoglycemia events:** In a Phase 3 study of islet transplantation, recipients' average HbA1c declined from 7.2% at baseline to 5.6% one year later. Subjects in this study had all suffered during the prior year at least one serious hypoglycemic event requiring assistance, even though they were under the care of an endocrinologist or diabetologist and had adhered to recommended glucose monitoring and insulin therapy (77% used CSII and 44% used CGM; n=48). Over the two year period following transplantation, recipients were virtually risk free of serious hypoglycemia events, as shown in Exhibit 18.<sup>122</sup>

**Exhibit 16**  
Islet transplantation partially restores glucagon counterregulation in T1D

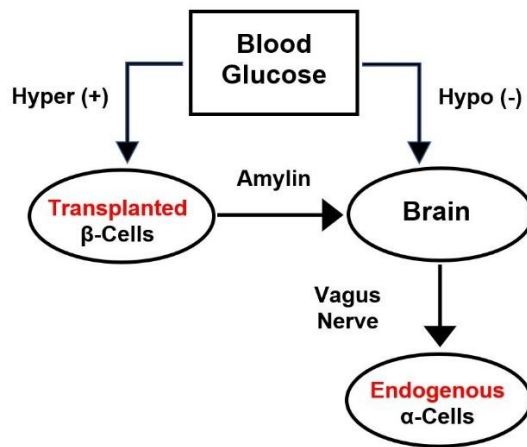
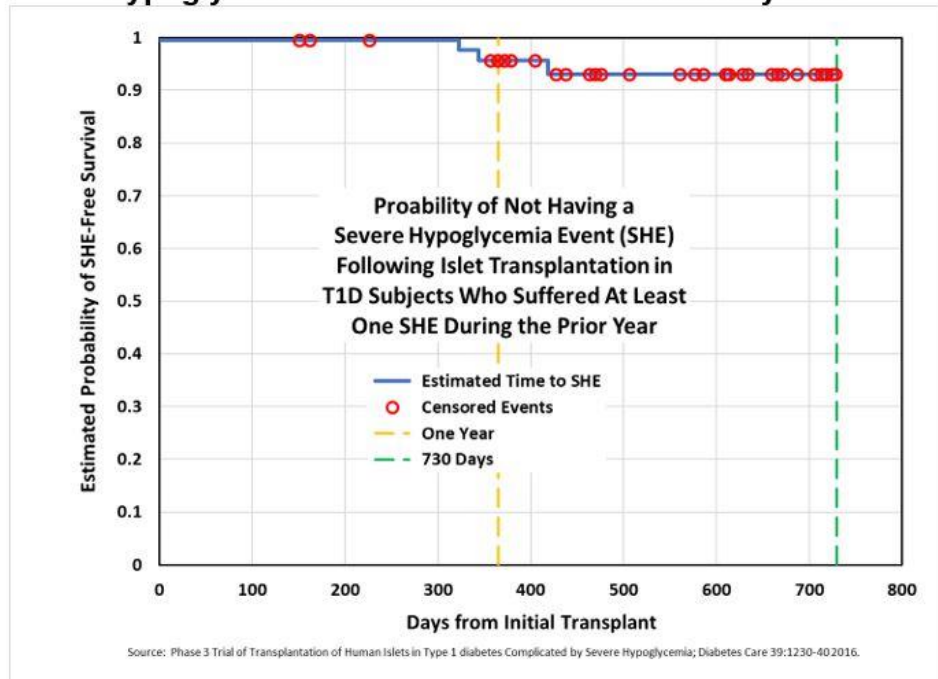


**Exhibit 17**  
Islet transplants increase hepatic glucose during hypoglycemia in T1D



Our view is that islet transplants partially restore the glucagon counterregulatory response because the transplanted beta-cells deliver a relatively normal diurnal profile of circulating amylin. At first blush, this proposal would appear to contradict our CNS-mediated circuit-breaker model, because the transplanted alpha-cells are NOT innervated and could not respond to tonic inhibition via the CNS. This apparent conflict can be easily resolved by assuming that the restored counterregulatory response occurred in the endogenous alpha-cells, not in the transplanted islets, as shown in this version of the circuit-breaker model:

**Exhibit 18**  
**Islet transplants virtually eliminate severe hypoglycemia events in T1D for at least two years**



We believe that, with respect to alpha-cell function, islet transplants are a physiologically correct way to deliver amylin replacement therapy, so that it restores counterregulation in patients' own pancreatic islets. And, we think there is reason to expect appropriate delivery of pramlintide could do an even better job of restoring counterregulation:

- In the studies cited above, the impaired glucagon response was attributed to the mass of surviving transplanted islets being less than normal as estimated by measurement of the beta-cell secretory capacity. If so, the tonic inhibition of alpha-cells may be muted. Correct dosing of pramlintide might resolve that deficiency.

- Because the transplanted alpha-cells are denervated and therefore not subject to tonic inhibition by amylin, their unrestrained glucagon secretion may interfere with full restoration of counterregulation. Pramlintide infusions would avoid this complication.

Bottom line, we think that islet transplant restoration of counterregulation is consistent with our circuit-breaker model, and that this observation underscores the need to test our model in the clinic with pramlintide infusions.

Why is there not yet evidence from clinical studies? Is it not be reasonable to expect that, after 15-some years of pramlintide patient use, there would be some signal that amylin plays a role in the glucagon counterregulatory response? In fact, an extensive literature search turns up no clinical study results that directly support the amylin circuit-breaker model, presumably because there have been no studies designed to test whether amylin replacement can restore glucagon counterregulation. As for anecdotal evidence among patients using pramlintide, we believe that the absence of supporting data can be attributed to inappropriate dosing of pramlintide, as will be discussed in Part 2.

After a decade on the market, pramlintide has not been considered for restoring glucagon counterregulation. In the early 1990s, preclinical studies suggested that *“an amylin agonist may have utility in protecting diabetic individuals from hypoglycemia. However, the spectrum of actions present in rodents was different from those in humans, and this indication was not pursued.”*<sup>123</sup> Prior to the development of the amylin circuit-breaker model, it was not plausible to propose that a hormone which suppresses glucagon secretion would be the key player in stimulating glucagon counterregulation.

\* \* \* \* \*

Nevertheless, the question remains: how could pramlintide be used to treat T1D since 2005 and not show any anecdotal evidence of restoring glucose counterregulation? We believe the answer to that question lies in the dosing of pramlintide. As described in Part 2, mealtime doses of pramlintide do NOT provide the tonic inhibition required for the amylin circuit-breaker model to work properly.

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<sup>122</sup> Phase 3 trial of transplantation of human islets in Type 1 Diabetes complicated by severe hypoglycemia; *Diabetes Care* 39:1230-40 2016.

<sup>123</sup> Amylin and the integrated control of nutrient influx; *Advances in Pharmacology* 52:67-77 2005.

## The Amylin Circuit-Breaker – Part 2 Getting the Amylin Dosing Right

The amylin circuit-breaker model is an hypothesis built on published knowledge about alpha-cell regulation and amylin physiology. It proposes that appropriately dosed amylin replacement therapy might not only correct prandial hyperglucagonemia, but also restore the glucagon counterregulatory response in T1D.

However, an amylin agonist – pramlintide – has been used to treat T1D since 2005 without any documented evidence of lowering the risk of iatrogenic hypoglycemia. Moreover, the poor benefit-to-burden tradeoff has discouraged widespread use of pramlintide. How, then, can the disappointing history of pramlintide be explained if the amylin circuit-breaker model is correct?

In this Part 2 we address the following topics:

- **Why do beta-cells make two hormones?** We believe this teleological question points to a novel hypothesis about amylin secretion: diurnal profiles of insulin and amylin need to be different to achieve glycemic homeostasis.
- **How could beta-cells generate two different diurnal profiles?** Differences in peptide clearance and secretion rates of amylin and insulin would result in different pulsatile diurnal profiles to optimize timing of signals that regulate glucose influx and efflux.
- **How do the plasma profiles of insulin and amylin compare?** Available data support the conclusions that circulating levels of these two hormones generate variable ratios over diurnal cycles, and that amylin exposure is primarily basal as opposed to the bolus exposure of insulin.
- **How well does pramlintide dosing mimic endogenous amylin?** Comparison of FDA-approved pramlintide dosing results *in vivo* to endogenous amylin diurnal profiles provides an explanation for the poor efficacy and tolerability of mealtime injections.
- **How could pramlintide dosing be changed to better mimic endogenous amylin profiles?** We propose that dual hormone AID systems using different basal and bolus ratios of pramlintide to insulin would optimize amylin replacement therapy. Other approaches using formulations and devices now under development might also restore some of the counterregulatory response in T1D.

In Part 3 we make a start at designing clinical research aimed at testing our hypothesis.

### BETA-CELLS SECRETE TWO HORMONES FOR A GOOD REASON

To continue our “outside the box” model building, we now ask a teleological question: why would beta-cells make two hormones, given that one hormone would be the more energy efficient way for beta-cells to exert their glucoregulatory authority? In this regard we quote William of Ockham: “*Pluralitas non*



*est ponenda sine neccesitate*” or “*Entities should not be multiplied unnecessarily.*” As we pointed out in Part 1, an amidated peptide hormone is an energy hill that Mother Nature doesn’t want to climb without good reason.

In other words, why couldn’t circulating insulin serve as the neuroendocrine message from beta-cells to the central nervous system? Beta-cell secretion of insulin has already been proposed as the alpha-cell inhibitor that controls glucagon secretion, and this “insulin switch-off model” necessarily assumes insulin’s secretion profile is appropriate for alpha-cell suppression. Couldn’t the CNS cells which respond to circulating plasma amylin instead detect plasma insulin, since plasma insulin levels rise at mealtimes when slowing of gastric emptying and suppression of glucagon secretion are needed? And, the hypoglycemia circuit-breaker could work just as well with insulin as the beta-cell signal to the CNS.

We believe the most plausible rationale for the second beta-cell hormone is that the diurnal plasma profile of insulin is not appropriate for regulation of blood glucose influx from the gut and liver. Insulin secretion is tightly correlated with circulating glucose and is aimed at controlling blood glucose efflux into liver, muscle, and fat tissues at mealtimes. If the diurnal profile that optimizes blood glucose efflux is different from the optimal influx profile, then a single hormone could not properly regulate both efflux and influx.

To test this theory, we start by asking: do the physiologies of insulin and amylin predict differences in circulating profiles?

## **PHYSIOLOGY POINTS TO DIFFERENCES IN HORMONE DIURNAL PROFILES**

How could the circulating concentrations of two hormones found in the same secretory granules display different diurnal profiles? We believe the explanation lies in two mechanisms: plasma clearance rates, and beta-cell secretion rates.

### **Different clearance rates**

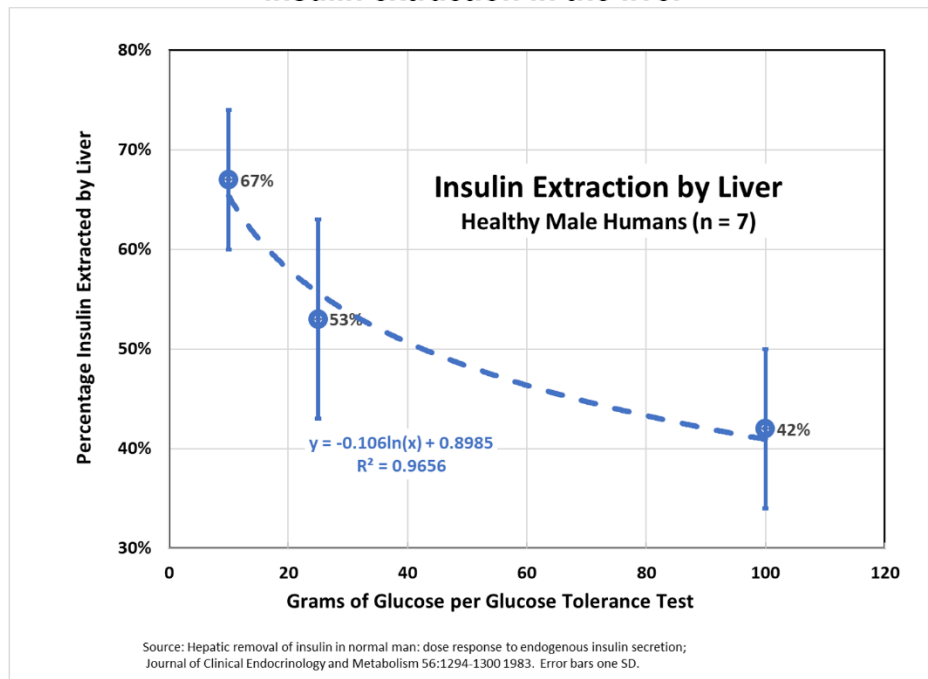
For diurnally pulsatile hormones, differences in plasma clearance rates would cause variations in circulating molar ratios. Following mealtime pulses, the hormone with a slower clearance rate would retain relatively higher plasma concentrations. In the present case, clearance mechanisms are quite different for the two beta-cell hormones.

Insulin is cleared by the liver, and studies have calculated an average hepatic extraction rate of about 50% of insulin appearing in the portal circulation.<sup>1</sup> It has also been shown that increasing liver exposure to insulin results in decreasing insulin extraction in the liver, which would result in amplifying post-hepatic insulin concentrations (boluses) at higher rates of beta-cell secretion (Exhibit 1).<sup>2</sup>

Amylin, in contrast, is cleared by the kidneys, and mathematical modeling has shown that amylin’s clearance rate is three- to four-fold slower than that of insulin.<sup>3</sup> Amylin’s longer half-life results in a slower rate of decline after mealtime bolus secretions, which would predict relatively higher basal, postabsorptive levels between meals.

These differences in clearance mechanisms and rates between the two beta-cell hormones predict that they should have differing profiles with respect to absorptive vs. postabsorptive states. As stated in a 1998 study of amylin distribution and kinetics: *“The lower clearance rate of amylin, which is close to that of C-peptide, as well as the higher mean residence time compared to insulin, indicate that the commonly used insulin-to-amylin ratio is not applicable under non-steady-state conditions.”*<sup>4</sup>

### Exhibit 1 Increasing insulin exposure causes decreasing Insulin extraction in the liver



### Different secretion rates

Beta-cell secretion rates of insulin and amylin may disconnect under certain circumstances. The literature is replete with contradictory evidence about whether the secreted ratio of amylin/insulin can vary; for example:

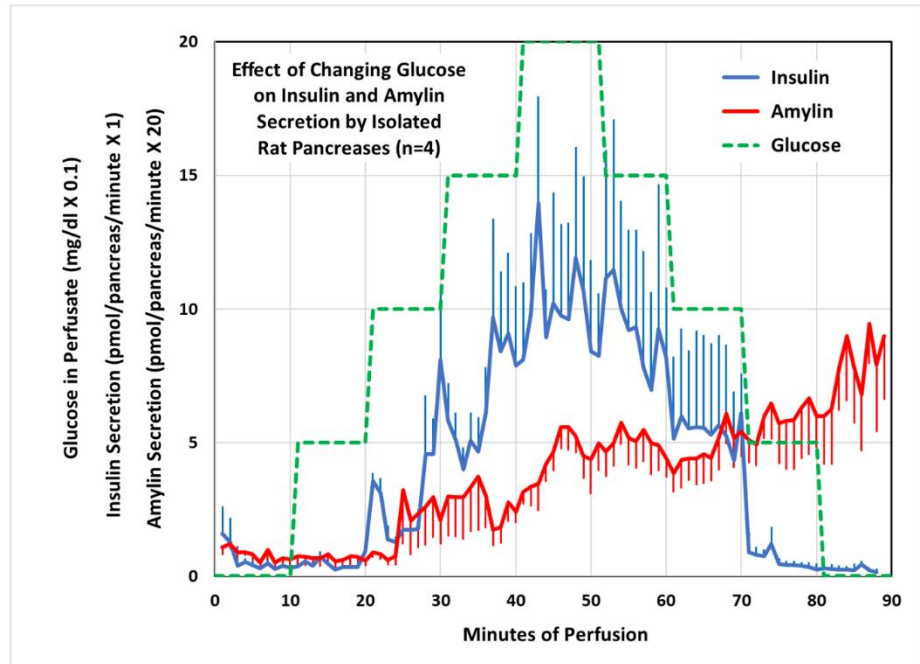
- *“We describe two conditions where the release of (amylin) and insulin are dissociated making it unlikely that (amylin) is always co-released from beta-cell granules that contain both peptides and participate only in regulated secretion.”*<sup>5</sup>
- *“Significant differences in the insulin-(amylin) ratios between experimental groups is consistent with the hypothesis that production of (amylin) and insulin are regulated differently in the beta-cell.”*<sup>6</sup>
- *“These results are consistent with the hypothesis that the regulation of (amylin) secretion from beta-cells of isolated rat pancreatic islets is essentially regulated by the same mechanisms as insulin.”*<sup>7</sup>
- *“In summary, it appears that, acutely, the secreted ratio of amylin:insulin is comparatively invariant, but long-standing hyperglycemia may favor induction of amylin synthesis and secretion over that of insulin.”*<sup>8</sup>

One study of perfused rat pancreases looked at the time course of insulin and amylin secretion when stimulated by stepwise rising and falling levels of perfusate glucose.<sup>9</sup> For normal, non-diabetic rats,

insulin secretion closely tracked glucose concentration, while amylin displayed a slowly rising secretion rate (Exhibit 2).

These different rates of response to rising blood glucose would act to vary the prandial peaks of insulin relative to those of amylin as a function of meal size. This study also demonstrated that amylin/insulin molar secretion ratios were different in diabetic rats compared to healthy, control rats:

### Exhibit 2 Secretion of insulin and amylin can be disconnected



Amylin (red) and insulin (blue) secretion (pmol/pancreas/min) in response to a stepped glucose profile (green) during one-minute intervals from the isolated perfused pancreases of control Wistar rats (n=4). Results are plotted as mean (±s.e.m.) of duplicate estimations at each point for each of the four rats.

Type of Rat	Amylin/Insulin Ratio
Healthy Wistar	8.9%
Diabetic Fatty Zucker	6.3%

*“These results are the first demonstration of the existence of a mechanism within the pancreas that enables differential secretion of amylin and insulin. In both normal and diabetic rat pancreases, amylin secretion continued to rise after glucose levels in the perfusate fell, whereas in both cases, insulin levels either fell towards basal (normal) or continued to decline (diabetic).”<sup>9</sup>*

While these results were in isolated rat pancreases, they are evidence that – under certain circumstances – amylin and insulin secretion rates may become disconnected. And, they suggest that diurnal insulin exposure should be more concentrated in prandial spikes, whereas diurnal amylin exposure should have a larger basal component.

\* \* \* \* \*

In summary, because diurnal beta-cell secretions are pulsatile, differences in clearance rates predict differing profiles of insulin and amylin circulating concentrations. Amylin’s much slower plasma clearance rate predicts relatively higher postabsorptive plasma concentrations. The variable rate of

insulin clearance in the liver would amplify prandial insulin peaks in circulation at higher rates of glucose influx without equal amplification of amylin's profile. Likewise, observed secretion differences predict that more of insulin's daily exposure would occur during prandial plasma peaks ("boluses"), while amylin's predicted exposure would be more spread out over the day, thus resulting in relatively higher basal levels of plasma amylin compared to plasma insulin.

What do the data show with respect to diurnal profiles of insulins and amylin?

### **AMYLIN IS THE MORE "BASAL" OF THE TWO BETA-CELL HORMONES**

First, a word about clinical assays for hormones. Peptides are measured with immunoassays which can be afflicted with specificity and calibration problems. Insulin and glucagon have been measured since the late 1960s, and it may be difficult to directly compare results over time as assay technology has evolved. Amylin has been measured since the early 1990s, and early assays were plagued by difficulties in working with the human amylin peptide, which tends to aggregate. So, the results reviewed in this section may be subject to adjustments when clinical research can validate them with the latest monoclonal immunometric technology. But these preliminary observations should be useful for defining more detailed hypotheses to be validated in the clinic.

Also, several amylin researchers have complained that, as of early 2020, there don't appear to be reliable amylin assays on the market. They report that amylin testing results are deemed not usable in publications. As a result, we are unable to find recent studies that present diurnal profiles of circulating amylin in healthy nondiabetics comparable to those reporting insulin and glucagon profiles. So, at this stage, we are reduced to presenting old, usually small studies that give, at best, a fragmentary picture.

\* \* \* \* \*

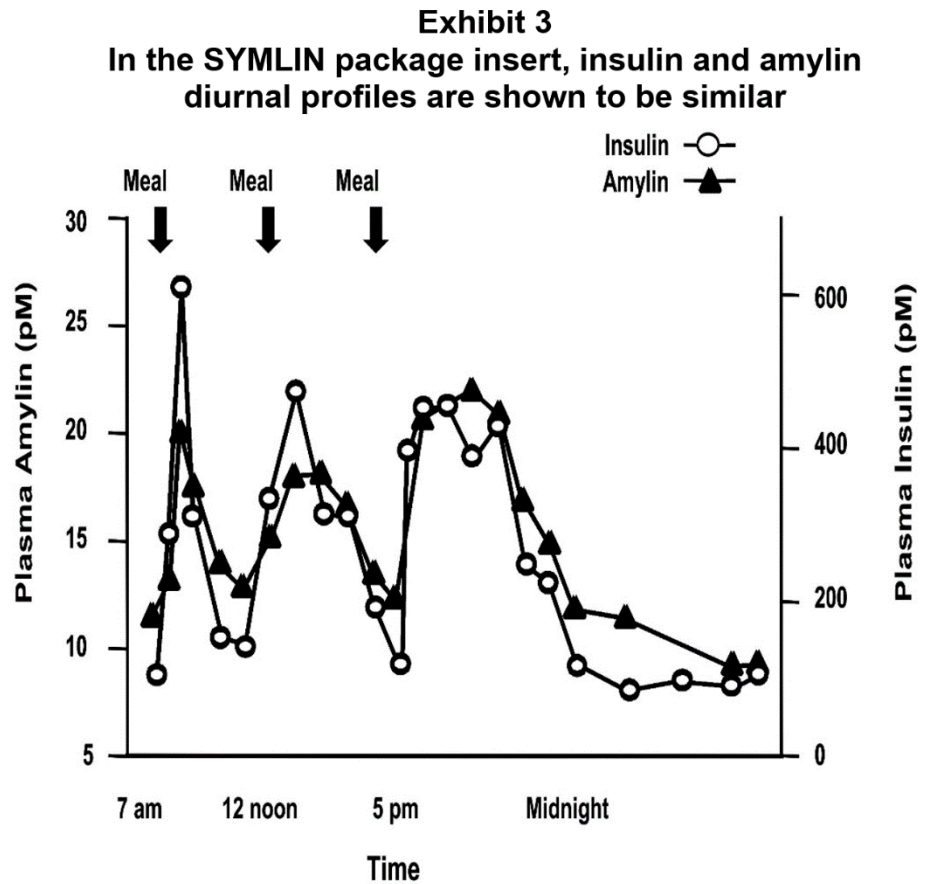
By 1989 it was known that insulin and amylin are colocalized in the secretory granules of beta-cells.<sup>10</sup> Shortly thereafter it was shown that glucose ingestion stimulates increases in circulating insulin and amylin, and that there is a correlation between insulin and amylin:<sup>11</sup>

<b>Correlation of Amylin with Insulin</b>	
Plasma concentration:	R-squared = 0.55
Change in plasma concentration:	R-squared = 0.42

These observations led to the general perception that insulin and amylin are co-secreted in parallel by beta-cells, resulting in similar diurnal profiles. An example appears in the FDA-approved SYMLIN Prescribing Information published by the manufacturer of pramlintide (Exhibit 3).<sup>12</sup>

Published literature generally echoes this view:

- A textbook example: “Amylin is secreted in equal proportions to insulin from beta-islet cells and causes a reduction in glycogenesis in skeletal muscle, a decrease in postprandial glucagon secretion, slows gastric emptying, and suppresses appetite.” (2013) <sup>13</sup>



- A review article example: “In general, amylin secretion from the b-cell correlates very tightly with insulin secretion.” (2015) <sup>14</sup> (Note that the R-squared values of 0.55 and 0.42 reported above can hardly be considered “very tightly.”)
- We have been unable to find any published sources that say the diurnal profiles of insulin and amylin differ in any important way.

But, a closer look at the chart in Exhibit 3 reveals a graphical trick: The vertical scales of the hormone concentrations have been graphed with separate vertical axes to allow both hormone profiles to be plotted on the same chart:

- **Scale of values:** The amylin scale of concentrations is about 4% of the insulin scale. The much lower plasma concentration of amylin is consistent with its role as a neuroendocrine hormone directed at brain receptors, while insulin’s peripheral actions regulate a greater tissue mass of liver, muscle, and fat. This scaling of the graph is legitimate for visually comparing the timing of diurnal patterns.
- **Range of values:** However, while the insulin concentration axis starts at zero, the amylin axis starts at 5 picomolar, thereby forcing an alignment of the insulin and amylin diurnal profiles. This distortion in the amylin range of values is misleading with respect to the magnitude of hormone basal and prandial levels.

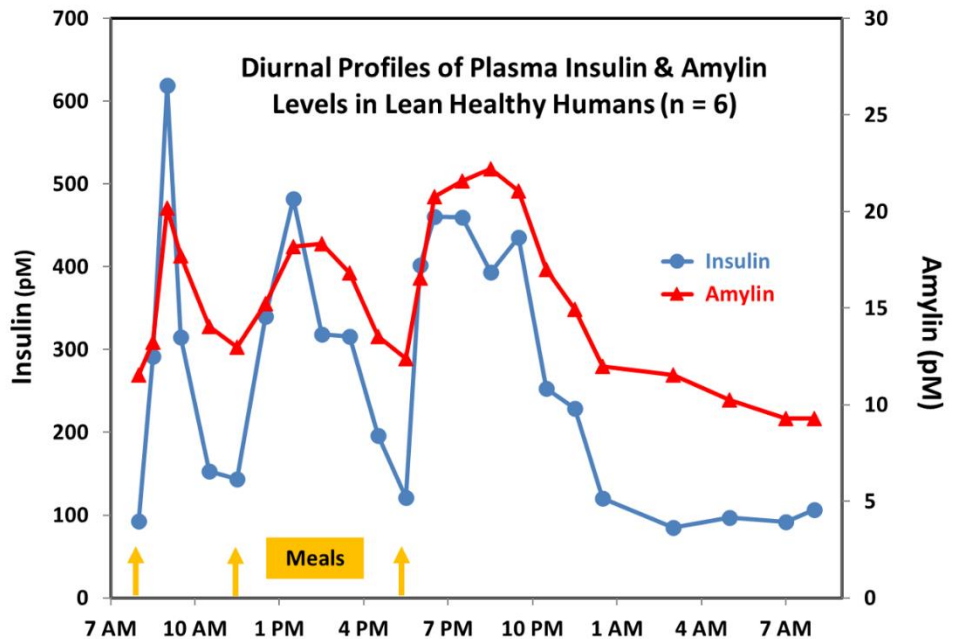
When Exhibit 3 is plotted correctly, the plasma profiles diverge (Exhibit 4).

While the plasma peaks are aligned at mealtimes, the molar ratio of the two hormones varies about 4-fold over a 24-hour period, from absorptive (mealtime) ratios of over 30:1 insulin:amylin, to postabsorptive (between meals) ratios of about 8:1 (Exhibit 5).

The sample size of human subjects that generated this data is relatively small (n = 6), so further study is warranted. However, these results are consistent with our hypothesis that, contrary to conventional wisdom, insulin and amylin diurnal profiles do NOT align precisely: while the timings of the diurnal pulses are in alignment, the ratios of resulting hormone circulating concentrations appear to vary widely.

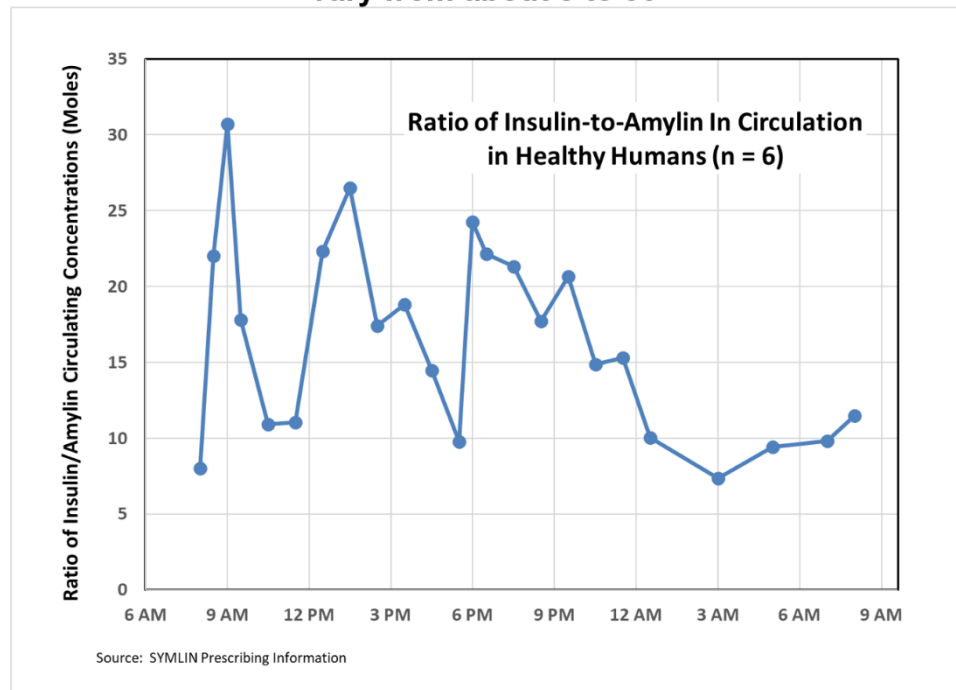
Several other studies support this conclusion. Exhibit 6 shows the molar insulin/amylin ratios measured for healthy, nondiabetic subjects following either mixed meals or 75 gram oral glucose tolerance tests.<sup>15 16 17 18 19</sup> It should be noted that these five studies were performed in the early

### Exhibit 4 Diurnal profiles of insulin and amylin are NOT the same



Source: FDA approved prescribing information for SYMLIN (pramlintide acetate) injection.

### Exhibit 5 Diurnal ratios of circulating insulin-to-amylin vary from about 8 to 30



Source: SYMLIN Prescribing Information



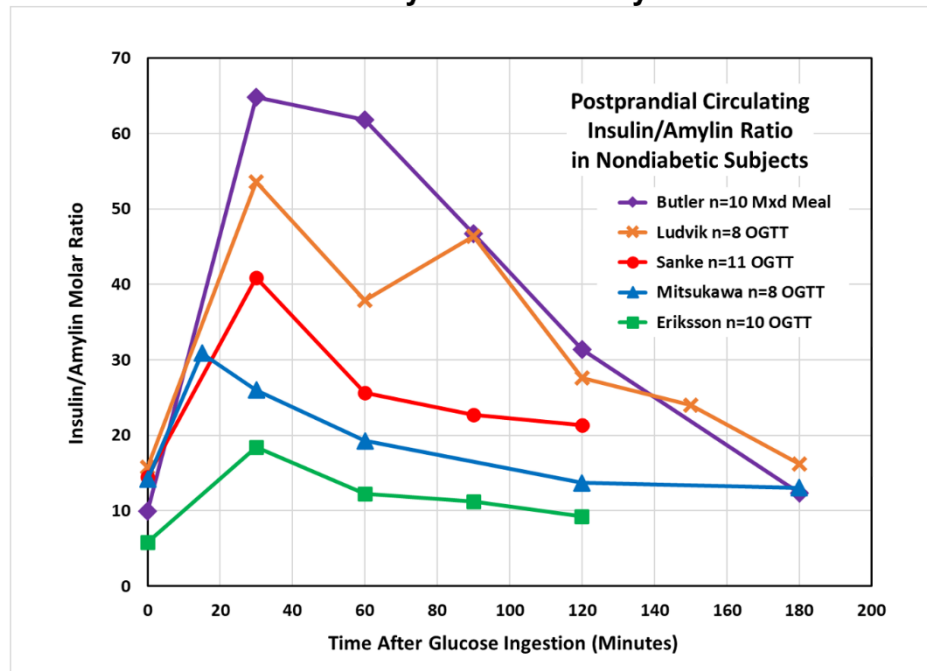
1990s before standardized commercial assays for amylin were available; consequently, the absolute ratios of insulin to amylin are not directly comparable.

These data demonstrate changes in insulin/amylin molar ratios of two- to four-fold following meals. This finding supports the hypothesis that the reason for two beta-cell hormones is that the appropriate diurnal profile for regulating blood glucose influx (amylin) is different from that regulating efflux (insulin).

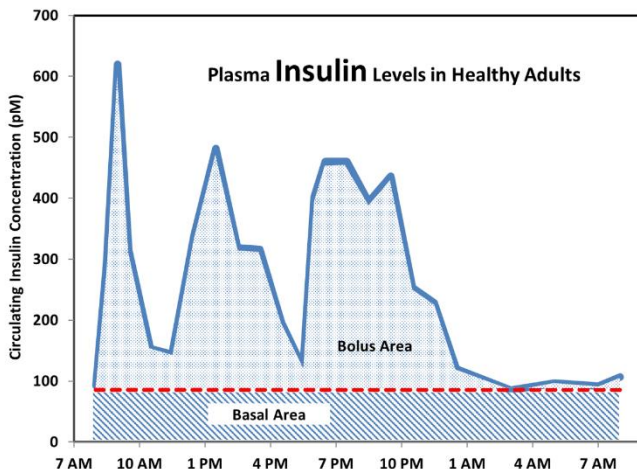
Quoting the conclusion in yet another study: “We conclude that the profile of plasma total and nonglycosylated amylin concentrations, as determined under similar steady-state hyperglycemic conditions in subjects with varying degrees of glucose tolerance, differ markedly from that of insulin. These differences could reflect either slower clearance of amylin than insulin or differences in the secretory dynamics of the two peptides.”<sup>20</sup>

Another way to visualize the difference in beta-cell hormone profiles is by separating the areas under the curves into basal (fasting) vs. bolus (mealtime) components, as shown in Exhibits 7 and 8 (Exhibits 10 and 11 of Part 1).

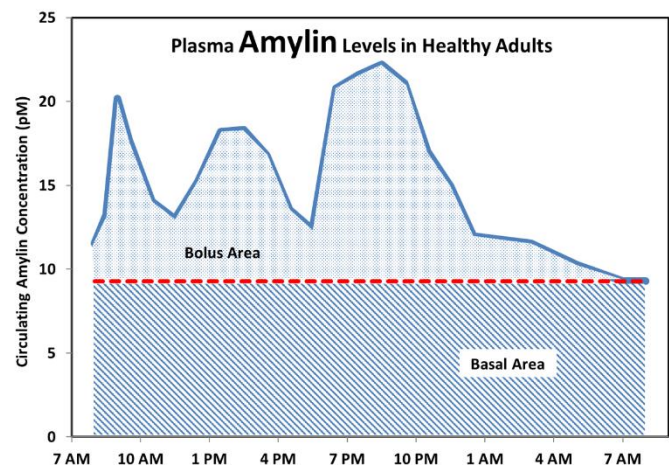
**Exhibit 6**  
Multiple studies indicate that the insulin/amylin ratio varies widely over diurnal cycles



**Exhibit 7**  
Circulating insulin is dominated by mealtime boluses



**Exhibit 8**  
Circulating amylin has a large basal component





These data suggest that, in healthy non-diabetics, about two-thirds of insulin’s total daily exposure is associated with mealtime peaks (boluses), whereas only about one-third of amylin’s daily exposure is bolus:

	Basal Level	Based upon AUCs:	
		Bolus/Basal Ratio	Basal as % Total
Insulin	85.2 pM	1.9	35%
Amylin	9.3 pM	0.6	62%

Insulin is well documented to be predominantly a bolus signal with relatively modest basal levels between meals. This is consistent with aggressively pushing circulating glucose into liver, muscle, and fat at mealtimes, while mostly shutting down insulin-stimulated efflux between meals when brain and other tissues are consuming plasma glucose without beta-cell stimulation. As discussed in Appendix A, correlation coefficients for the glucose-insulin diurnal profiles are in the 0.90+ range.

Amylin, in contrast, appears to be primarily a basal signal with relatively modest peaks at mealtime. This diurnal pattern is consistent (1) with maintaining tonic inhibition of alpha-cells between meals as predicted by the amylin circuit-breaker model, and (2) with moderating glucose influx at mealtime by slowing gastric emptying and suppressing glucagon secretion.

If the goal of amylin replacement therapy is to properly restore amylin’s regulatory role in T1D, how well do exogenous amylin agonist infusions mimic the healthy, nondiabetic diurnal profile of endogenous amylin in healthy nondiabetics?

### Exhibit 9

#### Pramlintide dosing is determined by patient tolerability

#### MEALTIME DOSING OF PRAMLINTIDE IS DYSFUNCTIONAL

The pramlintide dosing instructions for patients with T1D are shown in Exhibit 9.

Patients are advised to inject pramlintide before each meal at the maximum tolerated dose as determined by up-titrating dosing until nausea is experienced, then backing off to the next lower dose.

#### Initiation of SYMLIN therapy

In patients with type 1 diabetes, pramlintide should be initiated at a dose of 15µg and titrated at 15µg increments to a maintenance dose of 30µg or 60µg as tolerated:

- Initiate pramlintide at a starting dose of 15µg subcutaneously, immediately prior to major meals;
- Reduce pre-prandial, rapid-acting or short-acting insulin dosages, including fixed-mix insulins (e.g., 70/30) by 50%;
- Monitor blood glucose frequently, including pre- and post-meals and at bedtime;
- Increase the pramlintide dose to the next increment (30µg, 45µg, or 60µg) when no clinically significant nausea has occurred for at least 3 days. If significant nausea persists at the 45 or 60µg dose level, the pramlintide dose should be decreased to 30µg. If the 30µg dose is not tolerated, discontinuation of pramlintide therapy should be considered;

Adjust insulin doses to optimize glycemic control once the target dose of pramlintide is achieved and nausea (if experienced) has subsided.

The pharmacokinetics of pramlintide are comparable to rapid acting insulin. The SYMLIN package insert states that, in healthy subjects, the half-life of SYMLIN is about 48 minutes (Exhibit 10).<sup>21</sup>

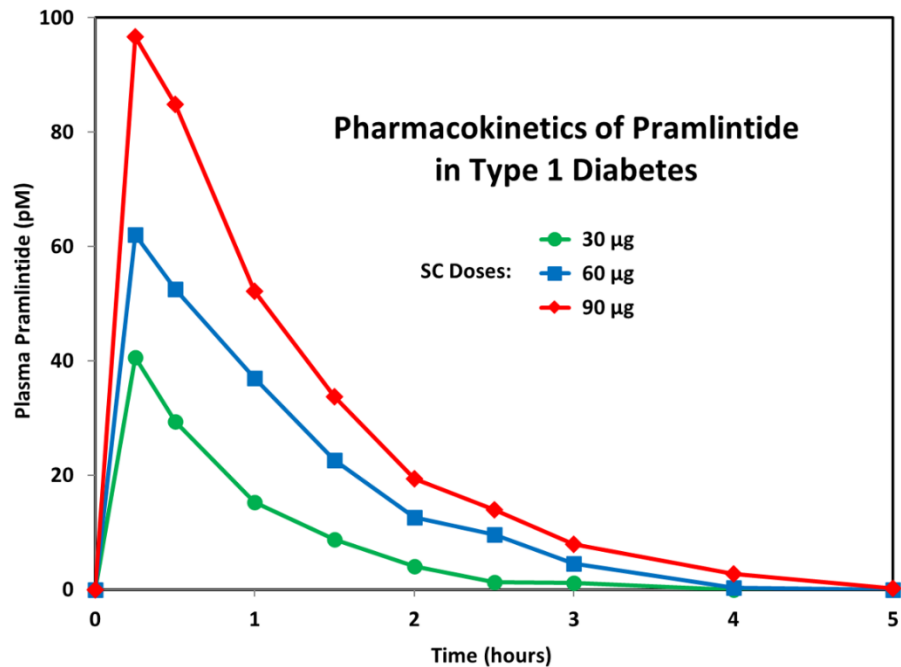
Insulin glulisine (APIDRA) has an apparent half-life of 42 minutes, and Exhibit 11 compares the pharmacokinetics of SYMLIN to HUMALOG (fast acting insulin) and HUMULIN (regular insulin).<sup>22</sup>

Because of pramlintide's relatively rapid pharmacokinetics, about 90% of each pramlintide injection is removed from circulation within 90 minutes post-injection. As shown in Exhibit 12, pramlintide's suppression of glucagon secretion at the physiologically relevant 30 µg dose has disappeared after about two hours.<sup>23</sup>

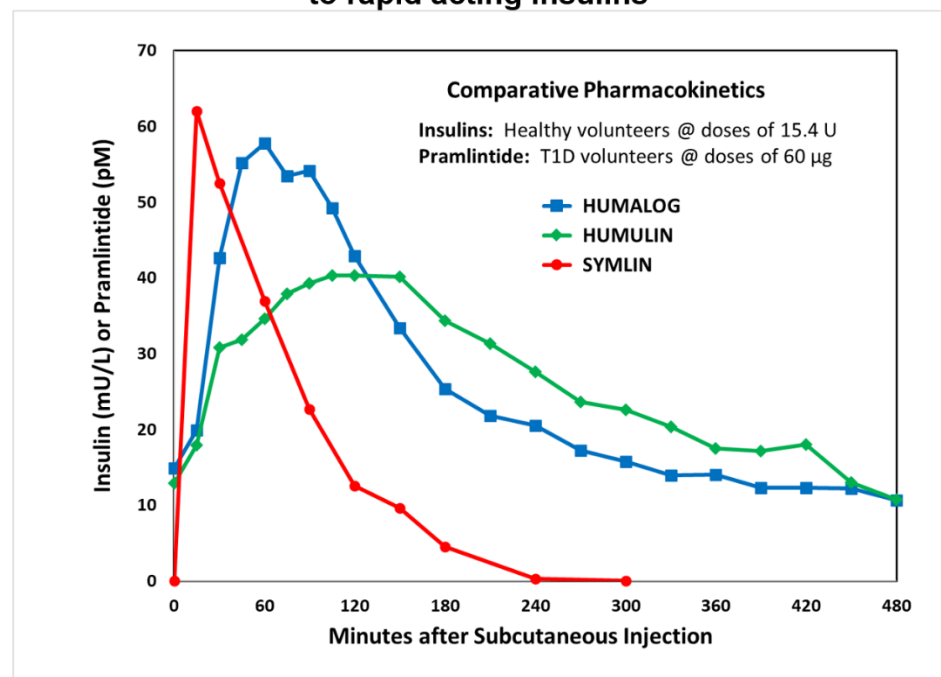
How well does pramlintide dosing mimic the healthy diurnal profile of amylin's plasma concentration? In Exhibit 13, the plot of pramlintide pharmacokinetics shown in Exhibit 10 is overlaid on the healthy plasma profile of amylin shown in Exhibit 4, assuming three mealtime doses daily.

Immediately following injections, pramlintide's plasma concentration spikes into pharmacological levels, which explains why nausea is the dose limiting adverse event, given that amylin receptors are in the

**Exhibit 10**  
Pramlintide's half-life following injection is about 48 minutes



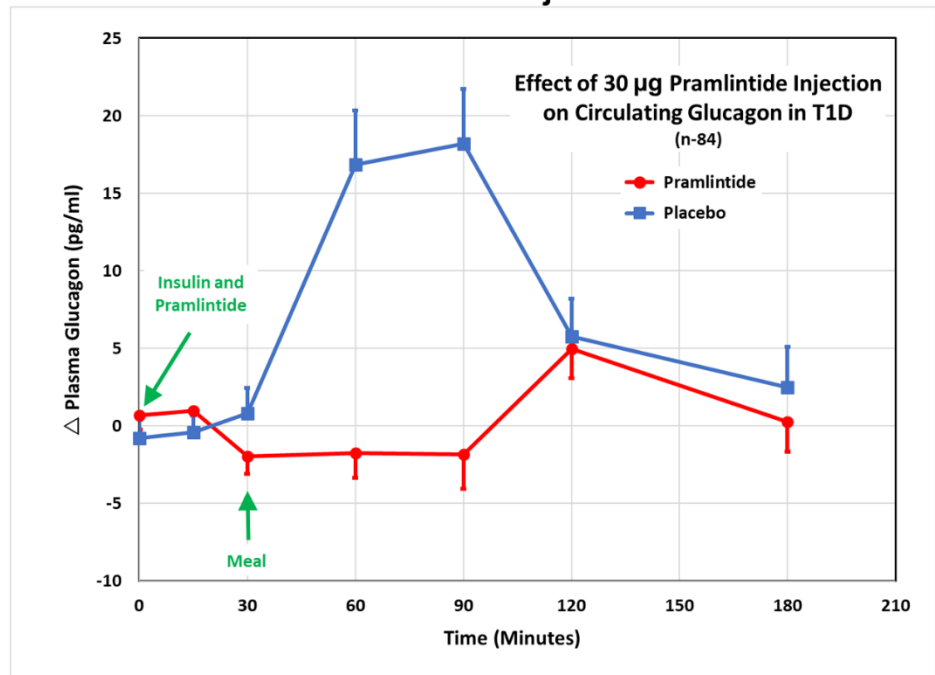
**Exhibit 11**  
Pramlintide's pharmacokinetics are comparable to rapid acting insulins



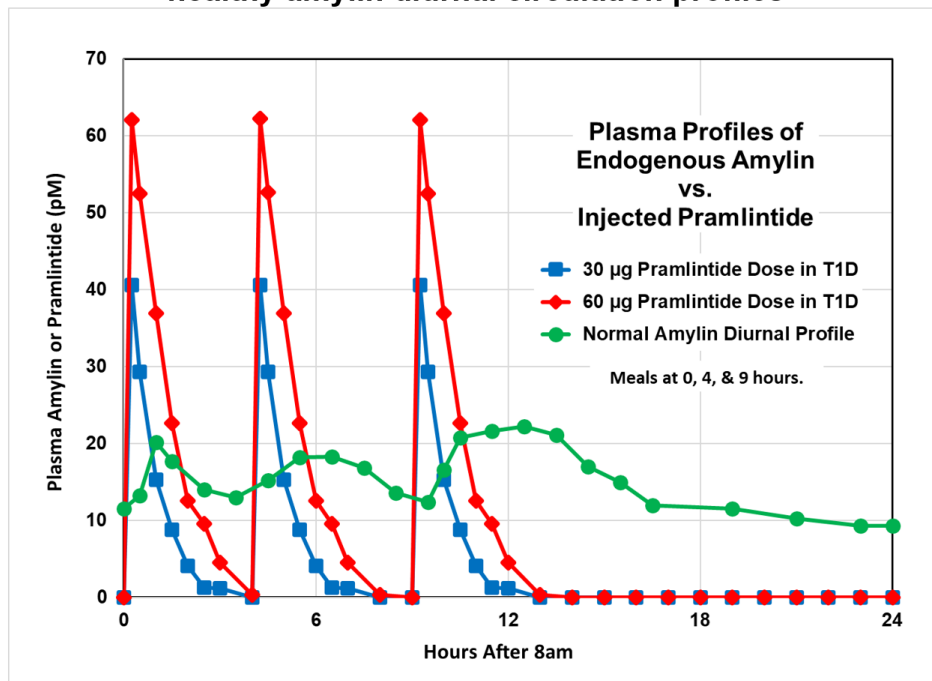
“vomit center” of the brain. Within a couple of hours after injections, pramlintide’s plasma concentration falls to undetectable levels, which eliminates the basal component of amylin’s daily profile, and disables the tonic inhibition of glucagon secretion.

At the highest recommended dose of 60 µg, the total daily pramlintide exposure is only about two-thirds the amylin exposure in healthy, non-diabetic individuals, based on measuring the areas under the diurnal profiles. At 30 µg injections T1D pramlintide delivers less than one-third of the normal daily amylin coverage (Exhibit 14).

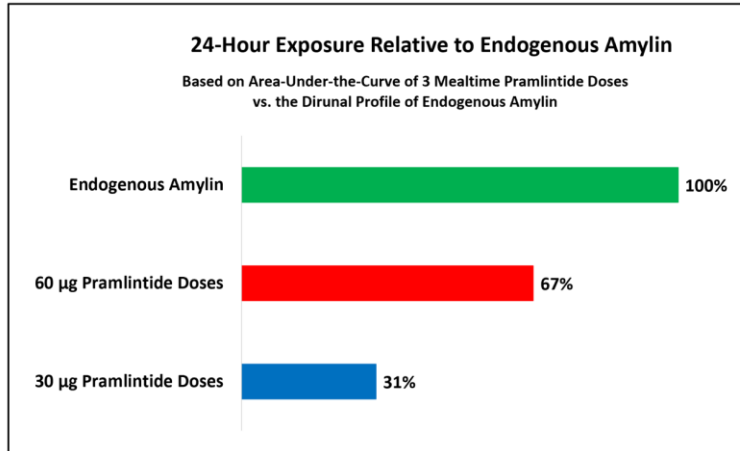
**Exhibit 12**  
**Pramlintide suppression of glucagon wears off about two hours after injections**



**Exhibit 13**  
**Pramlintide mealtime dosing does NOT mimic healthy amylin diurnal circulation profiles**



**Exhibit 14**  
**At the highest FDA-approved dose, daily exposure to pramlintide is only two-thirds of normal amylin exposure**

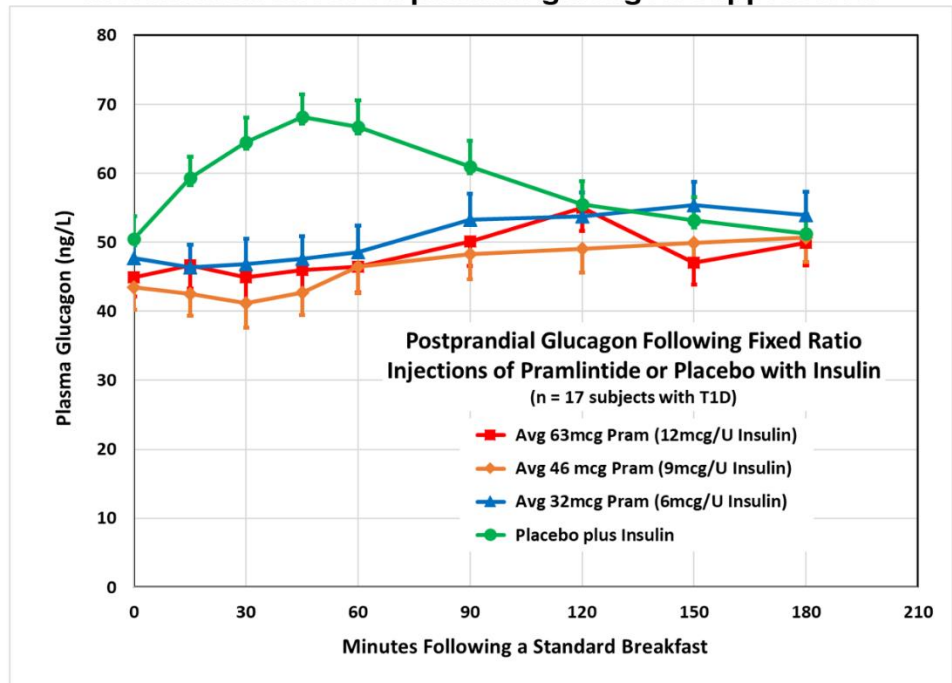


In light of the mealtime overdosing suggested by this analysis, it's interesting to consider the results of a 2015 study of fixed ratio dosing of insulin and amylin.<sup>24</sup> Three fixed ratios of pramlintide to regular insulin were tested in 17 T1D subjects. Results are shown in Exhibit 15, and they suggest that, on average, the ~30 µg pramlintide dose is at or above the top of the dose/response curve for mealtime glucagon suppression. Consequently, if patients follow the FDA-approved guideline for titrating doses upward until nausea sets in, then many patients are over-dosing pramlintide for prandial glucagon suppression.

In summary, a comparison of plasma levels for pramlintide injections with the diurnal profile of healthy endogenous amylin concentrations indicates that the FDA-approved dosing regimen does NOT mimic the natural hormone. Instead it causes:

- Over-dosing at mealtimes, which does not improve glucagon suppression and results in intolerable nausea, and
- Under-dosing between meals and especially

**Exhibit 15**  
**Doses above about 30µg appear to have very little incremental effect on prandial glucagon suppression**



overnight, which would deactivate the tonic inhibition of glucagon necessary for the amylin switch-off mechanism to activate the glucagon counterregulatory response.

No wonder most T1D patients have found pramlintide to be intolerable and ineffective!

## **THE SOLUTION IS DUAL HORMONE AUTOMATED INSULIN DELIVERY**

How should pramlintide dosing be altered to improve efficacy and tolerability? We've considered the following strategies:

- Dual hormone automated insulin delivery (AID) systems
- Other formulations and devices

The remainder of Part 2 will discuss each of these approaches.

### **Dual hormone AID systems**

Our view is that the way to administer both insulin and amylin in a fashion that most closely mimics normal human physiology is to use AID technology. Back in the 1990s, when development of pramlintide was started, the idea of pumping pramlintide never came up, because it would have been commercially impossible. Today the idea of a dual hormone AID system is not only a practical consideration, it is actively under development at Beta Bionics (Boston) and Inreda Diabetic (Netherlands), as illustrated in Exhibit 16.<sup>25 26</sup>

**Exhibit 16**  
Two companies are developing dual hormone pumps



Both pumps have been designed to infuse insulin and glucagon; the idea is that iatrogenic hypoglycemia can be mitigated by infusing glucagon to arrest declining blood glucose. The main challenge has been the availability of a stable glucagon formulation that could be stored in the pump cartridges. Not only has pramlintide been shown to be stable at room temperature while in use by patients, but pump studies have already demonstrated the feasibility of pumping pramlintide.

So, these dual chamber pumps could be repurposed to infuse pramlintide. Two alternative amylin infusion algorithms are plausible:

- **Independent amylin algorithm:** Pramlintide infusions would be determined by a separate dosing algorithm. A new amylin algorithm would be designed to reflect the differing magnitudes of basal and bolus levels required to correctly manage blood glucose influx.
- **Insulin-dependent amylin algorithm:** Pramlintide infusions would be determined as a ratio to insulin infusions, with the basal and bolus infusion ratios being different to reflect the differences in diurnal hormone profiles.

Our view is that the second, “Dual Ratio Amylin/Insulin” (DRAI) algorithm is likely to be the best approach to delivering an amylin analog via an AID system, as we now explain.

All AID systems incorporate dosing algorithms which deliver two types of infusions:

- **Basal:** A continuous, slow rate of insulin infusion interrupted only when blood glucose is projected to drop into hypoglycemia territory. During this period the ratio of circulating amylin/insulin should be highest to maintain the tonic inhibition of alpha-cells.
- **Bolus:** Short bursts of insulin infusion used at mealtimes to counteract the influx of glucose from the gut. During these periods the ratio of circulating amylin/insulin should be lower to avoid triggering nausea while suppressing glucagon and slowing gastric emptying.

Decreasing the amylin/insulin ratio during bolus infusions would prevent the nausea-inducing spikes at mealtimes; increasing the basal infusion rate would maintain the tonic inhibition of alpha-cells between meals. The ability to dial in different basal and bolus infusion ratios would be simple to implement, and the ratios could be tweaked to optimize the efficacy for individual patients.

Our calculations indicate that the following ratios would be a good starting point for clinical research (see Appendix B for derivation of these ratios):

Basal infusions:	6 $\mu\text{g}$ pramlintide per Unit insulin
Bolus infusions:	2 $\mu\text{g}$ pramlintide per Unit insulin

In silico modelling predicted an optimal ratio of 9  $\mu\text{g}/\text{U}$ , and a recently completed dual hormone AID study used a ratio of 6  $\mu\text{g}/\text{U}$ , and these ratios are consistent with our dual ratio calculations.<sup>27 28</sup>

Although there are not yet commercially available bihormonal pumps, clinical studies using separate pumps could begin immediately to validate the DRAI idea. The earliest amylin pump study was

reported in 2009 using separate pumps for pramlintide and insulin, so this approach has already been demonstrated as feasible.<sup>29</sup>

In addition to the DRAI system, the efficacy/tolerability problems with pramlintide dosing could probably be addressed in other ways, albeit with theoretically less efficacy:

- Insulin/pramlintide blends
- Long acting amylin agonists
- Osmotic pumps

### Insulin/pramlintide blends

Because the requirement for additional mealtime injections of pramlintide has been a significant barrier to patient acceptance, the idea of blending insulin with an amylin agonist for use with either multiple daily injections or in a pump has been a goal for a long time.

Several clinical studies have tested fixed ratios:

- **2009 Heptulla et al:** 24-hour basal-bolus infusions of 3, 4, or 5 µg/hour basal pramlintide was tested with bolus injections of 5 µg pramlintide per Unit of insulin. Glucagon was suppressed postprandially but not between meals, and postprandial hyperglycemia was reduced 26%.<sup>30</sup>
- **2018 Haidar et al:** 24-hour fixed ratio basal-bolus infusions of 6 µg pramlintide per Unit insulin (regular and rapid). With rapid acting insulin, time in range increased from 71% to 85%, and glucose variability decreased from 34% CV to 25% CV. No improvement was shown with regular insulin.<sup>31</sup>
- **2018 Riddle et al:** 24-hour fixed ratio basal-bolus infusions of 9 µg pramlintide per Unit rapid insulin. Postprandial increments in blood glucose were almost entirely suppressed when pramlintide was co-administered. Time in range (70 to 180 mg/dl) increased from 50% to 62%, and postprandial glucagon AUCs decreased between 7% and 16%.<sup>32</sup>

Exhibit 17 presents the glucose results from the Riddle et al study. Based on the AUCs in Exhibit 17, fixed ratio amylin infusions lowered the T1D estimated HbA1c from 8.0% to 7.3%. However, this smoothed profile remained hyperglycemic compared to normal, healthy subjects having an estimated HbA1c of 4.7%.

In the Riddle et al study, the total diurnal glucagon profile was smoothed but only lowered overall 4.3% based on AUCs (Exhibit 18). Adverse events were increased, suggesting supraphysiological concentrations of pramlintide were reached at mealtimes:

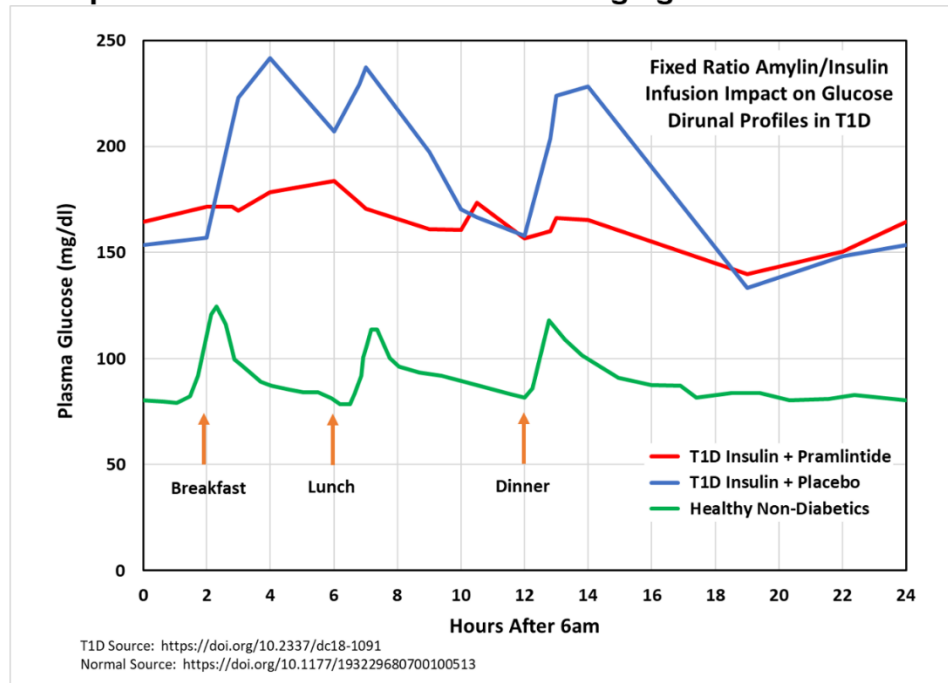
Adverse Event	Pramlintide	Control
Nausea	43.8%	7.1%
Headache	25.0%	3.6%
Vomiting	18.8%	0.0%



Two conclusions about this study seem reasonable:

- A 24-hour period is probably too short for the dual hormone therapy to reach a more beneficial equilibrium, and it is certainly too short to test the amylin circuit-breaker theory by measuring a reduction in hypoglycemic events.
- It's likely that the dosing regimen tested was not optimal. Bolus levels of pramlintide were probably too high, based on the adverse events, and basal levels may have been too low over night.

**Exhibit 17**  
**Fixed ratio amylin/insulin infusions smooth glucose profiles but do not normalize average glucose levels**



We believe a fixed ratio blend of insulin and amylin is not likely to achieve full efficacy in an AID system, especially the goal of restoring the glucagon counterregulatory response.

Two companies are known to be developing coformulations of insulin and pramlintide:

- **AstraZeneca:** In 2011 the JDRF and Amylin Pharmaceuticals (now merged into AstraZeneca) announced a collaboration to investigate coformulating pramlintide with insulin.<sup>33</sup> An October 2018 JDRF press release indicated that the Riddle et al study was a result of this collaboration, however, there is no visibility on AstraZeneca's recent progress or plans.
- **Adocia:** This French company is developing a coformulation of pramlintide and rapid insulin.<sup>34</sup> In September 2018 Adocia reported results of a study comparing their coformulation to separate injections (Exhibit 19). The results for slowing gastric emptying were equally equivalent comparing the coformulation to separate injections. Assuming the Adocia coformulation is usable in pumps, testing of a fixed ratio insulin/amylin blend in an AID system should soon be feasible.

Formulations with pharmacokinetics of short-acting insulin and pramlintide could be used in:

- **Single channel AID systems:** If the dosing ratio is designed to be optimal at mealtimes, clinical studies have demonstrated a dosing regimen that provides the amylin benefit of suppressing

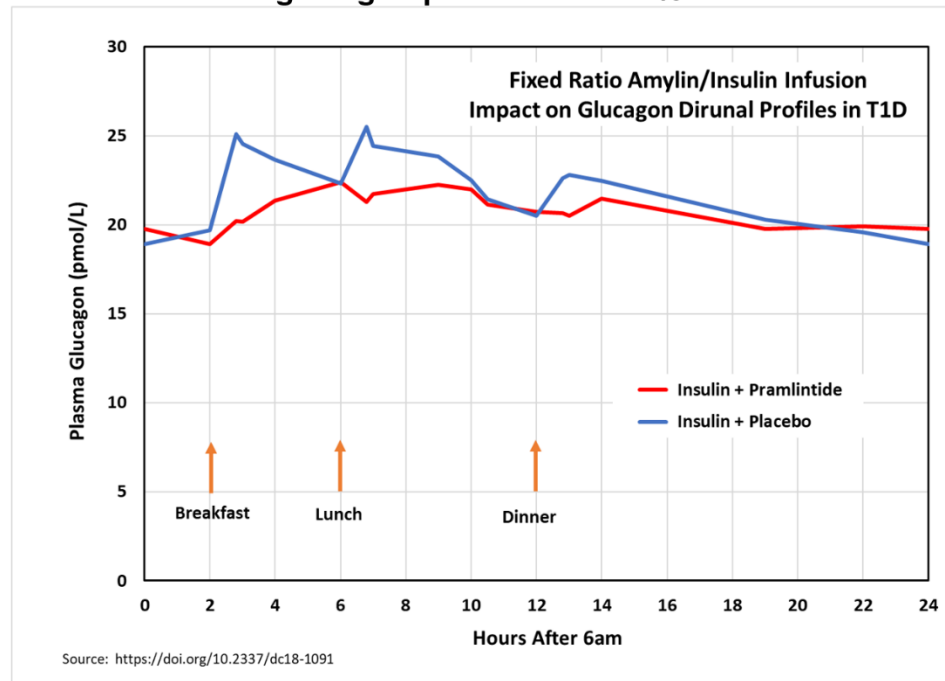
prandial glucagon secretion and slowing gastric emptying. However, it is unlikely that tolerable prandial boluses of amylin would provide enough basal coverage between meals to activate tonic inhibition of alpha-cells secretion and recharge the counterregulation response.

- **Multiple daily injections:**

Pramlintide dosing could be kept in the

physiological range to provide amylin bolus benefits without overdose-induced nausea. Data present above suggests 30 µg of pramlintide at mealtimes may be sufficient to suppress glucagon and slow gastric emptying, but insufficient to restore counterregulation without addition of a basal, long-acting amylin agonist.

**Exhibit 18**  
**Fixed ratio amylin/insulin infusions lower diurnal glucagon profiles about 4%**



We are pessimistic that short acting coformulations would be successful in restoring glucagon counterregulation without the addition of long-acting amylin agonists.

### Long-acting amylin formulations

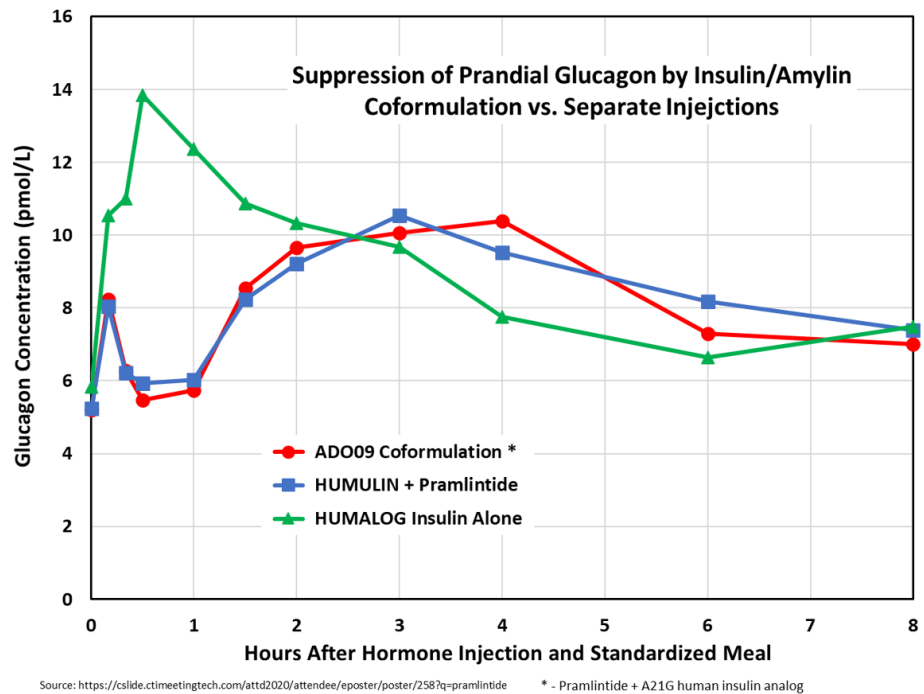
A long-acting amylin formulation might provide enough basal coverage to reactivate some or most of the glucagon counterregulatory mechanism. A long-acting amylin agonist would be analogous to insulin glargine (LANTUS), i.e. with once daily injections. Ideally the long-acting, basal amylin formulation could be used with a fixed-ratio, rapid-acting insulin/amylin blend at mealtimes. In this way the ratios of basal vs. bolus amylin/insulin could be adjusted to optimize efficacy and tolerability.

Several companies have reported projects to develop long-acting amylin analogues for weight loss:

- Novo-Nordisk reported that their long-acting amylin analog, AM833, was in Phase 2 as of February 2020, while a coformulation of AM833 and semaglutide (a GLP-1 agonist) was in Phase 1 testing.<sup>35</sup>

- As of March 2020, Zealand Pharma's website pipeline included a project to develop a long acting amylin agonist, BI-473494, which was projected to enter Phase 1 for an obesity indication during 2020.<sup>36</sup>
- In April 2019 Biozeus Biopharmaceutical SA (Brazil) published preclinical data for their long-acting amylin analog, BZ043.<sup>37</sup> However, as of March 2020 this compound was not shown in clinical development on the company's website.

**Exhibit 19**  
**A coformulation of insulin and amylin analogs suppresses prandial glucagon as well as separate hormone injections**



Hopefully, at least one of these projects will lead to a commercially available, long-acting amylin analog that could be tested for restoring the glucagon counterregulatory response in T1D.

**Implantable osmotic amylin pump**

For delivery of basal hormone profiles, the concept of an implantable pump is conceptually attractive. Intarcia (Boston) is working to commercialize an implantable osmotic pump that would deliver a GLP-1 agonist (exenatide) for six to twelve months. Clinical results are encouraging, presumably in part because patient compliance issues are eliminated.<sup>38</sup>

Intarcia's pump requires a high potency, body-temperature-stabilized formulation. If such an amylin agonist formulation can be developed, it could be an alternative to long-acting amylin agonists. However, in March 2020 the FDA rejected for the second time Intarcia's application to market the GLP-1 pump, so development of an amylin pump is unlikely any time soon.

\* \* \* \* \*

In summary:

- Beta-cells produce two hormones probably because influx and efflux diurnal profiles need to be different.

- The difference between insulin and amylin diurnal profiles is caused by differences in clearance and secretion rates.
- The amylin diurnal profile emphasizes basal exposure, compared to the more bolus-oriented insulin profile.
- Mealtime injections of pramlintide result in prandial overdosing and postabsorptive underdosing, which results in poor tolerability and efficacy.
- A bihormonal AID system using different amylin/insulin ratios for basal and bolus infusions would be the optimal way to correctly mimic both insulin and amylin diurnal plasma profiles.

These observations are readily tested with available technology. In Part 3 we begin the process of designing the research programs needed to validate the amylin circuit-breaker hypothesis.

## Endnotes

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# **The Amylin Circuit-Breaker – Part 3**

## **Where Do We Go From Here?**

As laid out in Parts 1 and 2, we believe that there is compelling evidence amylin plays a key role in the glucagon counterregulatory response, and that a dosing regimen which mimics the diurnal profile of circulating amylin may be the key to achieving euglycemia in T1D. And we think the payoff to patients and healthcare systems if this hypothesis is correct warrants clinical research aimed at testing its validity.

In this Part 3 we begin the process of translating the circuit-breaker hypothesis into specific objectives for clinical research. We do this by asking a series of questions that are raised by Parts 1 and 2. This list is a work-in-process, because we plan to use feedback about Parts 1 and 2 to expand the list and begin turning it into specific research proposals.

### **Questions Raised by the Amylin Circuit-Breaker Hypothesis**

#### **1. Diurnal hormone profiles**

- a. Are the Basu et al diurnal profiles representative of the general populations?
  - i. What do individual patient dose/responses look like?
  - ii. Would studies specifically designed to capture complete diurnal profiles substantiate the Basu et al findings?
  - iii. What would the diurnal profiles of amylin look like in these studies?
- b. What are the population basal/bolus relationships for insulin and amylin studies with large numbers of subjects?
  - i. How do meal types and exercise affect the hormone ratios?
- c. How do diurnal hormone ratios differ among individual subjects?
  - i. Do the differences correlate with differences in age or health?
  - ii. Do the differences imply that dual hormone dosing ratios should be personalized?

#### **2. Glucagon response to hyperglycemia**

- a. Why doesn't a complex carbohydrate meal affect glucagon secretion?
- b. Is there a meaningful time lag between rising glucose and glucagon suppression?

### **3. Glucagon response to hypoglycemia**

- a. Can imposing a basal level of circulating amylin restore the counterregulatory rebound in T1D?
- b. Over what time frame of therapy will basal amylin therapy restore the counterregulatory response?
- c. Can basal amylin therapy increase liver glycogen stores?

### **4. Non-glycemic forcings of alpha-cell secretion**

- a. Are there differences in glucagon diurnal profiles among individual T1D subjects?
- b. Do meals or exercise change the non-glycemic diurnal response of glucagon?

### **5. Optimizing amylin dosing**

- a. What basal and bolus ratios for pramlintide/insulin best mimic endogenous amylin profiles in individual patients?
- b. Can a long acting analog serve to cover the basal component of amylin replacement therapy?
- c. Should meals and/or exercise influence the choice of dual hormone dosing ratios?

### **6. Amylin and insulin secretion rates**

- a. Do different meal types result in different A/I ratios?
- b. Do individuals differ with respect to A/I ratios?
- c. Do morbidities influence A/I ratios, e.g. T2D or obesity?

### **7. Assay validation**

- a. Why do clinical researchers report problems with amylin assay results?

List of questions current as of 4/30/2020.



## Appendix A

### Alpha-Cell Response to Hyperglycemia in Health and Type 1 Diabetes

#### ABSTRACT

In this paper we report on the results of applying correlation analyses to the diurnal and postprandial profiles of circulating glucose, insulin, and glucagon in healthy subjects and in subjects with type 1 diabetes (T1D). We use the coefficient of determination (R-squared) to estimate the degree of linkage between variables, and we look for reoccurring patterns of deviations from the regression equation predictions (empirical models) to illuminate possible non-glucose forcings of glucagon secretion. Our results are consistent with the following conclusions about alpha- and beta-cell responses to rising blood glucose in the clinical studies evaluated:

- Regardless of the meal contents and schedules, in these studies changes in circulating glucose accounted for at least 90% of the observed changes in circulating insulin based on measuring glucose and insulin at the same points in time. The remaining 10% of insulin variation displayed a reoccurring pattern of deviations from the regression predicted levels, suggesting some of it was caused by changes in beta-cell sensitivity to insulin changes.
- In nondiabetics taking the simple carbohydrate meals, there is a negative correlation between circulating glucose and glucagon levels, with R-squared values of 0.59 in the diurnal study and 0.49 in the simple carb study. Thus, about half of glucagon postprandial variation was explained by alpha-cell response to hyperglycemia.
- However, complex carbohydrate meals showed no correlation between postprandial glucose and glucagon in healthy subjects. The data from these studies suggest that alpha-cell suppression is not triggered unless the postprandial rate of glucose increase is high enough.
- There also appear to be non-glucose regulatory mechanisms which result in relatively consistent postprandial deviations of circulating glucagon from levels predicted by the dose-response to circulating glucose. These nonglycemic forcings appear to account for about one-third of the measured diurnal variation in healthy subjects. The observation that T1D subjects show similar patterns of diurnal glucagon variation without any glycemic response suggests these deviations are unlikely to be artifacts of alpha-cell secretory delay, but rather reflect nonglycemic diurnal forcings of alpha-cell secretion.
- Subjects with T1D have circulating glucagon levels that are similar to nondiabetic levels immediately before meals and depressed compared to nondiabetics between meals. Over a complete diurnal period, T1D subjects are about 18% hypoglucagonemic compared to nondiabetics.
- In comparison to levels predicted by the glycemic model, T1D subjects are relatively hyperglucagonemic between meals. The excess is about 35% based on the AUCs of the diurnal

profiles. This hyperglucagonemia would be expected to amplify the hyperglycemia characteristic of T1D.

- In subjects with T1D there is no diurnal correlation between circulating glucose and glucagon, indicating that the mechanism by which postprandial glucose increases suppress alpha-cell secretion is completely missing:
  - These data are consistent with the theory that direct alpha-cell sensing of circulating glucose is NOT the primary glucagon regulatory mechanism, since alpha-cells in T1D otherwise appear normal.
  - These data are also consistent with the hypothesis that the primary T1D defect – loss of beta-cell function – is the cause of alpha-cell insensitivity to postprandial glucose.
- The observation that complex carbohydrates stimulate an insulin response without causing a change in circulating glucagon implies that insulin is NOT a paracrine regulator of glucagon secretion.
- The 18% absolute hypoglucagonemia in T1D suggests that alpha-cell depletion of glucagon is NOT a cause of counterregulatory malfunction. Rather, the counterregulatory failure must be caused by a failure of the hypoglycemia sensing mechanism.
- These findings support the idea that a drug aimed at restoring glucose dependent control of postprandial alpha-cell secretion should address the primary defect caused by loss of beta-cells. Since these data indicate insulin is not a paracrine regulator of alpha-cell secretion, it makes sense to focus pharmaceutical efforts to regulate postprandial glucagon in T1D on the other beta-cell secreted hormone which is known to suppress alpha-cell secretion: the neurohormone amylin.

## INTRODUCTION

Unrestrained alpha-cell secretion of glucagon is thought to be a central factor in the pathogenesis of T1D. To quote a 2016 review: “*Hyperglucagonemia is present in every form of diabetes. Glucagon is essential for hyperglycemia in T1D.*”<sup>1</sup>

In addition to the canonical role of glucagon in glucose homeostasis, there is long established evidence that glucagon plays a role in energy homeostasis by enhancing satiety, increasing energy expenditures, and inducing thermogenesis.<sup>2</sup> Alpha-cells are known to respond to nonglycemic plasma signals, e.g. arginine.<sup>3</sup>

It is the purpose of this paper to use correlation analysis to quantify the glycemic and nonglycemic glucagon diurnal patterns caused by postprandial glucose in populations of nondiabetic, healthy subjects. It is well understood that there is regulatory linkage between circulating glucose and glucagon, so the cause-and-effect basis for the resulting correlation is established. Our hypothesis is that there is, first, a dose-response of glucagon secretion to rising blood glucose, and that there are second, nonglycemic mediating effects on alpha-cell secretion that are independent of circulating glucose concentrations.

We do not consider comparable alpha-cell responses to hypoglycemia, since none of the underlying studies generated data in the hypoglycemic range.

The data is sourced from studies that were not designed specifically for the goals in this paper, so there are limitations to the analysis. For example, the studies don't permit considering short (several minutes) time lags in responses. However, the results provide interesting perspective on average glucagon secretory patterns in the study populations, as well as offer insight into the separate glycemic and nonglycemic mediators of glucagon secretion. They also permit quantifying the disturbances in postprandial alpha-cell secretion caused by T1D.

In this paper we use the term “model” to refer to the correlation equations and deviations therefrom derived from the clinical studies. This approach differs from simulation modeling aimed at estimating the impact of multiple parameters interacting in dynamic systems (see, for example, *Modelling the Effects of Glucagon During Glucose Tolerance Testing; Theoretical Biology and Medical Modelling*, <https://tbiomed.biomedcentral.com/> 2019.) Our goal here is to mathematically describe the diurnal profiles from the top down – an approach that we term “empirical modeling” – in order to better understand the interactions among three plasma parameters, circulating glucose, insulin, and glucagon. From our modeling we make observations about the endocrine factors that force alpha-cell secretion, and we illustrate an approach to modeling alpha-cell secretion that with appropriately designed clinical studies could be extended to hypoglycemia and amylin secretion.

## DATA SOURCES

Three articles by a Mayo Clinic team under the leadership of Andy Basu provide the database for analyzing glucagon diurnal plasma profiles:

- *Diurnal pattern to insulin secretion and insulin action in healthy individuals; Diabetes 61:2691-700 2012.*
- *Diurnal pattern of insulin action in type 1 diabetes; Diabetes 62:2223-9 2013.*
- *A novel natural tracer method to measure complex carbohydrate metabolism; Am J Physiol Endocrinol Metab 317:E483-93 2019.*

The first two “diurnal studies” were designed to determine whether there is a daily pattern of changing glucose tolerance following mixed meals which should be incorporated in the design of dosing algorithms for Automated Insulin Delivery systems. The third “carbohydrate study” was designed to test a new way of measuring insulin sensitivity and beta-cell responsivity to simple and complex carbohydrates. The study parameters relevant for the modeling in these papers are summarized in Exhibit 1.

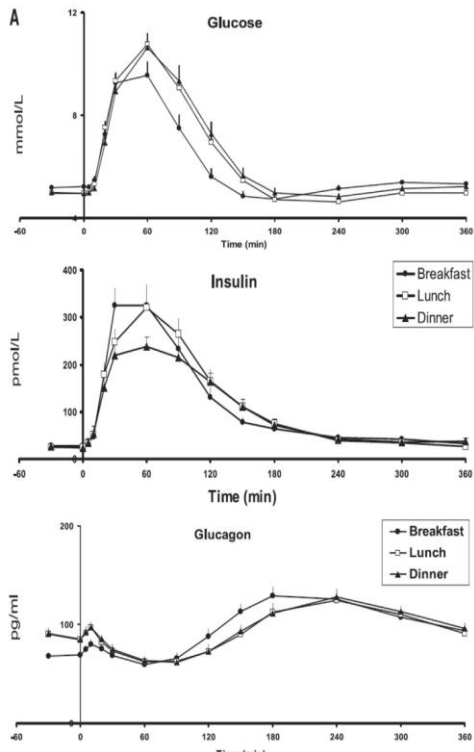
**Exhibit 1**  
**Summary of study parameters**

	Diurnal Studies	Carbohydrate Studies
Objective	Measure diurnal cycles of insulin sensitivity for purposes of designing dosing algorithms to control Automated Insulin Delivery systems	Compare insulin sensitivity and beta-cell responsivity to simple vs. complex carbohydrates
Subjects	20 <u>nondiabetic</u> ; 19 T1D using CSII	8 <u>nondiabetic</u>
Duration	Three full day in-patient study visits	Two morning in-patient visits
Meals	Three daily equal calorie, standardized meals containing ~50 grams carbohydrate (Jell-O) 6 hours apart at time 0	Breakfasts containing ~50 grams of simple (Jell-O) or complex (rice or sorghum) carbohydrate
Between meals	Uniform physical activity protocols	None
Study meals	One of each meal type (B, L, and D) was randomly selected from each patient's 9 meals to measure blood levels	Breakfast only
Blood draws	Times -30, 0, 5, 10, 20, 30 min; every 30 min to 180 min; every hour to 360 mins	Same <u>except</u> : no 5 min; added 40, 50,75, 105, 135, and 165 min
Preprandial insulin adjustments	T1D subjects continued insulin pump therapy according to their customary, personal regimens. To avoid significant hyperglycemia during the testing protocol, T1D subjects were required to have starting glucose values below 150 mg/dl. To achieve this limit, subjects were allowed small insulin boluses up to one hour prior to time zero. Subjects who did so lowered their glucose concentrations and elevated their insulin concentrations pre-meal.	None
Glucagon assay	Double antibody radioimmunoassay (Linco Research)	Same

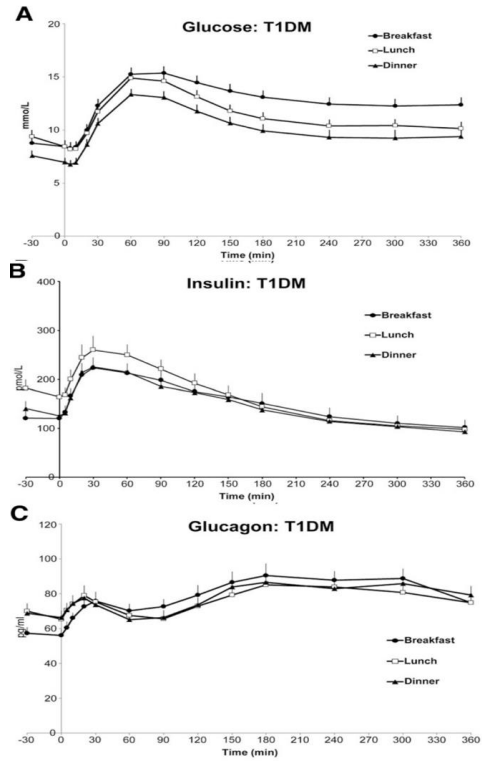
A Java program called Plot Digitizer <sup>4</sup> was used to convert the data points shown in the article charts into tables of circulating concentrations. This process introduces small random variations between the actual study data and the results used in this analysis, and it could be expected to lower the R-squared values slightly.

The diurnal study profiles of glucose, insulin, and glucagon are shown in Exhibits 2 and 3 (copied directly from the charts of the referenced articles). The carbohydrate study profiles of these analytes are shown in Exhibit 4 (also copied directly from the charts of the referenced article). The error bars in the diurnal studies are smaller than those in the carbohydrate study, presumably because of the difference in the number of subjects (20 and 19 vs. 8).

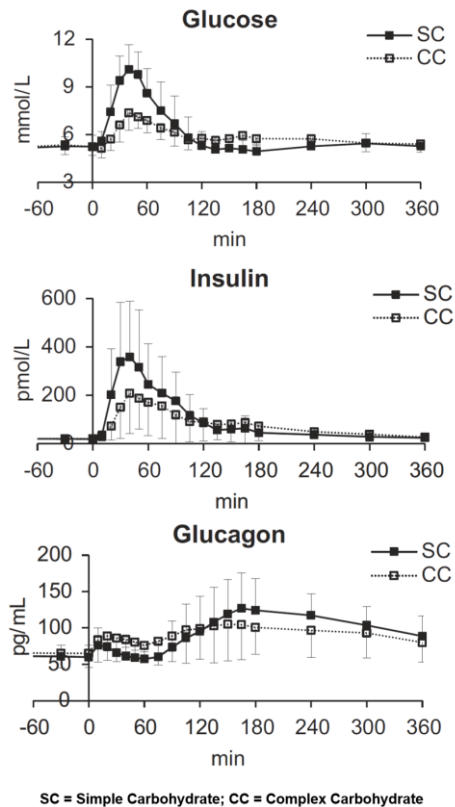
**Exhibit 2**  
Diurnal profiles in healthy, nondiabetic subjects (n=20)



**Exhibit 3**  
Diurnal profiles in T1D subjects (n=19)



**Exhibit 4**  
Postprandial profiles in carbohydrate study (n=8)



## **CONSTRUCTING DIURNAL PROFILES**

To simulate 24-hour profiles, the diurnal study data were assembled end-to-end. At the connections between meal cycles, time 0 minutes for the subsequent cycle was used in place of time 360 minutes for the previous cycle. Concentrations between hours 18 and 24 were estimated based on a straight-line extrapolation and shown as dotted lines.

Correlation analyses are based on 38 diurnal data points from -0.5 to 18 hours.

### **Meal profile misalignments caused by experimental factors**

For the diurnal study, the profile alignments between breakfast/lunch and lunch/dinner were evaluated by comparing the analyte levels at times 360 and 0 minutes to the peak analyte levels measured during each meal cycle. Alignment for nondiabetics was quite good: the starting and ending plasma concentrations of all three analytes were close together, with misalignments of less than 4% of peak concentrations.

Alignment for T1D subjects was not as close as for nondiabetics:

- Time 0 average insulin concentrations were elevated 10-27% of peak concentrations above time 360 concentrations, presumably in part because of the pre-meal adjustment boluses by some subjects.
- Time 0 average glucose concentrations were depressed 22-25% of peak concentrations below time 360 concentrations, perhaps because of the pre-meal insulin adjustments.
- Time 0 average glucagon concentrations were elevated about 11% concentrations over time 360 concentrations; we can offer no mechanistic hypothesis to explain this.

These alignment differences should be considered when evaluating the composite 24-hour diurnal profiles. For the T1D subjects, they distort somewhat the linkages between breakfast/lunch and lunch/dinner, so chart connections between meals for T1D subjects are shown as dotted lines. However, the alignment differences should be irrelevant for the correlation analyses.

### **Perspective on healthy, nondiabetic diurnal patterns**

Exhibit 5 combines all three analytes for healthy nondiabetics on a scale indexed to levels at thirty minutes before breakfast (the basal level). The delta in insulin from basal has been divided by seven to render visually comparable spikes to glucose and insulin.

As expected, there is a direct relationship between glucose and insulin concentrations. Also as expected, the glucagon profile is largely contrary to the glucose (and insulin) profile: rising glucose causes a

postprandial depression in glucagon, then falling glucose causes a rise in post absorption glucagon, followed by a return of glucagon toward basal levels before the next meal.

### Perspective on T1D diurnal patterns

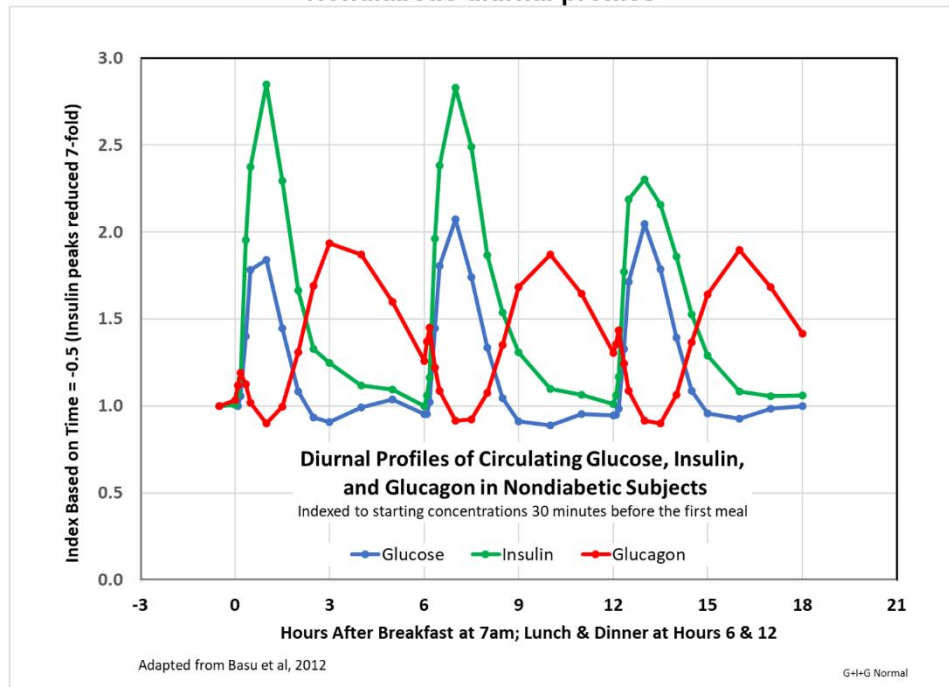
Exhibit 6 combines the three analytes for T1D subjects on the same scale as Exhibit 5. Mealtime glucose spikes are proportionally similar to those in nondiabetics, albeit from a higher starting

concentration (Exhibit 7). Mealtime insulin spikes are relatively muted because of constraints associated with subcutaneous delivery. Also, insulin returns toward basal more slowly than in nondiabetics, probably because of continuing diffusion from the subcutaneous infusion site.

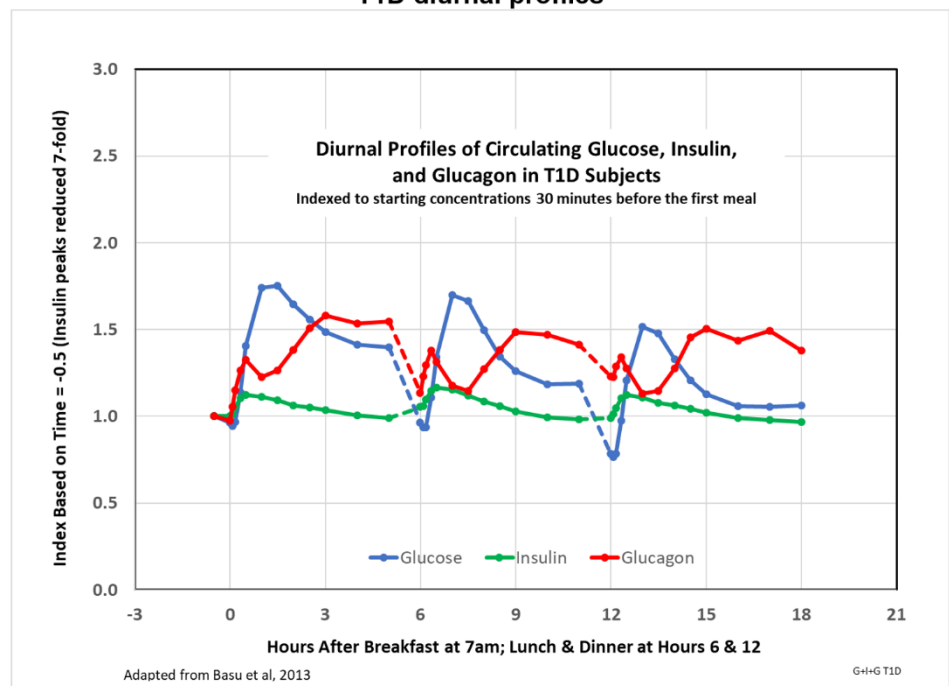
### DIURNAL PROFILES COMPARED: HEALTHY VS. T1D

As expected, individuals with T1D had higher blood glucose levels than healthy, nondiabetics throughout the meal cycles (Exhibit 7). Based on area-under-the-curve (AUC) for the diurnal glucose profiles, T1D exposure to blood glucose was 80% higher than that of nondiabetics. The AUCs of these diurnal profiles translate into HbA1c levels of 5.4% for nondiabetics and 8.5% for T1D subjects.<sup>5</sup>

**Exhibit 5**  
Nondiabetic diurnal profiles



**Exhibit 6**  
T1D diurnal profiles





T1D subjects have higher average insulin in circulation than nondiabetics receive from endogenous beta-cell secretions (Exhibit 8). Based on the insulin AUCs, T1D subjects are exposed to 65% more circulating insulin than nondiabetic subjects. It is well documented that T1D patients are insulin resistant, i.e. display lower sensitivity to the effects of insulin in both hepatic and muscle tissue. Also, higher circulating exogenous insulin may be needed to compensate for subphysiological levels in the liver; because about 50% of endogenous insulin is extracted by the liver before reaching circulation, endogenous insulin secretion results in intraportal concentrations about twice those in peripheral circulation.

**Exhibit 7**  
Diurnal glucose profiles

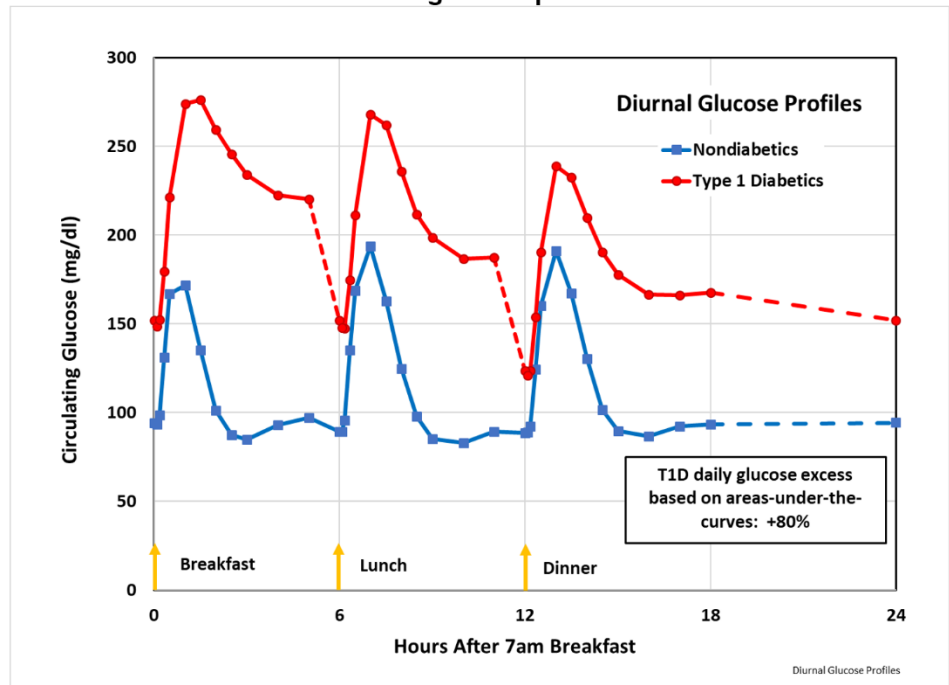
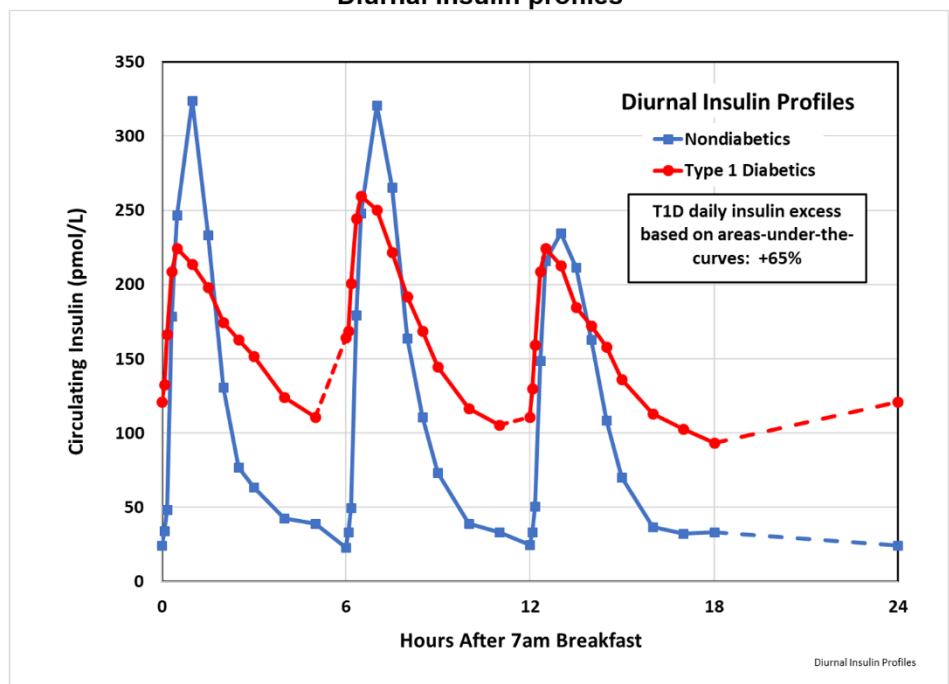


Exhibit 8 also demonstrates that insulin pump infusions do a poor job of mimicking the diurnal profile of endogenous secretions.

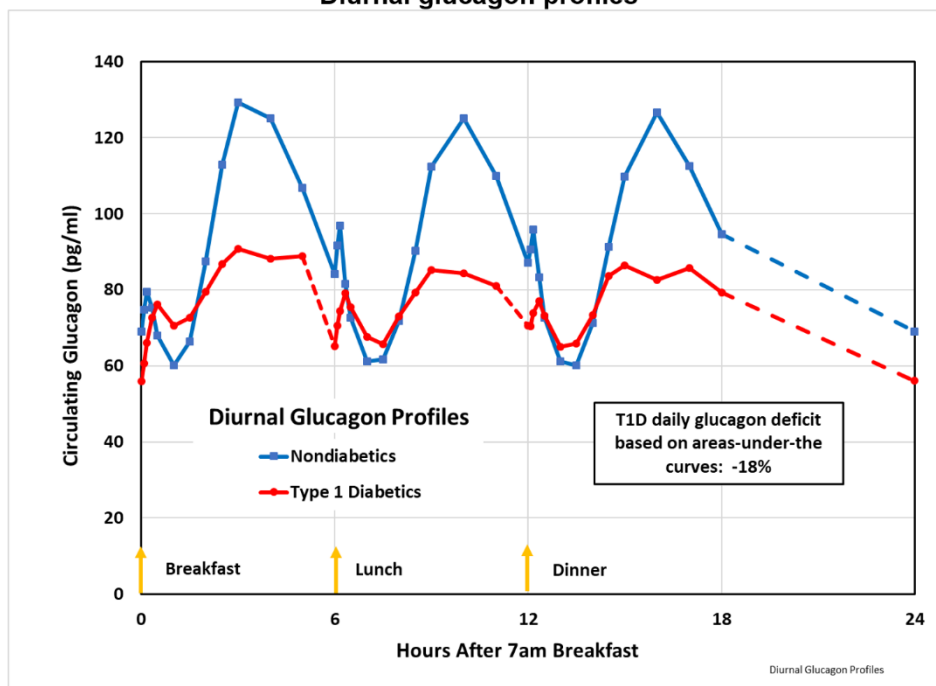
In this study, the prandial rate of exogenous insulin increase in T1D appears similar to that of endogenous insulin in nondiabetics, but the rate of clearing infused insulin is slower in T1D. As a result, T1D insulin levels remain elevated at times when nondiabetic insulin concentrations have fallen to basal levels. Breakfast and lunch peak concentrations are subnormal in T1D, probably because the risk of iatrogenic hypoglycemia constrains mealtime bolus amounts.

**Exhibit 8**  
Diurnal insulin profiles



Because T1D patients are on average both hyperglycemic and hyperinsulinemic, current thinking in the diabetes field supposes they should demonstrate lower average circulating glucagon levels than nondiabetics do, if their alpha-cell regulation is normal. As shown in Exhibit 9, this hypothesis is correct: T1D patients have roughly normal levels of glucagon at mealtime, but postabsorption levels are depressed. Over a complete diurnal cycle, T1D patients are exposed to 18% less daily glucagon than nondiabetics: they are hypoglucagonemic in ABSOLUTE terms.

**Exhibit 9**  
**Diurnal glucagon profiles**



Since alpha-cells appear to be relatively normal in T1D, this finding that T1D subjects secrete less daily glucagon than healthy subjects points to the following conclusion: the defective counterregulatory response to hypoglycemia characteristic of T1D is NOT caused by depletion of glucagon stores in alpha-cells. Rather, the counterregulatory defect must be intrinsic to the glucose sensing mechanisms which stimulate the alpha-cell response to hypoglycemia.

Is the T1D postabsorption deficiency in glucagon consistent with levels predicted by their hyperglycemia, if their glycaemic regulation of alpha-cells were normal? More specifically, is an 18% reduction in glucagon exposure a healthy response to an 80% increase in blood glucose exposure? To answer this question, we need to measure the healthy, nondiabetic dose-response of glucagon to postprandial changes in blood glucose.

The healthy diurnal glucose and glucagon profiles provide the basis for considering two parameters by which circulating glucagon concentrations are determined:

- **Glycemic Alpha-Cell Regulation Model:** We first estimate the degree to which circulating glucagon levels correlate to glucose levels. If conventional wisdom is correct, higher blood glucose should result in lower blood glucagon. (The “glycemic model.”)
- **Nonglycemic Alpha-Cell Regulation Model:** We then analyze the diurnal deviations of circulating glucagon from levels predicted by the glycemic model. If consistent meal cycle deviations from the glycemic model are detected, these will be interpreted as indicative of non-glucose sensitive regulation of alpha-cell secretion. (The “nonglycemic model.”)

Finally, we will combine these two models into an **Integrated Alpha-Cell Regulation Model** to demonstrate whether the glucagon diurnal profile can be predicted by our empirical modelling. (The “integrated model.”)

## THE GLYCEMIC ALPHA-CELL REGULATION MODEL

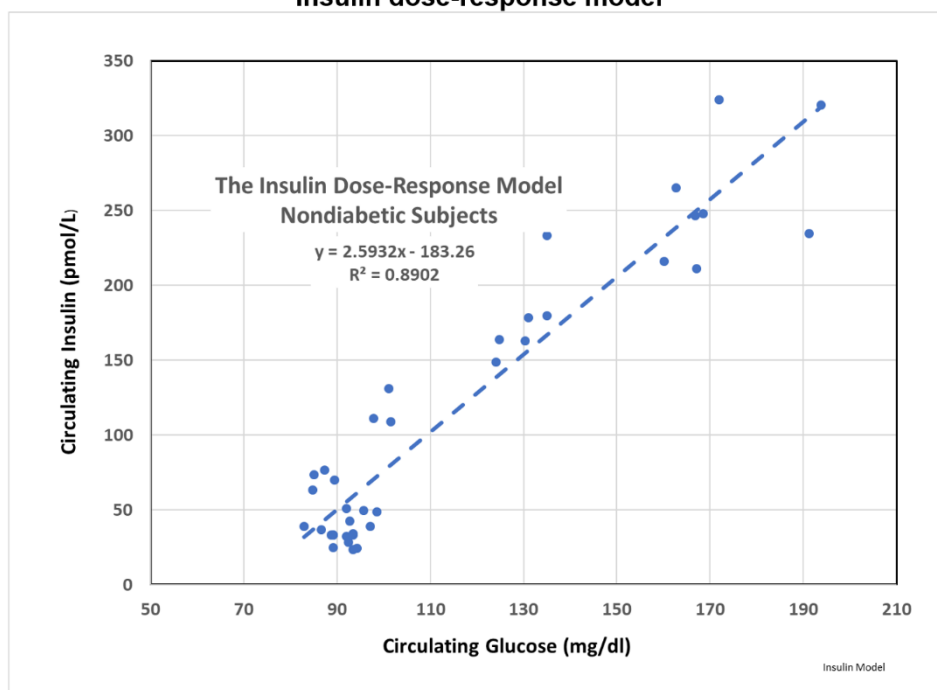
In this section we test the idea of developing an empirical dose-response model using insulin, we apply this methodology to the glucose-glucagon data for nondiabetics, and we then examine glucagon response in T1D subjects.

### Insulin responds closely to circulating glucose

As a preliminary test of our methodology for measuring the glucagon dose-response to glucose, we looked at the equivalent insulin dose-response. As shown in Exhibit 10, the correlation with a linear regression analysis is excellent: the R-squared is 0.89 for healthy subjects over three meals in the diurnal study. A logarithmic curve fit gives almost exactly the same R-squared value.

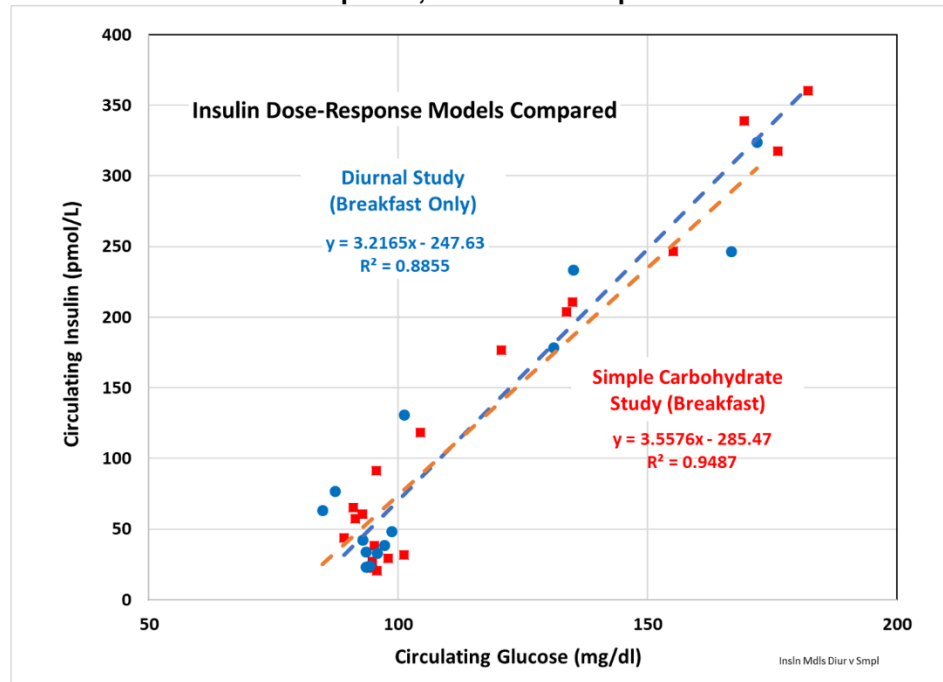
As a check on whether the diurnal study is representative of a more general insulin dose-response, we compared the correlation provided by the simple carbohydrate study to that of the diurnal study following breakfast, as shown in Exhibit 11. The R-squared for the simple carb data is 0.95, even higher than that from the diurnal study. The dose-response slope from the simple carb data is 11% higher than the diurnal data slope; a possible explanation might be that the diurnal study missed the actual peak of the insulin response by not taking measurements at 40 and 50 minutes after the meal, as suggested by Exhibit 16.

**Exhibit 10**  
**Insulin dose-response model**



**Exhibit 11**  
**Insulin dose-response, diurnal vs. simple carb studies**

As a further check on the insulin dose-response, we compared the results for the simple carb study to the those of the complex carb study. The complex carb subjects achieved an average increase in postprandial glucose level over basal of about half the increase over basal in the simple carb study. Nevertheless, the R-squared for the complex carb dose-response was 0.94, almost identical to that of the simple carb dose-response at 0.95 (Exhibit 12). The calculated slope of the complex carb dose-response was 36% higher than the simple carb; this robust dose-response for the complex carb study suggests that glycemic control of insulin secretion was fully active, even though the postprandial glucose increase was muted by the delay in complex carbohydrates raising blood glucose. This observation will be revisited during the discussion of the glucagon dose-response in the complex carb study.

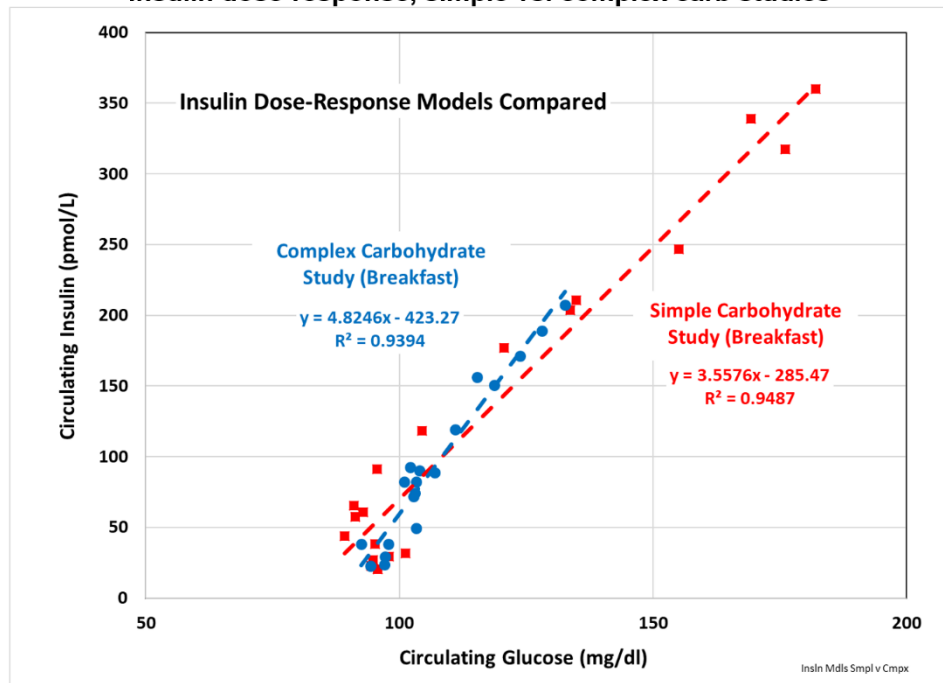


In Exhibit 13 we use the insulin dose-response model from the diurnal study to predict a profile of circulating insulin, and we compare it to the actual profile of circulating insulin.

The glucose-mediated model accounts for about 80% of the actual insulin profile ( $R$ -squared = 0.80), and it diverges from reality in two ways:

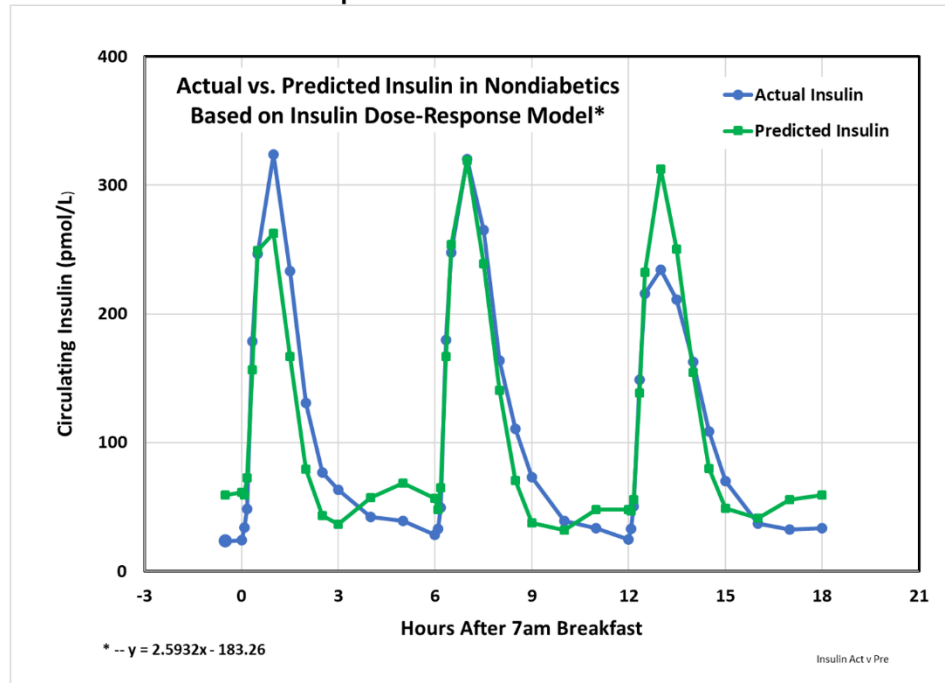
- The actual insulin response to

**Exhibit 12**  
**Insulin dose-response, simple vs. complex carb studies**



breakfast is more robust than predicted, and the actual response to dinner is blunted. The breakfast difference is reflected in the dose-response slopes of the breakfast (3.2) and three-meal (2.6) equations of the diurnal study: the breakfast slope is 24% greater than the slope generated by the profiles from three meals (see Exhibits 10 and 11 for the linear regression equations).

**Exhibit 13**  
**Actual vs. predicted insulin in nondiabetics**



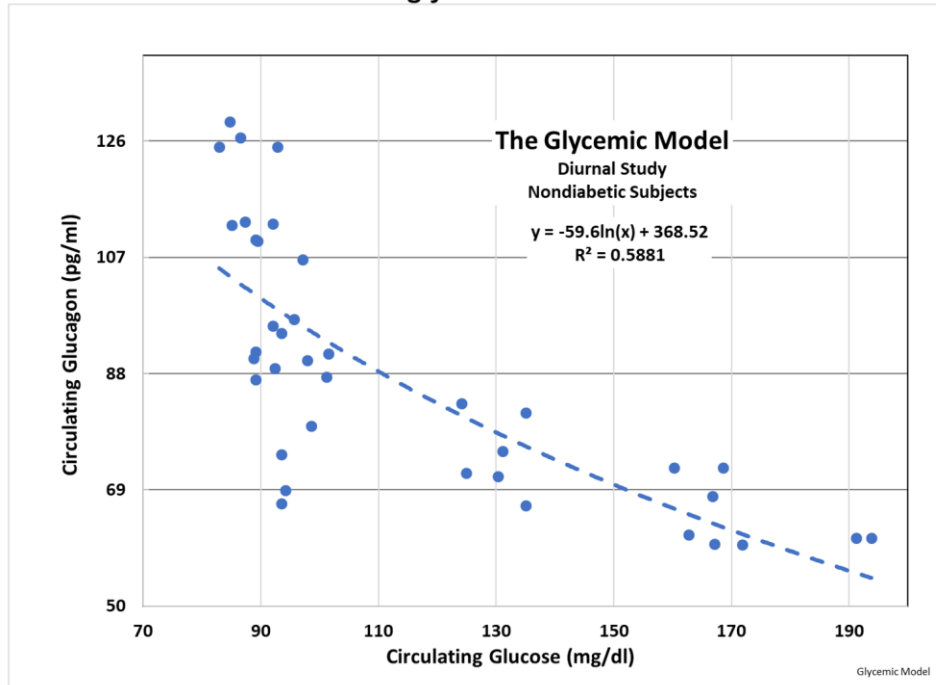
- By about three hours postprandial actual circulating insulin has somewhat disengaged from the predicted dose-response relationship. As can be seen in Exhibit 5, following its postprandial spike, circulating glucose declines past its preprandial level and then recovers. Circulating insulin does not follow this glucose overshoot/snapback pattern, but simply returns to its preprandial level.

A plausible mechanism for nonglycemic insulin deviations could be variations in beta-cell sensitivity. Meanwhile, the 90%+ correlation between circulating glucose and insulin levels is consistent with general knowledge that beta-cell secretion is mostly driven by response to rising blood glucose levels. Thus, we move on to using this methodology to explore the glucagon dose-response with increased confidence that our empirical modeling is consistent with general knowledge about islet hormone response to rising blood glucose.

**Glucagon response is only partially glucose driven in nondiabetics**

Exhibit 14 plots glucose vs. glucagon blood concentrations for the diurnal study data of nondiabetics.

**Exhibit 14**  
**The glyceimic model**



The best fit is a logarithmic curve described by this equation:

$$[\text{Glucagon concentration}] = -59.6 * \ln[\text{glucose concentration}] + 368.52.$$

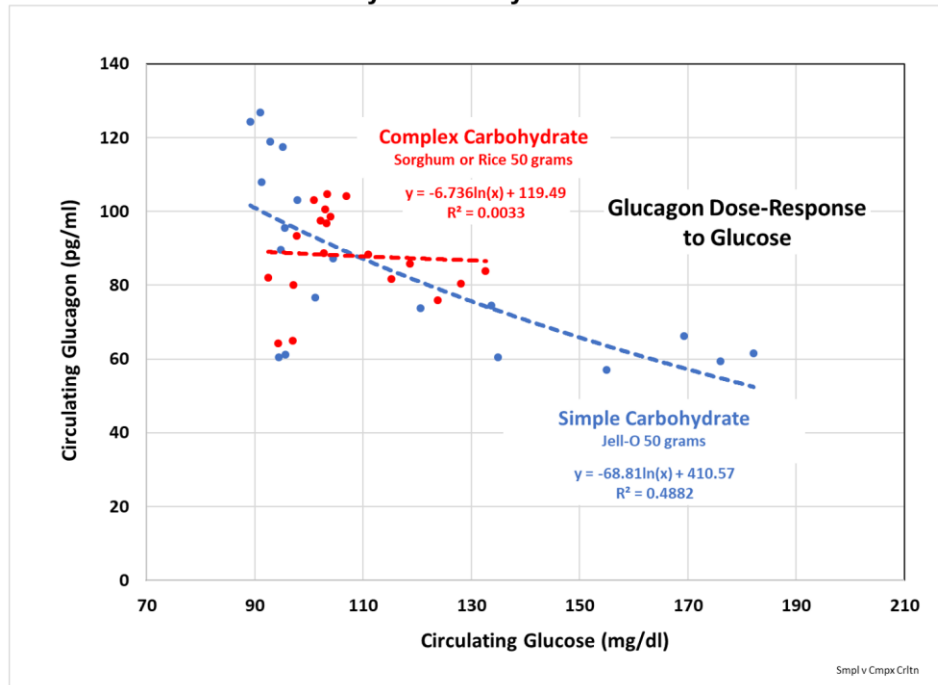
This equation will be termed the “glycemic model.”

The R-squared value is 0.59, which implies that – in this population of nondiabetics under the conditions tested – close to 60% of the diurnal variation in circulating glucagon is explained by variations in circulating glucose. The first derivative of the dose-response is  $Y = -59.6/x$ ; as glucose concentration increases, the rate of glucagon decline decreases.

As a rough check on whether there is a lag between glucose and glucagon, the correlation was run by shifting glucagon forward one time period (e.g. the glucagon value at 60 minutes was correlated to the glucose value at 30 minutes); the slope of the regression declined about 5%, and the R-squared dropped from 0.59 to 0.54. Thus, it appears that, for the time intervals tested, using simultaneous circulating concentrations of glucose and glucagon is appropriate for the glyceimic model.

Exhibit 15 plots glucose vs. glucagon blood concentrations for the carbohydrate studies.

**Exhibit 15**  
**Carbohydrate study correlations**



While the simple carb shows an R-squared of 0.49, the complex carb slope and correlation are both close to zero. This suggests that, in this study design, the simple carb activated glycemic regulation of glucagon, but the complex carb did not produce a strong enough glycemic effect to show up in the correlation analysis.

For both simple and complex carbs, glucagon displayed the first phase postprandial glucagon increase (Exhibit 4), but suppression of this peak was substantially stronger for the simple carb, as was the subsequent decline from that peak:

	<b>Simple Carb</b>	<b>Complex Carb</b>
Glucagon rise to 1 <sup>st</sup> peak from basal:	27%	38%
Glucagon decline to nadir from 1 <sup>st</sup> peak:	26%	15%

Three mechanistic explanations for the complex carb failure to regulate glucagon are plausible:

- **Insufficient glucose was absorbed from the complex carbohydrate:** Exhibit 16 shows the postprandial glucose profiles of the nondiabetic diurnal and carbohydrate studies. (Note that the diurnal study may have missed the peak glucose level by not testing at 40 and 50 minutes.) The complex carb AUC above the basal level at time 0 is about 80% of that for the simple carb; thus, the complex carb resulted in a somewhat lower glucose loading than did the simple carb. The insulin AUC above basal in the complex carb study was 79% of the simple carb AUC; thus,



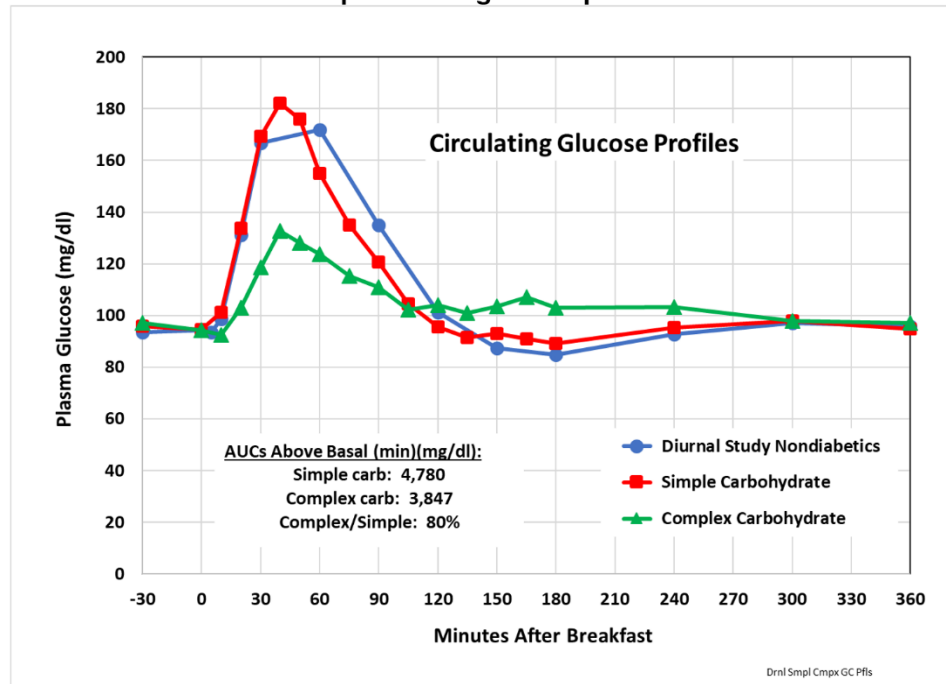
glycemic regulation of insulin reflected response to glucose in both studies. This suggests that something other than the 20% reduction in net carb load accounts for the complete loss of glycemic correlation to glucagon.

- **Glucose did not reach a threshold level for activation:** When the four highest glucose levels (above ~135 mg/dl) are deleted from the

simple carb correlation, the R-square declines somewhat to 0.36 and the slope of the dose-response becomes much steeper. This is consistent with the conclusion that glycemic regulation of glucagon was occurring at the lower glucose levels for the simple carb study, and that there is no concentration threshold for activation.

- **Glucagon stimulation is sensitive to the rate of glucose increase:** The simple carb meal resulted in a rate of glucose increase that was 143% faster than that of the complex carb meal (data from Exhibit 16):

**Exhibit 16**  
**Comparison of glucose profiles**



Study	Data Times Postprandial (minutes)	R-Squared of Linear Regression	Rate of Glucose Increase per Minute
Simple Carbohydrate	10 – 20 – 30	0.9993	3.4 mg/dl
Complex Carbohydrate	10 – 20 – 30 – 40	0.9936	1.4 mg/dl

Since the glucagon dose-response appears to have occurred in the simple carb study below the maximum level achieved in the complex carb study, it seems likely that a threshold rate of glucose increase is required to activate glycemic regulation of alpha-cells.

One hypothesis proposed for glycemic control of alpha-cell secretion is that insulin exerts a paracrine effect between the beta- and alpha-cells. However, these observations about the glucagon dose-response to rising glucose are consistent with the following conclusion: insulin secretion does NOT activate glucagon secretion.

- As shown in Exhibit 12, insulin levels are under tight, 90%+ control by glucose regardless of the magnitude and timing of postprandial glucose increases.
- In contrast as shown in Exhibit 15, postprandial glucagon response is only about 50% explained by rising glucose in the simple carb study, and there is no glucagon response to glucose in the complex carb study.

**Exhibit 17**  
**Glucagon response to glucose in two studies**

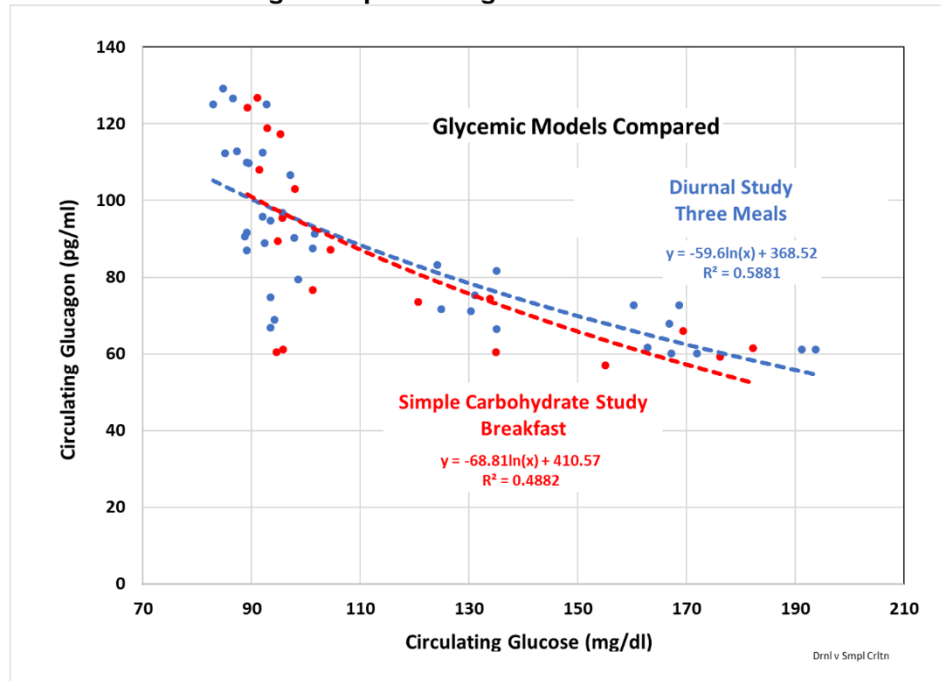
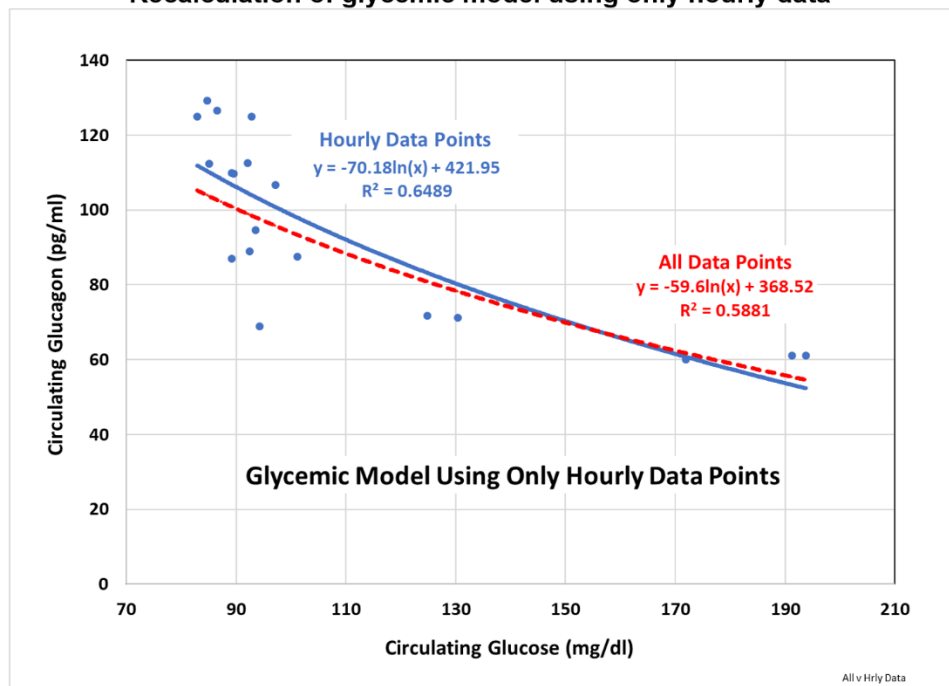


Exhibit 17 compares the glucagon dose-response resulting from the simple carb study to that of the diurnal study (the glycemic model).

Both studies included mixed meals with the same amount of Jell-O; however the diurnal study blended averages for selected breakfast, lunch, and dinner meals, whereas the simple carb study was only breakfast. The similarity of glucagon correlation equations between the two different studies increases our confidence that, for the conditions tested, the diurnal glucose model is a good reflection of the postprandial glucagon dose-response to circulating glucose. Clearly this model does NOT apply to meals

**Exhibit 18**  
**Recalculation of glycemic model using only hourly data**



based on complex carbohydrates.

In both the diurnal and simple carb studies, more frequent data points were collected early in the postprandial period. To test whether this skewed the regression analysis, a correlation was done using only the hourly diurnal data, as shown in Exhibit 18.

The slope of the logarithmic curve increased about 16% and the R-squared increased to 0.65. Most of the deviation between the all-data and hourly-data glycemic correlations occurred at the lowest glucose concentrations, i.e. at 90 mg/dl, glucose-predicted glucagon based on hourly-data exceeded that predicted by the all-data by about 6%, while there was virtually no deviation between models at 190 mg/dl. This relatively small deviation suggests that the pattern of blood tests did not distort the correlation analyses.

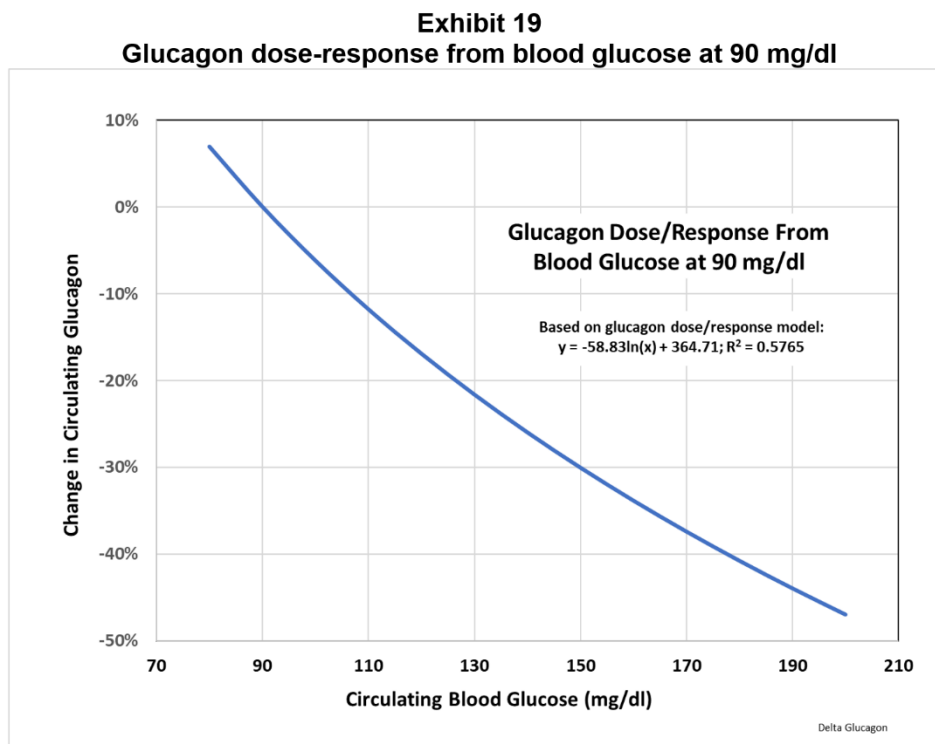
Interestingly, the use of only hourly data in the diurnal study resulted in a correlation equation very close to that of the simple carb study:

	<b>Slope</b>	<b>Intercept</b>	<b>R-Squared</b>
Diurnal Study Hourly Data Only	-70.18	421.95	0.6489
Simple Carbohydrate Study	-68.81	410.57	0.4882

For the remainder of this paper we use the glycemic model derived from all the data points in the diurnal study.

When 90 mg/dl is set as a “basal” level of blood glucose, Exhibit 19 shows the projected decline in circulating glucagon as blood glucose rises above basal level.

At 190 mg/dl glucose (about the peak level of glucose twice per day in nondiabetics following lunch and dinner), this glycemic model predicts glucagon will decline 44% to about 56 pg/ml. As shown in Exhibit 9, glucagon concentrations in nondiabetics do reach a nadir of about 60 pg/ml after meals.



In Exhibit 20, the glycemic model is used to predict the glucose-dependent component of glucagon levels in healthy nondiabetics, which is then compared to their actual glucagon circulating concentrations.

There are three times of deviations in the profiles, as shown in Exhibit 21.

- **Prandial (red):**

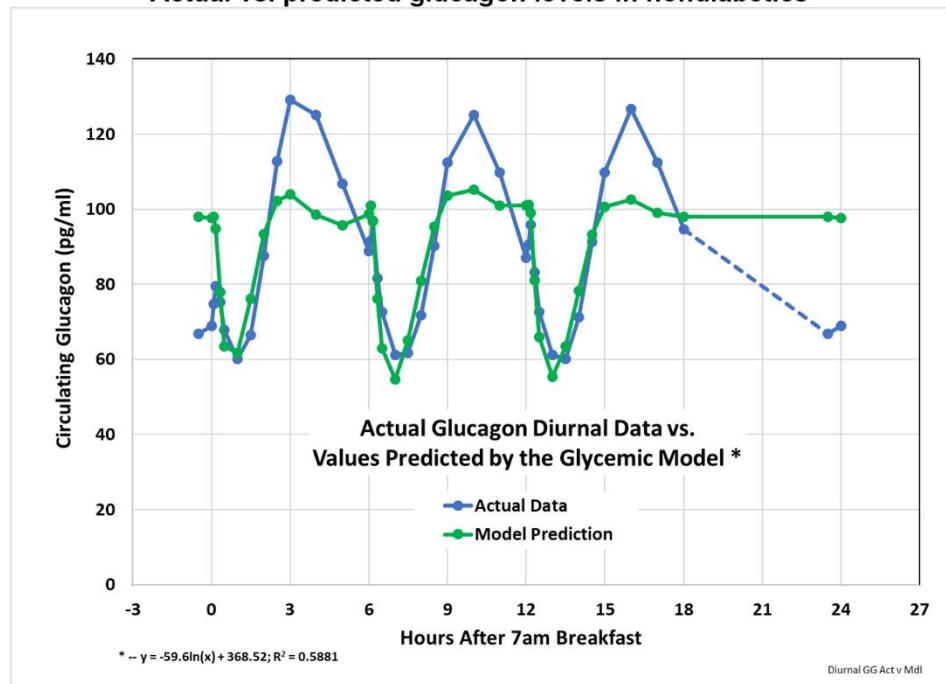
During the first ten minutes following the start of mealtimes, glucagon spikes briefly before

glucose begins rising and glycemic control of alpha-cell secretion kicks in, presumably because of protein stimulation of alpha-cells.

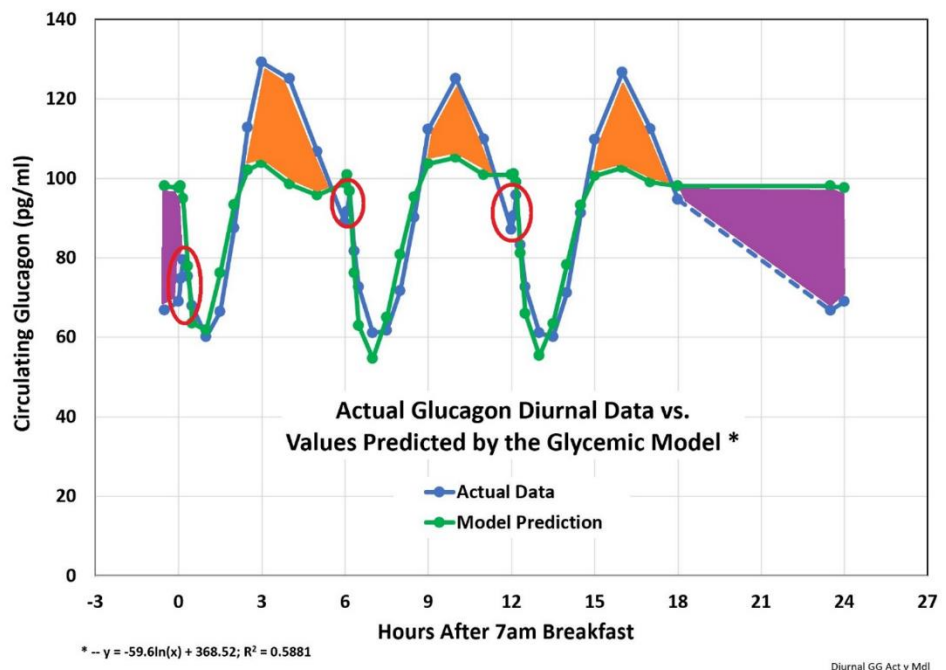
- **Postabsorption (orange):** About 1.5 hours postprandial when blood glucose has returned to basal concentrations, circulating glucagon begins to rise and peaks at 3-4 hours postprandial before beginning to decline toward fasting levels, perhaps to suppress hunger and accommodate a negative energy balance during exercise.

- **Fasting (purple):** Starting about six hours after dinner, circulating glucagon declines below levels predicted by glycemic regulation, presumably to compensate for the sleep cycle.

**Exhibit 20**  
Actual vs. predicted glucagon levels in nondiabetics



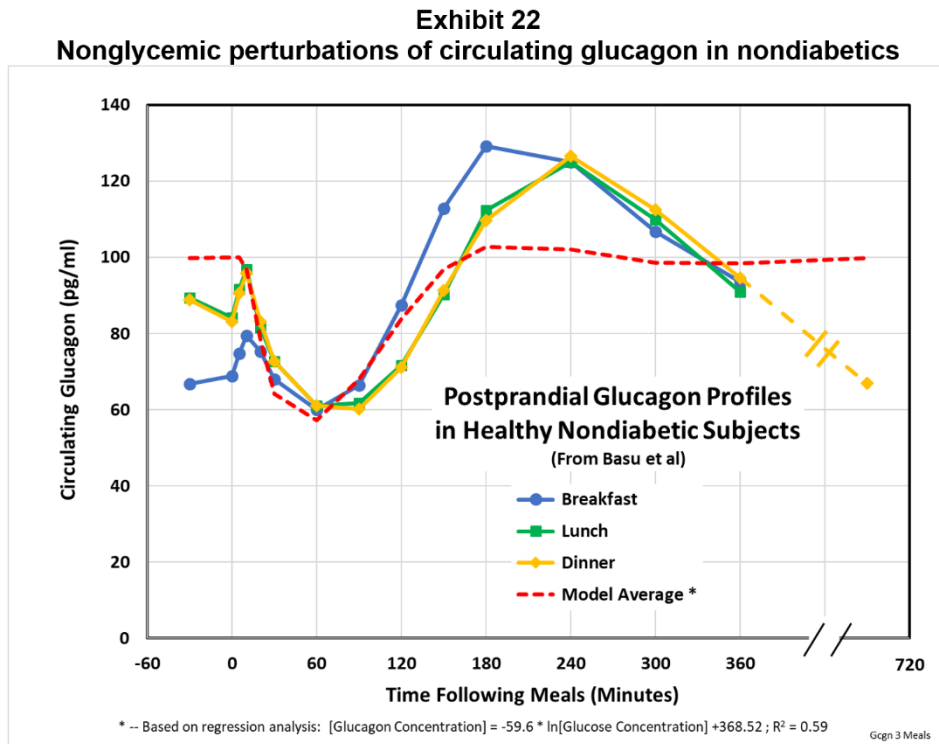
**Exhibit 21**  
Nonglycemic regulation of circulating glucagon in nondiabetics



These deviations from the glycemic model predictions will be addressed by the nonglycemic model.

Another way to visualize the nonglycemic perturbations in circulating glucagon is shown in Exhibit 22.

The three postprandial averages for circulating glucagon (blue, green, and orange lines) are shown in the context of the glucagon levels predicted by the correlation between glucose and glucagon (red dashed line) using the three-meal average glucose profile.



The overnight fast changes the breakfast profile from the lunch and dinner profiles somewhat, but the patterns are the same. During the time when glucose is driving the glucagon levels, the three meals show virtually the same minimum, with the postabsorption breakfast profile peaking an hour before the lunch and dinner profiles. From four hours postprandial the glucagon profiles are in perfect alignment, except that breakfast and lunch declines toward basal are interrupted by the next meal.

From these profiles it is evident that the only time glucose has an impact on alpha-cell secretion is during the postprandial period when exogenous glucose influx is occurring. At this time, alpha-cells secretion is suppressed to minimize glucose influx from the liver.

### **There is no glucagon dose-response to glucose in T1D**

In Exhibit 23 the glucagon dose-responses for nondiabetics and T1Ds are compared based on data from the diurnal study.

In T1D there is NOT a correlation between glucose and glucagon:

- R-squared equals 0.04, which is consistent with a lack of a direct regulatory connection between glucose and glucagon in T1D. In other words, the mechanism which suppresses glucagon secretion as blood glucose rises is missing in T1D.
- The regression equation shows a slightly positive slope that is contrary to the well documented effect of rising glucose on glucagon levels in nondiabetics; hence, the true value of this slope is probably zero.

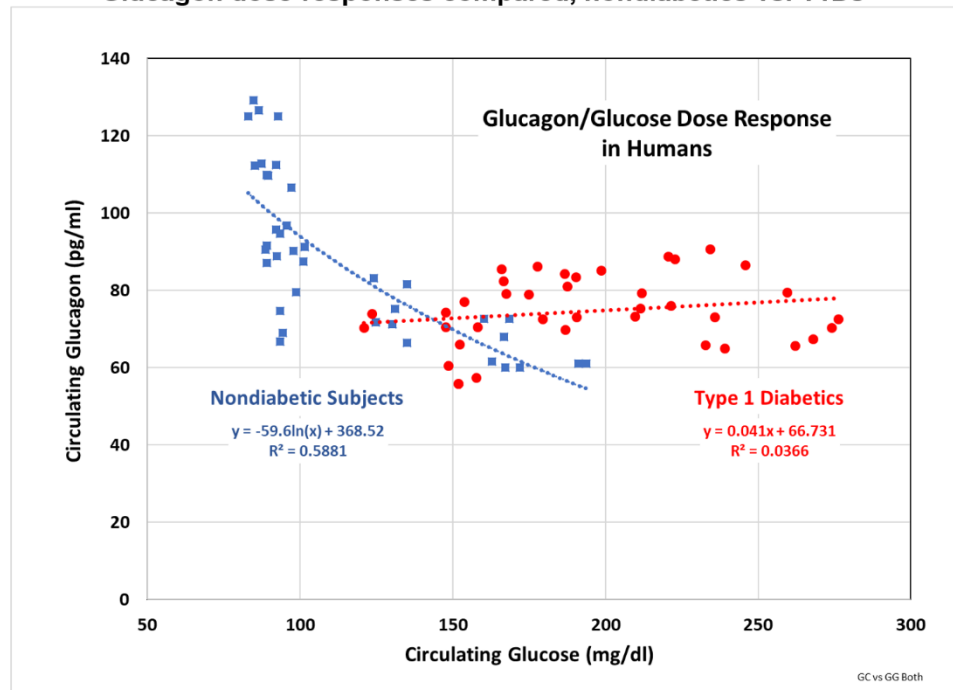
It is unlikely that this lack of alpha-cell response to rising glucose is caused by a rate of change in T1D below the necessary threshold. As seen in Exhibit 7, the immediately postprandial slopes of the nondiabetic and T1D glucose profiles are essentially parallel.

This finding is inconsistent with the idea that glucose concentration is directly sensed by alpha-cells, because alpha-cells in T1D otherwise appear to be relatively normal. Rather it supports the hypothesis that the deficit in glycemic regulation of glucose is caused by the primary underlying etiology of T1D, which is the deletion of beta-cells. I.e., beta-cells have the glucose sensory mechanisms that regulate alpha-cell response to circulating glucose.

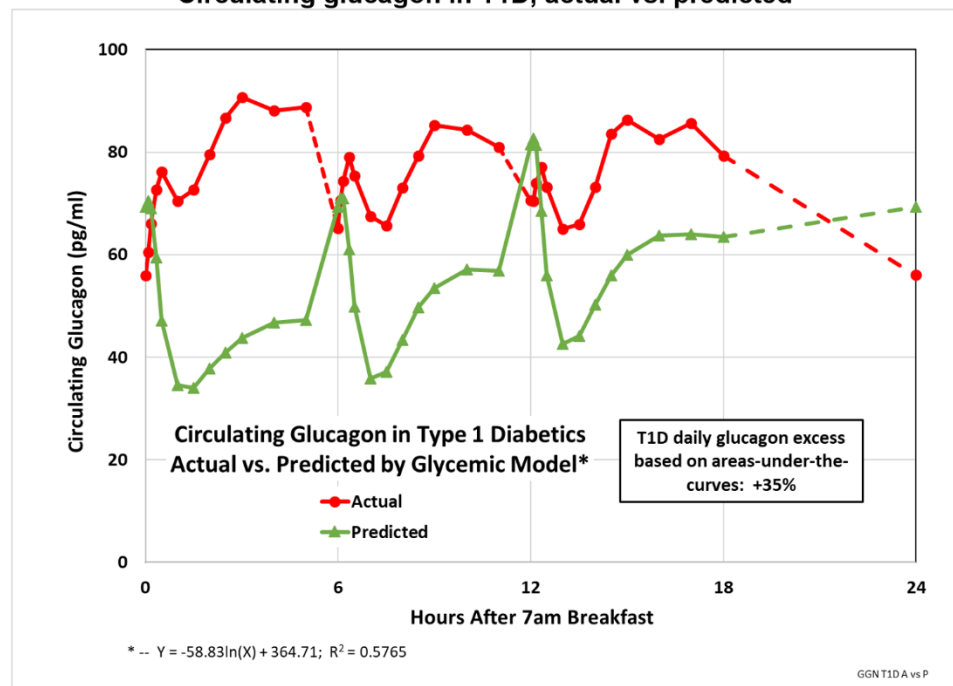
In Exhibit 24 we use the glycemic model to predict circulating glucagon in T1D.

The green triangles show what glucagon concentrations would be if the glycemic model was effective in T1D; the red circles show actual glucagon concentrations in T1D. The results indicate that T1D subjects are relatively hyperglucagonemic between meals, because glucagon levels fail to decline as predicted by the glycemic model derived from healthy subjects. Based on AUCs, the resulting excessive daily

**Exhibit 23**  
Glucagon dose-responses compared, nondiabetics vs. T1Ds



**Exhibit 24**  
Circulating glucagon in T1D, actual vs. predicted





exposure to circulating glucagon is about 35% above what normal glucose regulation of glucagon would be expected to achieve.

### THE NONGLYCEMIC ALPHA-CELL REGULATION MODEL

Based on the glycemic model, we can now examine the half of the glucose-glucagon diurnal profile that is not explained by changes in glucose. The question we ask: is there a clear pattern of diurnal glucagon changes which would imply nonglycemic regulation of alpha-cells?

**Exhibit 25**  
**Deviation of actual glucagon levels from Glycemic Model predicted values**

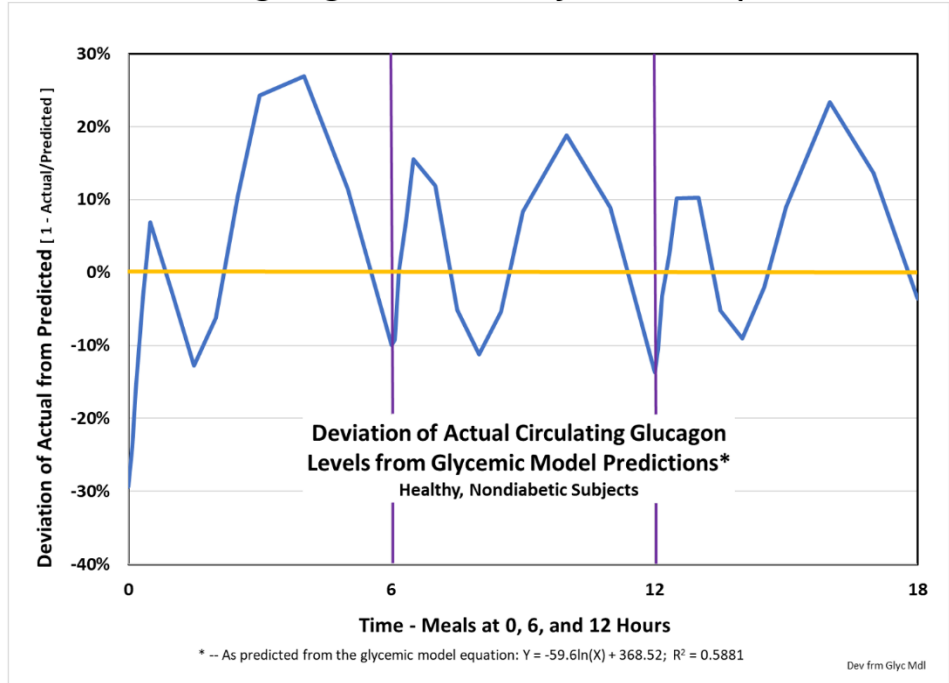
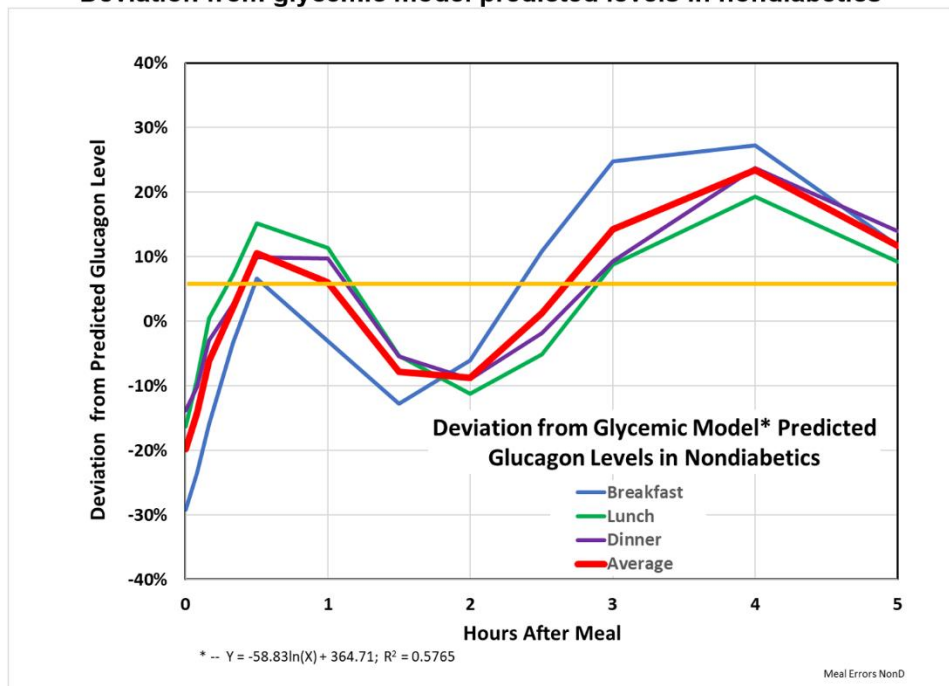


Exhibit 25 shows the percentage deviation of actual glucagon levels in nondiabetics from the levels predicted by the glycemic model (Actual / Predicted – 1).

The diurnal pattern of deviations is repetitive, as shown in Exhibit 26, which superimposes the three meal profiles, plus the average profile, on one five-hour scale.

These deviations do not appear to be random, but rather suggest systematic forcings of glucagon levels associated with mechanisms different from those that drive the glycemic correlation. The breakfast profile appears to deviate from the other two meals, with about three-fold greater suppression at time zero (basal level), and

**Exhibit 26**  
**Deviation from glycemic model predicted levels in nondiabetics**





a shorter time to the postprandial nadir; this suggests that the longer overnight fast results in amplified suppression of circulating glucagon.

In Exhibit 27 we show a plot of the average postprandial glucagon deviations shown in Exhibit 26. The values along this plot will be termed the “**Nonglycemic Alpha-Cell Regulation Model.**” (The nonglycemic model.)

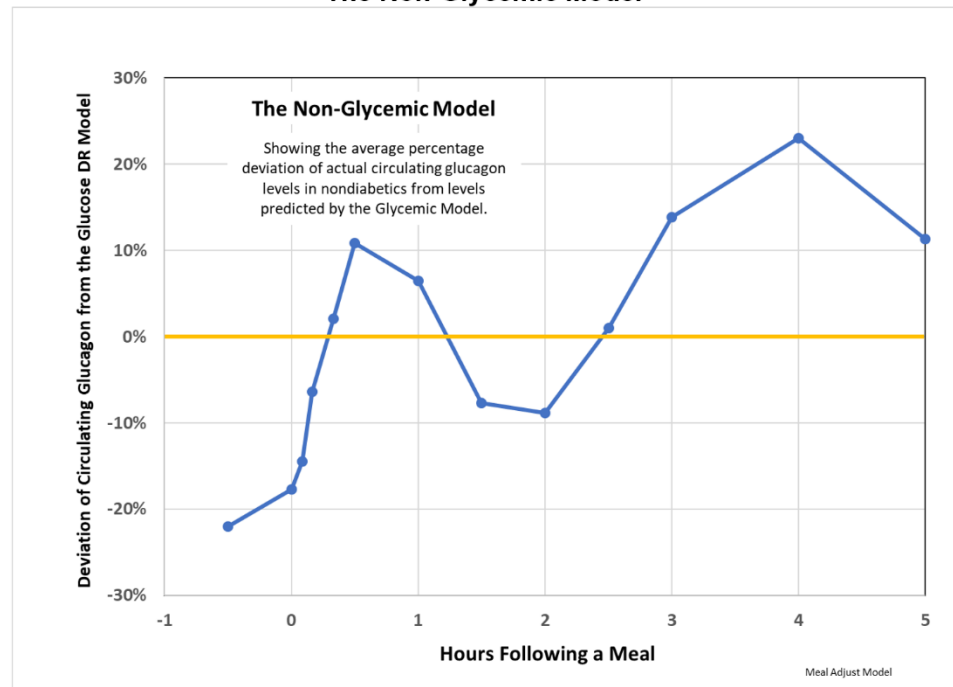
To consider whether this nonglycemic model is likely to be validated beyond the current diurnal study, we used the same approach with the data from the simple carb study. The result is shown in Exhibit 28, which compares the nonglycemic models from the diurnal and simple carb studies.

The resulting profiles are similar, except that the simple carb pattern starts lower and reaches its first nadir about one hour ahead of the diurnal study; this is consistent with the breakfast profile shift in the diurnal study (Exhibit 26).

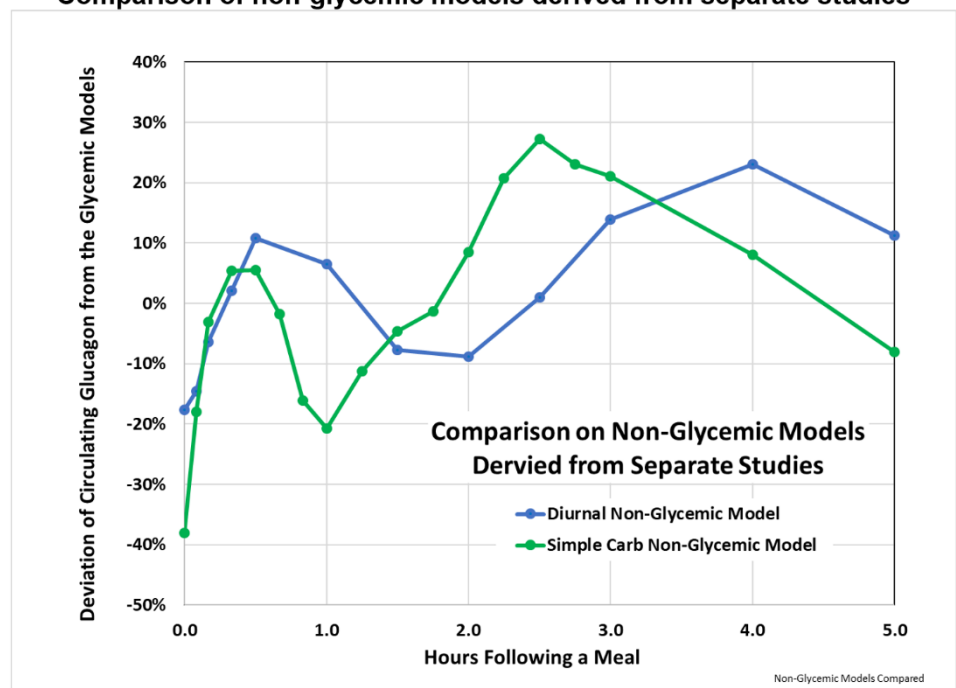
We interpret the similarity of profiles as preliminary evidence that the pattern of peaks and valleys seen in the deviation of the glycemic model predictions from the diurnal study data may be indicative of a universal nonglycemic model.

Exhibit 29 proposes that the postprandial nonglycemic model may be segmented into four distinct phases.

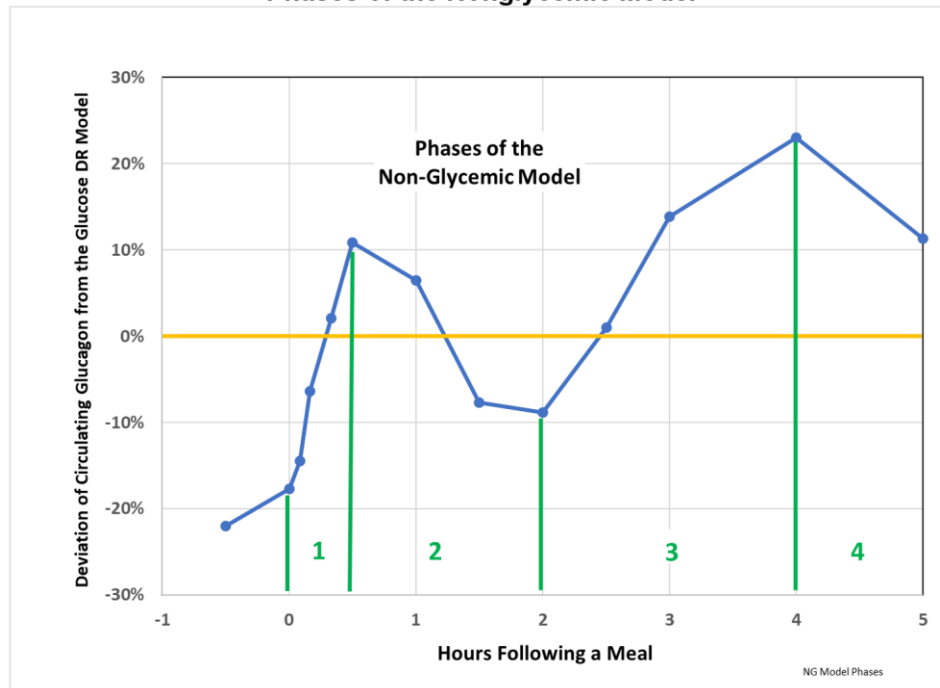
**Exhibit 27**  
**The Non-Glycemic Model**



**Exhibit 28**  
**Comparison of non-glycemic models derived from separate studies**



**Exhibit 29**  
**Phases of the Nonglycemic Model**



In this table we speculate about possible mechanisms to explain this profile:

Phase	Glucagon Deviation from the Glycemic Model	Etiology?
Basal	Preprandial glucagon is substantially below that predicted by the glycemic model.	Suppression of alpha-cell secretion by some glucose independent forcing agent during sleeping?
1	Rapid rise from the basal deficit to a modest overshoot by 30 minutes postprandial.	Stimulation of alpha-cell secretion by ingested nutrients (arginine) and gut-derived hormones?
2	Decline from the prandial overshoot to a slight (~10%) deficit by 2 hours postprandial.	Dissipation of the non-glucose alpha-cell stimulation?
3	Rise to a 20%+ overshoot by 4 hours.	Anorexic signal to hold off the urge to eat until the next scheduled meal, plus response to exercise?
4	Decline back toward the fasting deficit.	Shift to sleeping metabolism, plus dissipation of the anorexic signal to encourage eating?

The over- and under-shoot glucagon values relative to the glycemic model might also be consistent with a regulatory system that requires some time to settle toward the equilibrium determined by the glycemic regulation after being perturbed by the prandial glucose forcings, i.e. as would be seen in a damped oscillating system. However, as we will discuss shortly, the presence of roughly the same four-phase postprandial pattern in T1D argues against this hypothesis, since we showed in Exhibit 23 that there are no glucose forcings of glucagon in T1D.

An interesting question is why the pre-breakfast basal levels are hypoglucagonemic relative to the glycemic model prediction. Perhaps there is some glucose-independent background alpha-cell suppression signal that is overridden by the return to glucose driven suppression and postabsorption nonglycemic increases?

### THE INTEGRATED ALPHA-CELL REGULATION MODEL

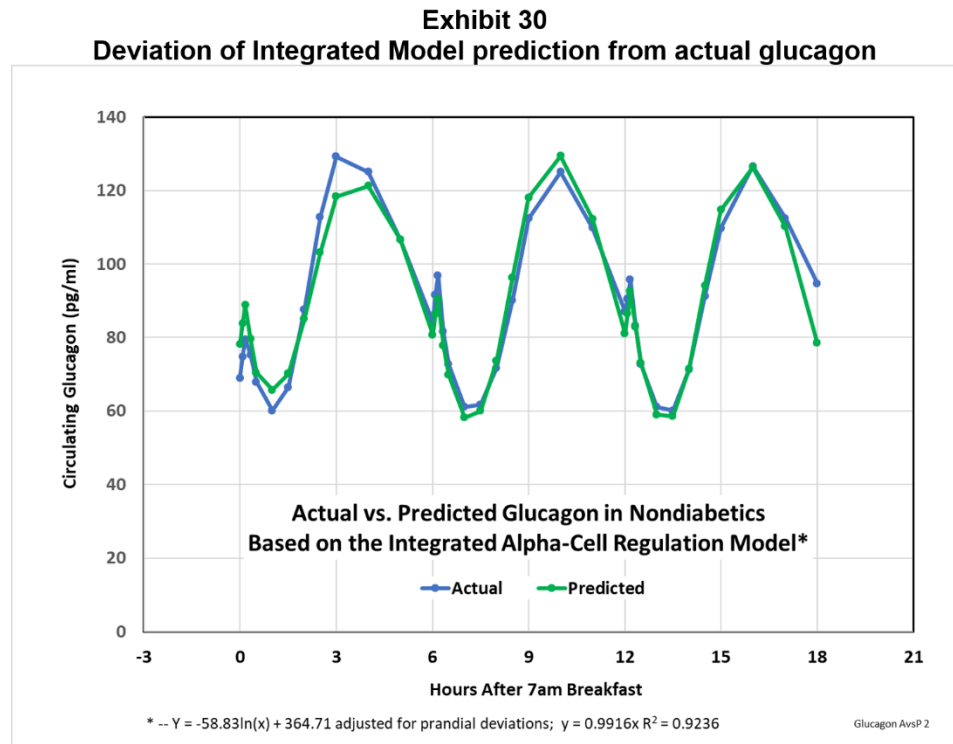
We can now construct a model that considers both the glucose-dependent response and the nonglycemic regulation by multiplying the two models. First, we use the glucose concentration in the glycemic model to calculate a preliminary glucagon concentration. Then, we multiply this preliminary glucagon concentration by the adjustment factor in the nonglycemic model at each point in time post-meal to generate a predicted glucagon concentration.

This combination of models is termed the “**Integrated Alpha-Cell Regulation Model.**” (The “integrated model.”)

In Exhibit 30 we compare for nondiabetics the actual glucagon concentrations to those predicted by the integrated model.

The fit is quite good, as shown in Exhibit 31 which compares the integrated model predictions of circulating glucagon to the measured levels.

The slope is close to 1.0 with an intercept of 6 pg/ml, and the R-squared is 0.93; thus, the integrated model accounts for over 90% of the variation in glucagon for this study, a



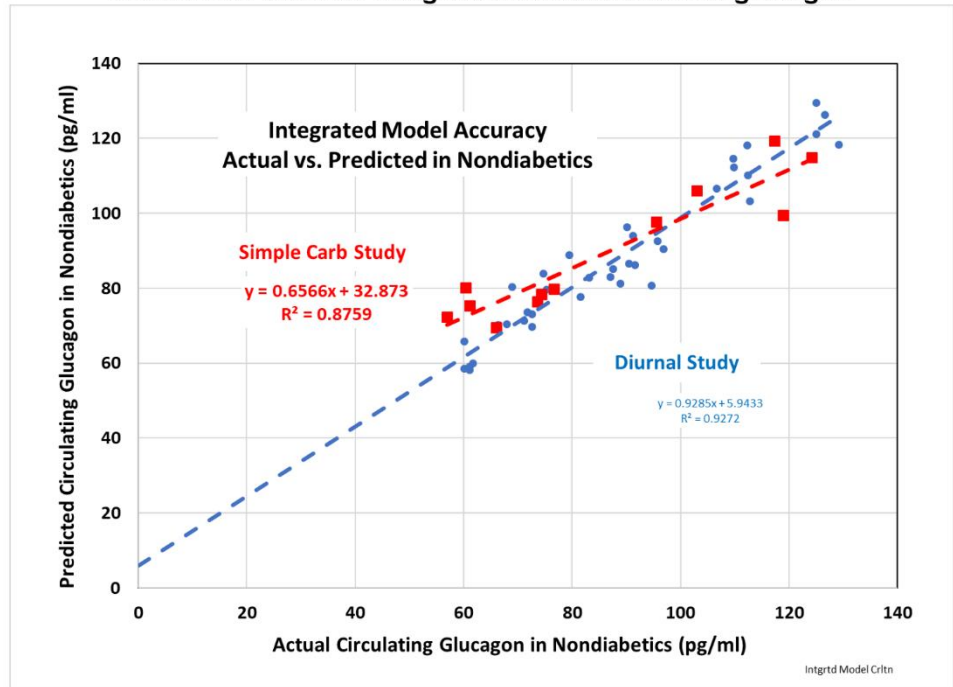
level similar to the R-squared value for the insulin model (Exhibit 10).

We expected that the integrated model would do a good job of predicting the data from which it was derived. To test the general applicability of the model, we used the integrated model to predict glucagon levels for the simple carb study and correlated the predicted values with actual simple carb results as shown in Exhibit 31. The R-squared was 0.88; however, the slope of that linear correlation equation was 0.66, and the intercept was 33 pg/ml. Presumably this deviation from a slope of 1.0 and an intercept of 0.0 reflects the slight differences in the dose-response curves shown in Exhibit 17.

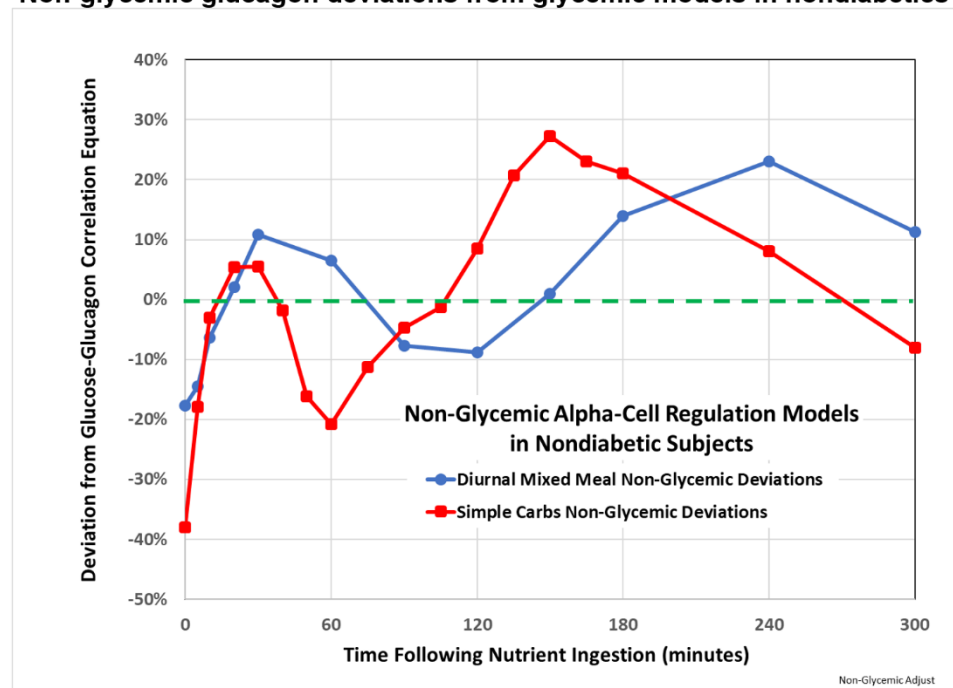
To determine whether the nonglycemic effects on glucagon secretion differed between studies, we used the glycemic equations in Exhibit 17 to calculate the deviations from the glucose correlation equations. The simple carb deviations mimicked the breakfast deviations in the diurnal study, as shown in Exhibit 32.

- The basal fasting deviation of the simple carb study is almost three times as deep as that of the diurnal study glycemic model prediction. As shown in Exhibit 26, the breakfast deviation for the diurnal study was 30%, which is similar to the simple

**Exhibit 31**  
Correlation between Integrated Model and actual glucagon



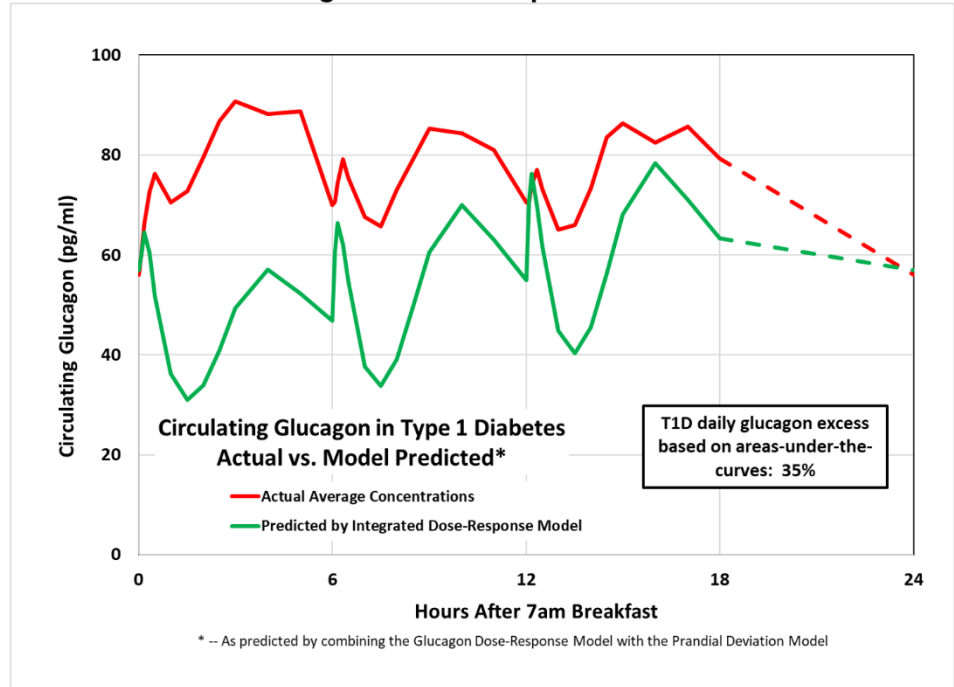
**Exhibit 32**  
Non-glycemic glucagon deviations from glycemic models in nondiabetics



carb study in Exhibit 28.

- The two studies show similar four-phase patterns of nonglycemic variations.
- However, Phase 2 of the simple carb study is shorter, reaching bottom about 30 minutes earlier than the diurnal study. The simple carb study also showed a deeper deviation below the glycemic prediction at the end of Phase 2.

**Exhibit 33**  
Diurnal glucagon in T1D, actual vs. predicted by the Integrated Dose-Response Model

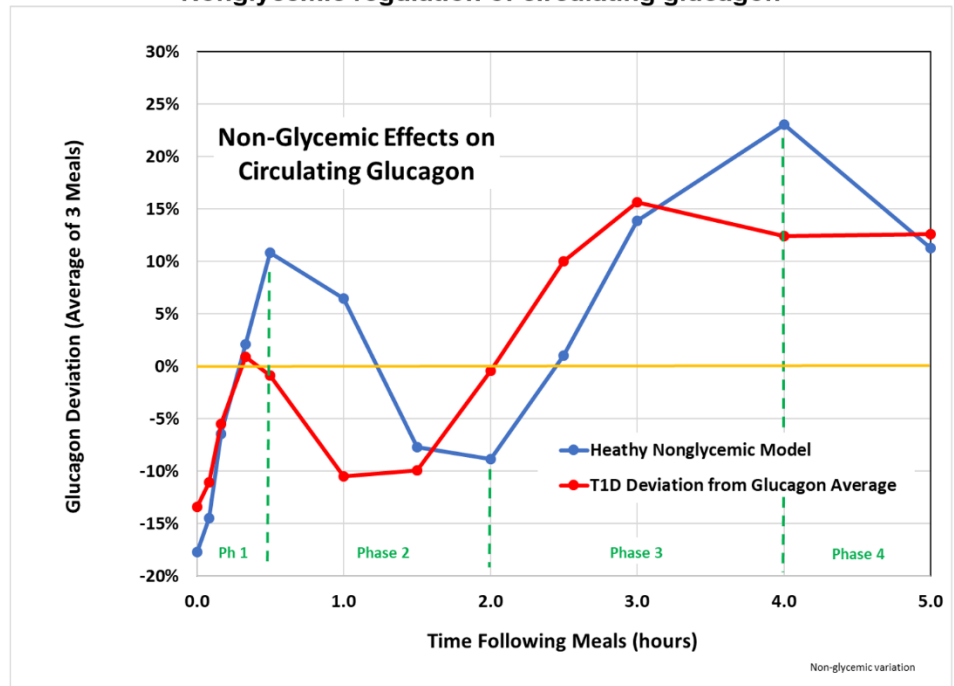


We then applied the integrated model to analyzing the degree and timing of hyperglucagonemia in the T1D population. Exhibit 33 compares the actual diurnal glucagon profile of the T1D subjects to the profile predicted by the Integrated Model.

At the start of mealtimes, T1D glucagon levels are briefly in the “normal” zone for the relevant glucose levels and prandial timing, but between meals T1D glucagon levels are excessive, with a total daily over-exposure of about 35% based on AUCs.

Since T1D patients have lost the glucose-driven regulatory mechanism, their pattern of postprandial glucagon variations could be expected to mimic those predicted by the nonglycemic model, if the non-glucose forcings on

**Exhibit 34**  
Nonglycemic regulation of circulating glucagon



alpha-cells are relatively normal in T1D. To test this idea, for each of the three diurnal study meal periods for T1D subjects, we calculated the five-hour glucagon average concentrations, then calculated for each meal period the percentage deviations of actual glucagon levels from these averages (Actual / Projected – 1), and finally averaged the three deviations for each time reading. This index is equivalent to assuming that circulating glucose has no effect on circulating glucagon in T1D.

As shown in Exhibit 34, the T1D deviation from average pattern is remarkably similar to the nonglycemic model for healthy subjects.

Following are the differences between nonglycemic-driven glucagon in healthy vs. T1D subjects by phases of the nonglycemic model:

- **Phase 1:** The T1D rate of increase is the same as the healthy rate, except that the T1D rise peaks at about the average concentration.
- **Phase 2:** Both T1D and healthy deviations decline to about the same level, except that the T1D nadir is reached about 30 minutes earlier.
- **Phase 3:** Again, the T1D rate of increase is about the same as the healthy rate, except that the peak is reached about 60 minutes earlier.
- **Phase 4:** Both T1D and healthy glucagon deviations have converged at just over 10% by five hours.

It appears from the profiles in Exhibit 34 that, while T1D subjects have lost glycemic regulation of alpha-cells, that the nonglycemic regulatory mechanisms remain largely intact. This also suggests that the nondiabetic deviations from the glycemic model are not caused by time lags in alpha-cell response to circulating glucose levels.

## Endnotes:

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<sup>1</sup> -- Glucagon is the key factor in the development of diabetes; *Diabetologia* 59:1372-5 2016.

<sup>2</sup> -- Glucagon Control on Food Intake and Energy Balance; *International Journal of Molecular Sciences* 20:3904-16 2019.

<sup>3</sup> -- Arginine-Stimulated Acute Phase of Insulin and Glucagon Secretion in Diabetic Subjects; *Journal of Clinical Investigation* 58:565-70 1976.

<sup>4</sup> -- <http://plotdigitizer.sourceforge.net/>

<sup>5</sup> -- Translating the A1C Assay Into Estimated Average Glucose Values; *Diabetes Care* 31:1473-8 2008.



## Appendix B

### Derivation of Appropriate Dual Ratio Amylin/Insulin Dosing

Since the purpose of hormone replacement therapy is to mimic healthy endogenous plasma levels, it follows that subcutaneous dosing of hormone agonists should be designed to achieve these differences in plasma basal and bolus ratios. Since T1D patients with diabetes adjust their daily insulin dosing to reflect exercise and eating patterns, the best way to accomplish this is to determine amylin agonist doses based on amylin’s healthy physiologic relationship to insulin. In this way patients would have a simple algorithm for deciding how much pramlintide to take based upon their individual insulin doses.

In this appendix we use the following process to estimate ratios for the Dual Ratio Amylin/Insulin (DRAI) dosing:

- **Estimate exogenous dosing ratios to mimic plasma profiles:** Based upon the mass ratios of insulin and amylin plasma levels, we first estimate the amounts of amylin agonist that should be infused subcutaneously calculated as ratios to the basal and bolus insulin doses.
- **Convert mass ratios to convenient guidelines for mixing with insulin:** We then convert the mass ratios to  $\mu\text{g}/\text{U}$  ratios and illustrate how these would dictate pramlintide dosing as a function of insulin dosing.

#### ESTIMATE EXOGENOUS DOSING RATIOS TO MIMIC PLASMA PROFILES

Insulin-dependent dosing can be achieved by calculating two separate components of pramlintide dosing: (1) the weight ratio of the daily basal component of amylin-to-insulin; and (2) the weight ratio of the daily bolus

component of amylin-to-insulin. Table 1 shows this calculation: column two shows the calculated molar AUCs for endogenous plasma insulin and amylin; column four shows endogenous gram AUCs after correcting for molecular weight differences; and column six shows exogenous gram AUCs after correcting for bioavailability of subcutaneous (SC) injections. The exogenous ratios of pramlintide-to-

**Table 1**

	Calculation of Dosing Ratios Based on Endogenous Hormone Ratios				
	<i>In Vivo</i>				<i>Ex Vivo</i>
	AUC pM x 24hr	Molecular Weight	AUC $\mu\text{g}/\text{L} \times 24\text{hr}$	Bio- Availability*	AUC $\mu\text{g}/\text{L} \times 24\text{hr}$
Basal Amylin	222.79	3949	879.82	35%	2513.76
Basal Insulin	2045.34	5808	11879.36	70%	16970.52
<b>Basal Amylin/Insulin Ratio</b>	<b>10.9%</b>		<b>7.4%</b>		<b>14.8%</b>
Bolus Amylin	135.19	3949	533.88	35%	1525.37
Bolus Insulin	3813.31	5808	22147.72	70%	31639.60
<b>Bolus Amylin/Insulin Ratio</b>	<b>3.5%</b>		<b>2.4%</b>		<b>4.8%</b>

Note: This calculation assumes approximately equal absorption and clearance rates for insulin, amylin, and pramlintide.  
\* -- Based on package insert data.



insulin are highlighted in grey.

These calculations indicate that, to mimic healthy endogenous hormone levels, basal SC infusions of insulin should be accompanied by about 15% pramlintide by weight to insulin, and bolus SC injections of insulin should be accompanied by about 5% pramlintide by weight to insulin.

In the example above absorption and clearance rates for insulin, amylin, and pramlintide are assumed to be approximately equal. For use in a dual hormone pump, the most likely choice of insulin would be from among the rapid acting varieties, e.g. APIDRA with an apparent SC half-life of 42 minutes compared to the apparent SC half-life of 48 minutes for SYMLIN; in this case the assumption of about equal absorption and clearance rates is probably appropriate. In practice, actual dosing ratios should be determined by taking into account the specific pharmacokinetics of the insulins and amylin agonists being used.

### CONVERT MASS RATIOS TO CONVENIENT GUIDELINES FOR MIXING WITH INSULIN

Because insulin is traditionally dosed in Units rather than  $\mu\text{g}$ , it is more useful to express these ratios as  $\mu\text{g}$  of pramlintide per Unit of insulin. In Table 2 the WHO standard for insulin is converted into  $\mu\text{g}$  per Unit of insulin, which is then multiplied times the basal and bolus ratios highlighted in grey in Table 1.

Thus we have the DRAI ratios:

- **Basal:** 5.7  $\mu\text{g}$  pramlintide per Unit basal insulin.
- **Bolus:** 1.8  $\mu\text{g}$  pramlintide per Unit bolus insulin.

### A REAL LIFE EXAMPLE OF DUAL RATIO AMYLIN/INSULIN DOSING

To put some flesh and blood around the above analysis, following is the experience of a 50-year-old man with T1D who has tried and abandoned pramlintide. This individual is well above average in education and medical skills: he is an MD who is presently CEO of a biotech firm in the molecular biology area and who has remained on the faculty of a top tier university. He has collaborated in this

Table 2

Calculation of Discrete Basal/Bolus Hormone Ratios	
1987 WHO standard for Units per gram insulin: <sup>1</sup>	26,000
Grams per Unit of insulin (inverse of standard):	0.00003846
$\mu\text{g}$ per Unit of insulin (inverse $\times 10^6$ ):	38.5
<b>Basal pramlintide <math>\mu\text{g}</math> per Unit of insulin:</b>	<b>0.148 <math>\times</math> 38.5 = 5.7</b>
<b>Bolus pramlintide <math>\mu\text{g}</math> per Unit of insulin:</b>	<b>0.048 <math>\times</math> 38.5 = 1.8</b>

analysis of pramlintide dosing from the perspective of someone who recognizes the theoretical benefits but found the cost/benefit ratio to be unacceptable.

He started with 48 µg mealtime doses of pramlintide while backing off insulin from 40-50 U/day to 30-40 U/day, a reduction of 20-25%. Because of nausea he reduced pramlintide to 24 µg doses, or 72 µg daily (this was before vials were replaced by prefilled syringes with set amounts). During the nine months he was on pramlintide he experienced significant though short-lived (30-45 minutes) nausea even at the lower doses, although he never felt close to vomiting. Because he had already been maintaining tight blood sugar control (HbA1c at 5.7-6.4), he was concerned about the time it took to pull out of hypoglycemic episodes. Over the nine month period he lost 15-20 pounds, so discontinuing was a tough call. In spite of his recognition of the theoretical benefits of pramlintide therapy, its burdens simply did not justify continuing because he could achieve his HbA1c target with insulin alone.

How would he dose pramlintide using an AID system and the DRAI algorithm? Let's run the numbers...

At the upper range of our subject's daily insulin dose with 50% as bolus, his average basal/bolus doses would be as shown in Table 3.

**Table 3**

<b>Example Calculation of Pramlintide Dosing</b>					
<b>Infusion Component</b>	<b>Daily Insulin Dose</b>		<b>Ratio: Pramlintide per U Insulin</b>		<b>Daily Pramlintide Dose</b>
Basal	20 U	X	5.7	=	114 µg
Bolus	20 U	X	1.8	=	36 µg
Daily	40 U				150 µg

The indicated daily dose of pramlintide would be 150 µg:

- **Basal dose:** 114 µg would be infused as a continuous basal level over 24 hours, i.e. at an infusion rate of about 4.8 µg per hour.
- **Bolus doses:** 36 µg would be infused with the three insulin boluses, i.e. 12 µg at mealtimes, assuming equal mealtime insulin boluses.

Following package insert instructions, he was taking mealtime injection boluses of 24 µg for a daily total of 72 µg. In other words:

- **Mealtime overdosing:** During a 2-hour post prandial period he should have received  $[12 + (2 \times 4.8)] = 21.6$  µg, ideally with the 12 µg as a square wave bolus to better match the natural amylin profile. Thus his mealtime injections of 24 µg were about 10% over this starting ratio; either his

continuing nausea was explained by this overdosing, or his ideal bolus ratio to avoid nausea might be lower than 1.8 µg/U.

- **Daily underdosing:** During a 24-hour period he should have received 150 µg, or twice the amount that resulted from following the package insert instructions. This would explain the disappointing impact on HbA1c, and the lack of any noticeable impact on his hypoglycemia experience.

The doses calculated from the *in vivo* plasma profiles are different from current clinical practice. However, the most dramatic difference is not dosing size, but rather dosing profile: it is changed so that only about one quarter to one third of the daily dose is administered as mealtime boluses, rather than 100% of the daily dose. By emphasizing the basal component of amylin's plasma profile, efficacy should be maximized without triggering nausea, and the more natural plasma profile will reduce insulin dosing, which could be expected to correct the problem of hypoglycemia stickiness caused by too much insulin onboard.

In actual clinical practice, the DRAI ratios would be adjusted to (1) avoid any mealtime nausea while suppressing postprandial glucagon and (2) achieve a high enough basal level to restore glucagon counterregulation.

## Appendix C

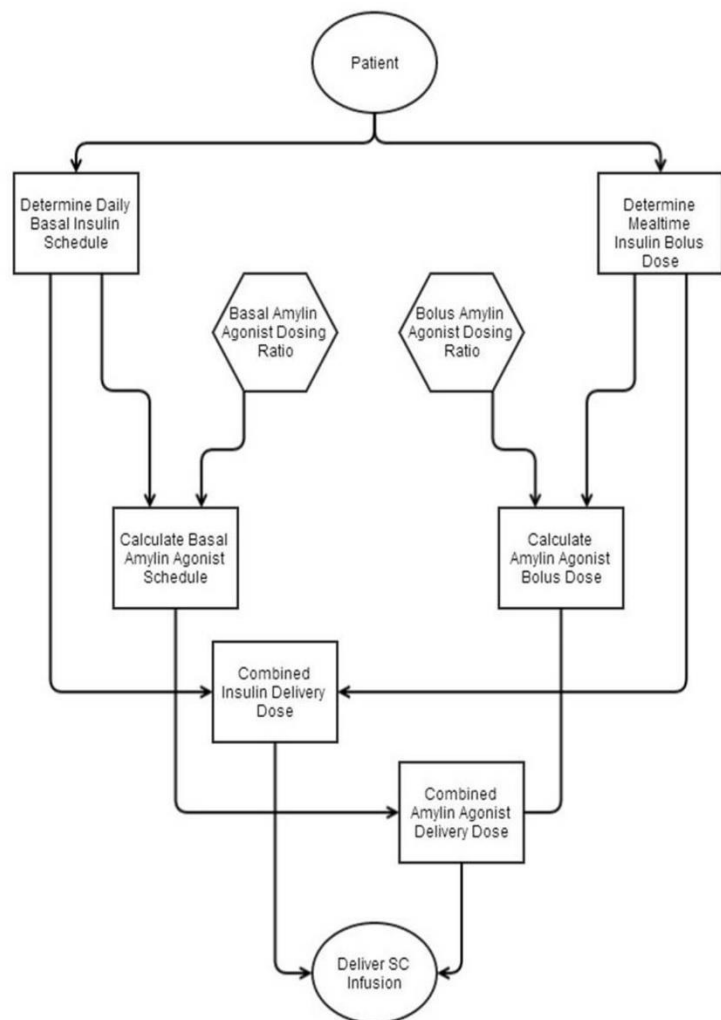
# US Patent 9,656,017 INFUSION DELIVERY DEVICES AND METHODS

### ABSTRACT

Devices that include multi-reservoir infusion devices and systems for dispensing compositions for the treatment of subjects with an amylin agonist (e.g., the amylin agonist analog, pramlintide), wherein amylin agonists are administered in certain differential bolus and basal ratios to an administered insulin, as well as methods, compositions, and kits and articles of manufacture comprising said compositions for use in the treatment of responsive patients with an amylin and an insulin in ratios thereof that are distinct for bolus and basal administration.

### FIRST CLAIM

1. A medical infusion pump system for delivering an insulin and an amylin agonist analog to a patient, said system comprising a user interface, an insulin drug reservoir, an amylin agonist analog drug reservoir, and independent pumping mechanisms for said drug reservoirs, wherein said pumping mechanisms can be regulated by the patient and/or one or more computer algorithms via a processor that (a) sets the basal and bolus rates of insulin infusion to stabilize glucose levels and (b) calculates the basal and bolus rates of amylin agonist analog infusion in ratios to said basal and bolus insulin infusion rates, wherein the amylin agonist analog/insulin basal ratio is different from and higher than the amylin agonist analog/insulin bolus ratio.



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