Type 1 Diabetes and the Insulin Problem

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Interests: Type 1 Diabetes Mellitus

Introduction

I am honored to have the opportunity to present Medicine Grand Rounds, for the first time since joining the faculty at UTSW. I plan to use this occasion to introduce myself to you, share my research background and interests, and give you my perspective of type 1 diabetes as a physician scientist, hoping that this will lead to collaborative scholarly endeavors.

Case Presentation

Chris is a 19 year old male college student, engineering major, who has had type 1 diabetes of 9 years duration.

Chris is a smart and motivated patient who manages his diabetes intensively using a rapid acting insulin analog via insulin pump. His pump delivers insulin at a basal rate of 0.8 to 1.1 units per hour, depending upon the time of day. He sort of counts carbohydrates at each meal and administers a bolus of insulin, 1 unit for every 8 grams of carbohydrate consumed. For meals that are high in protein or fat he uses additional insulin, above what is required for the carbohydrates, and administers this extra insulin bolus over a prolonged period of time, typically 2 to 6 hours. He also administers insulin to correct for pre-meal hyperglycemia, using 1 unit of insulin for every 40 mg/dl that his blood glucose concentration is elevated above normal. Before, during and for several hours after exercise, he temporarily reduces his basal insulin to 80% of the normal rate, administers less insulin for food, and uses less insulin to correct hyperglycemia, all to prevent hypoglycemia resulting from exercise-induced increases in insulin sensitivity. He does all of this every day, several times per day.

Chris self monitors his blood glucose 8 to 10 times per day. He is often seen eating glucose tablets to quickly normalize blood glucose values that are trending low. He recently purchased, out-of-pocket, a continuous glucose monitor (CGM) which has helped him manage his diabetes better than ever and with reduced occurrence of hypoglycemia. Yet, he goes for periods of time without wearing his CGM because the technology is imperfect and adds to the daily burden of managing his diabetes. This frustrates his parents who worry that he will not wake up from hypoglycemia after drinking alcohol while away at college.

His diabetes is well controlled with glycosylated HbA1c values in the 6.5% to 7% range. Yet, as can be seen by a download of his home glucose meter, blood glucose values are highly variable (**Figure 1**). Each black dot on this Figure 1 represents a single result of self-monitored blood glucose. The glucose concentration (y-axis) is plotted according to the time of day that it was obtained (x-axis). This graph represents values obtained in the 2 weeks prior to a clinic visit. **High glucose variability characterizes type 1 diabetes,** with frequent values as low as 50mg/dl and as high as 300mg/dl, even in well controlled patients.

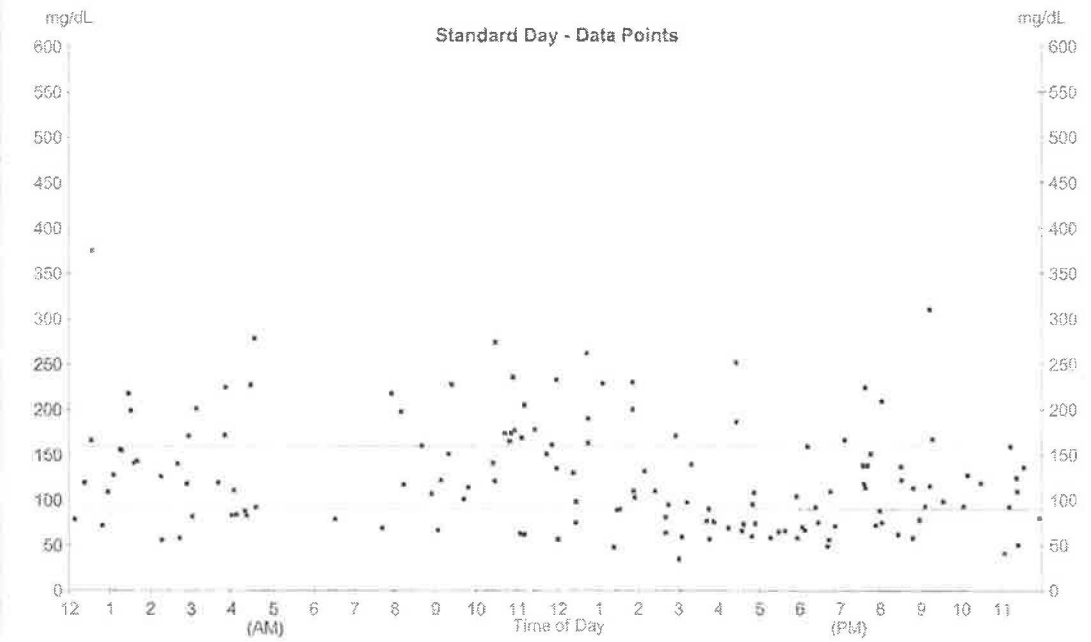


Figure 1. *Chris' glucose meter download with 2 weeks' worth of values plotted according to blood glucose (mg/dl, y-axis) and time of day it was obtained (x-axis).*

Reviewing his meter download in clinic, we observe a trend towards hypoglycemia in the late afternoon, before dinner. He is very active with fencing practice after lunch (note: this is a patient of mine from the East Coast, not Texas). Knowing that the fast acting insulin analog that he uses in his pump starts to work quicker than regular insulin, and that it peaks in glucose lowering activity after about 2 hours and lasts 4 hours, we reduce his basal insulin by 20% starting one hour before practice. He also adjusts his pump to deliver less insulin per carbohydrate at lunchtime. Aside from this trend to go low after regular exercise, there is no pattern to blood glucose values taken at any time of the day.

Keep in mind that Chris' case is about as good as it gets in terms of managing diabetes. He is technology savvy and good with numbers. He understands concepts related to the pharmacodynamic profile of insulin activity and is always considerate of where he is on the "insulin activity curve", balancing this with the effects of food, exercise and stress. He has been part of a novel program of coordinated transition from the pediatric to adult endocrinology clinic and has always seen endocrinologists focused on T1DM. He is happy and has been able to "find the calm in the storm" of living with T1DM. His family is supportive of him and together they are active in the JDRF and ADA. Yet Chris' blood glucose values are still all over the place all of the time which is why Chris volunteers in a laboratory at his university focused on curing diabetes.

Let's talk about type 1 diabetes and the insulin problem.

Type 1 diabetes is an autoimmune disease

Unlike the more common type 2 diabetes which is associated with obesity and insulin resistance, type 1 diabetes is an autoimmune disease that leads to destruction of the insulin producing beta cells of the islets of Langerhans (**Figure 2**). As a result of absolute insulin deficiency, patients are rendered dependent upon insulin for life.

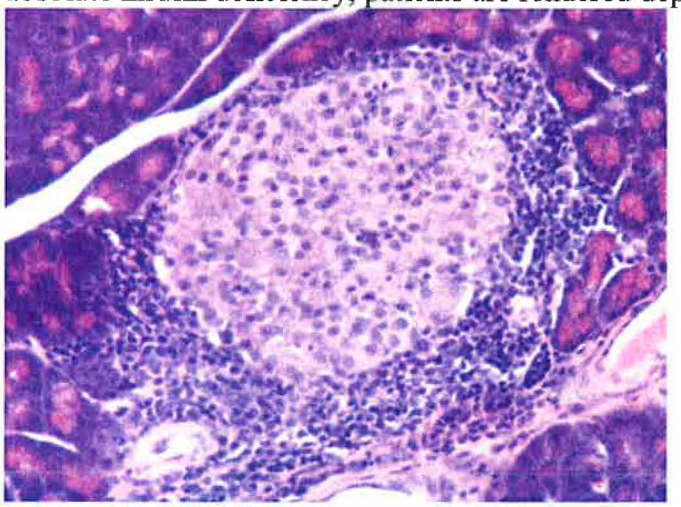


Figure 2. *Lymphocytic peri-insulitis. Here you can see T lymphocytes invading the insulin producing beta cells of a panctreatic islet of Langerhan's.*

In contrast to type 2 diabetes, type 1 diabetes is not usually considered a familial disease, meaning that most patients with T1DM do not have a family member who is likewise afflicted. There is, however, a genetic predisposition to development of T1DM with the most important genes being located within the Major Histocompatibility Complex (MHC) HLA Class II region[1]. Specific HLA-DR and HLA-DQ haplotypes provide both susceptibility towards, and protection from, the disease. Non-HLA genes have also been associated with T1DM [2].

The Eisenbarth model for how T1DM develops is depicted in **Figure 3** [3]. The genetic predisposition to disease development, when acted upon by a putative environmental trigger, leads to a process of T-cell mediated destruction of the insulin producing beta cells. These environmental triggers have not been clearly defined but 3 different groups have been implicated which include 1) viruses (eg, enteroviruses, coxsackie, congenital rubella, 2) early infant diet (eg, cow's milk, cereals, gluten), and 3) toxins (eg, nitrosamines). In addition to the T-cell mediated process of insulitis, there is a humoral response with B-cell production of antibodies to beta cell antigens. Although the presence of these autoantibodies is not believed to be involved in the pathogenesis of the disease, their presence is used as a surrogate marker for the autoimmune process and aids in establishing the clinical diagnosis or to predict future disease onset in high risk individuals [4]. As beta cell mass is destroyed, there is first a loss of the first phase insulin response which can be detected by intravenous glucose tolerance test. As beta cell mass declines further, perhaps to 10% of normal, patients present with symptoms leading to clinical diagnosis. The autoimmune process continues and ultimately most patients are rendered with absolute insulin deficiency. There is a period of time, called the honeymoon, which occurs after the initiation of insulin therapy and is notable for

restoration of endogenous insulin production to beta cells that are not yet destroyed. The disease is relatively easy to treat with insulin during this time period and many efforts are aimed at halting the disease process at this time [5].

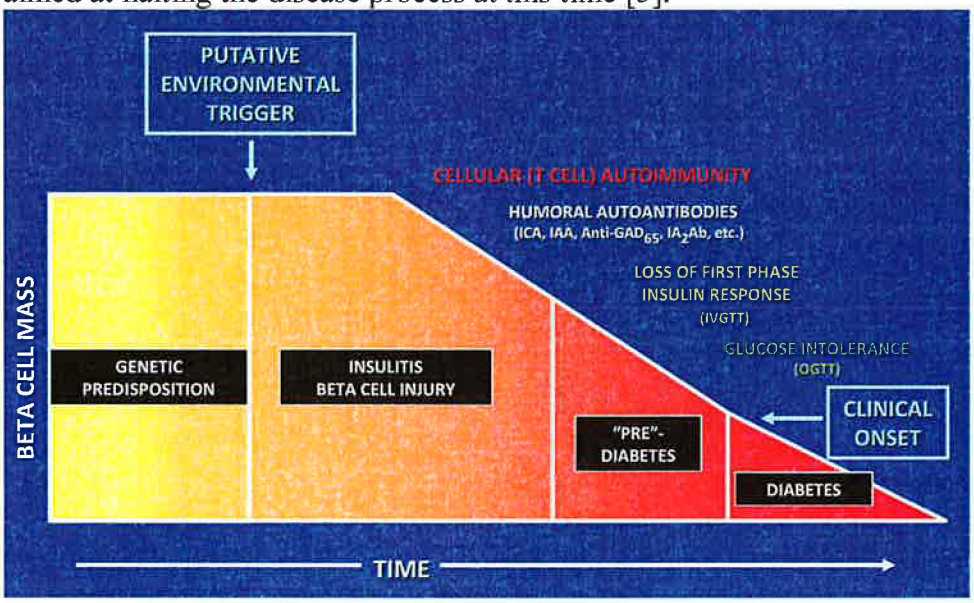


Figure 3. *The Eisenbarth model of T1DM development showing a linear time course of beta cell loss with progression from 100% of normal beta cell mass at birth to absolute insulin deficiency as a result of the autoimmune process.*

Epidemiology

Much less common than type 2 diabetes, type 1 diabetes accounts for only about 5% to 10% of all cases of diabetes. There are approximately 1 million persons in the United States who carry the diagnosis of T1DM. There are potentially another million patients, mostly adults, who have been misdiagnosed as having type 2 diabetes (**Figure 4**).

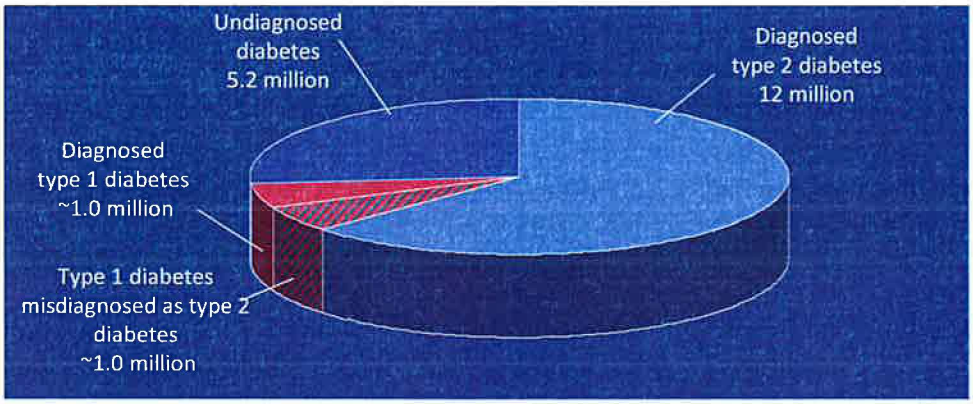


Figure 4. *Prevalence of Diabetes in the United States based on US Population: 275 million in 2000.* (Available at: http://www.cdc.gov/diabetes/pubs/estimates.htm; EURODIAB ACE Study Group. *Lancet.* 2000;355:873-876; Harris MI. In: National Diabetes Data Group. *Diabetes in America.* 2nd ed. Bethesda, Md: NIDDK; 1995:15-36; U.S. Census Bureau Statistical Abstract of the U.S.; 2001)

The incidence of T1DM is increasing at a rate of 3 to 4% per year. The reason for this increase is not clear but is likely related to environmental factors. T1DM was previously referred to as Juvenile Diabetes, as its onset was mostly confined to the pediatric population. Currently, however, 50% of incident cases are diagnosed after age 20. This late-onset T1DM is characterized by an autoimmune process that is slower than that which manifests in childhood. Adults with new onset T1DM often present only with hyperglycemia, without ketoacidosis. As a result, they are often misdiagnosed as having type 2 diabetes. Oral agents are usually ineffective in managing this late-onset T1DM and insulin is eventually required.

Treatment

The Diabetes Control and Complications Trial (DCCT) clearly showed that intensive therapy, compared to conventional therapy, could be used to reduce average blood glucose and lead to a reduced incidence and progression of microvascular complications [6]. Intensive therapy consists of administering insulin in a basal/bolus fashion, using a long acting peak-less insulin such as glargine or detemir or continuous insulin pump delivery of rapid acting insulin to create the basal insulin profile. This is combined with rapid acting insulin (lispro, glulisine, apidra) administered before each meal with frequent self monitoring of blood glucose to guide adjustments in therapy (**Figure 5**). In the DCCT, the intensively treated group had a reduced incidence of retinopathy by 76 percent compared to the conventionally treated group. Intensive therapy also slowed the progression of pre-existing retinopathy by 54 percent, reduced the occurrence of microalbuminuria (urinary albumin excretion of > or = 40 mg per 24 hours) by 39 percent, albuminuria (urinary albumin excretion of > or = 300 mg per 24 hours) by 54 percent, and clinical neuropathy by 60 percent.

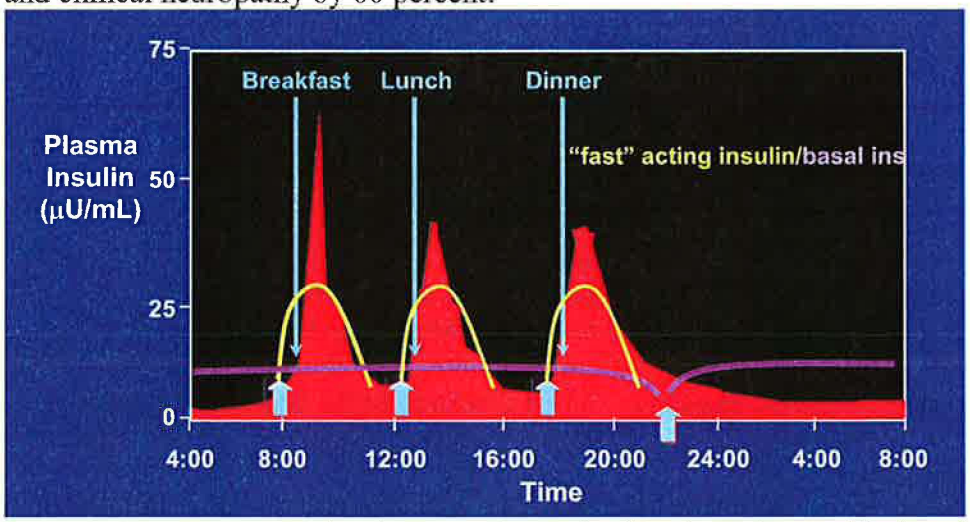


Figure 5. *Intensive insulin therapy using a flat basal insulin profile (purple) with prandial insulin delivery (yellow) superimposed on the insulin profile of a normal individual (red). This regimen requires four injections per day (arrows).*

Intensive insulin therapy and normalization of blood glucose is limited by hypoglycemia which occurs more often as average glucose is reduced, owing to the high glucose variability that is inherent in T1DM. As a result, most patients with T1DM remain inadequately controlled even in the post-DCCT era.

Outcomes

Despite the remarkable results of the DCCT, the morbidity and mortality associated with T1DM remain high. Recent estimates suggest that someone such as Chris in the case presentation, who was diagnosed with diabetes at the age of 10, will lose 19 life-years and 32 quality-adjusted life years [7].

Much of the excess morbidity and mortality is related to the development of cardiovascular disease (**Figure 6**) [8]. In fact, it has been difficult to indicate a benefit from improved glycemia on cardiovascular outcomes. Four large prospective studies in patients with type 1 diabetes do not show that glycemia predicts coronary artery disease occurrence [9-12]. Only recently, have studies resulting from long term follow-up of the DCCT subjects suggested that glycemia is related to heart disease outcomes [13]. Factors that are associated with CVD in T1DM include duration of diabetes, nephropathy, and insulin resistance [9, 14-15]. Since I cannot promise Chris that his good glycemic control will reduce his risk of macrovascular disease, I encourage him to exercise regularly.

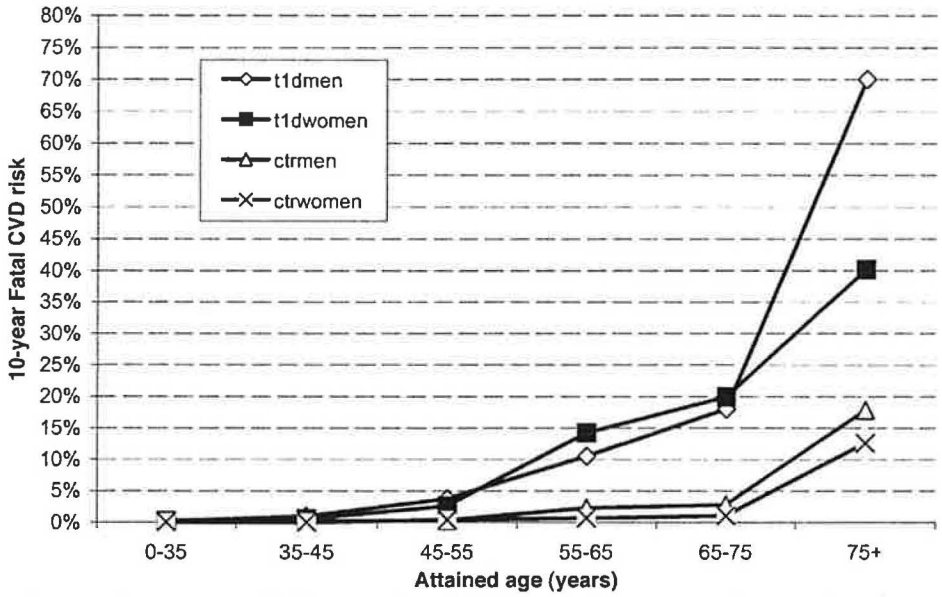


Figure 6. *Estimated 10-year fatal CVD risk by current (or attained) age in type 1 diabetic (t1d) men and women compared with nondiabetic comparison group (ctr)* [8].

The high rate of cardiovascular disease in T1DM is related to the insulin problem.

Problems with insulin

The discovery of insulin in 1922 [16] transformed type 1 diabetes mellitus (T1DM) from a uniformly fatal disease into a manageable, albeit burdensome, disorder. This almost miraculous transformation of a dreaded disease of children and young adults has for the past 87 years endowed insulin treatment with a near-mythical aura of essentiality and harmlessness, despite the fact that life span is shortened [17-18]. Traditionally, all of the disease morbidity, including the shortened life expectancy of well-contolled T1DM patients is attributed to the disease rather than to its therapy.

Insulin normally released from the beta cells of pancreatic islets of Langerhan's reach the islet alpha cells at very high concentration before reaching the liver via the portal vein, still at a high concentration relative to peripheral tissues. These high concentrations of insulin are required to suppress the hyperglucagonemia and increased hepatic glucose production that characterize both type 1 and type 2 diabetes (**Figure 7**) [19].

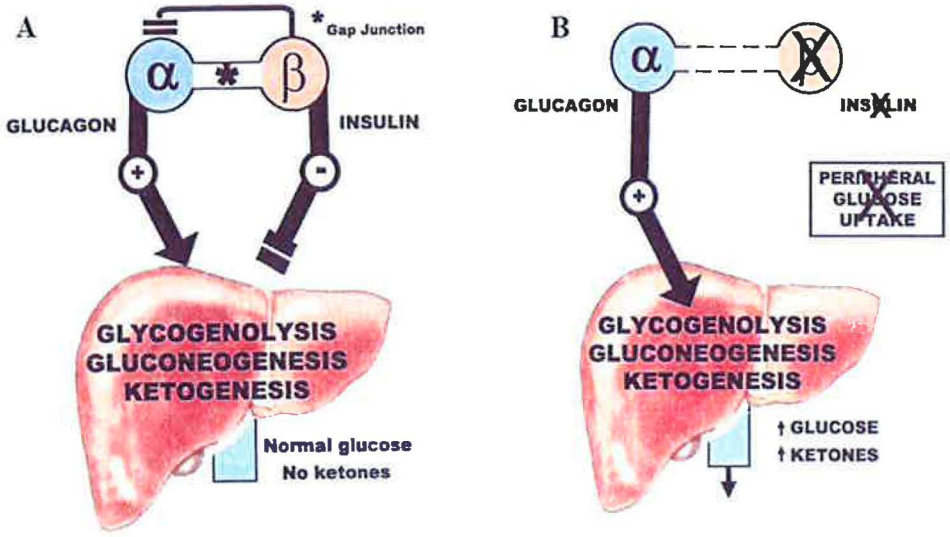


Figure 7. ***A.*** *Interrelationships of alpha- and beta-cells in normal regulation of hepatic fuel production.* ***B.*** *Destruction of β-cells in type I diabetes permits unregulated hyperglucagonemia and unopposed glucagon-mediated overproduction of fuels by the liver.*

Short-term Insulin Issues

Glycemic Instability: The main short-term issue with insulin monotherapy is the instability of the daily glucose profiles achieved by peripheral injections of insulin. Even optimally controlled patients with at target HgbA1c values have daily spikes of hyperglycemia, with occasional hypoglycemic dips **(Figure 8)** [20].

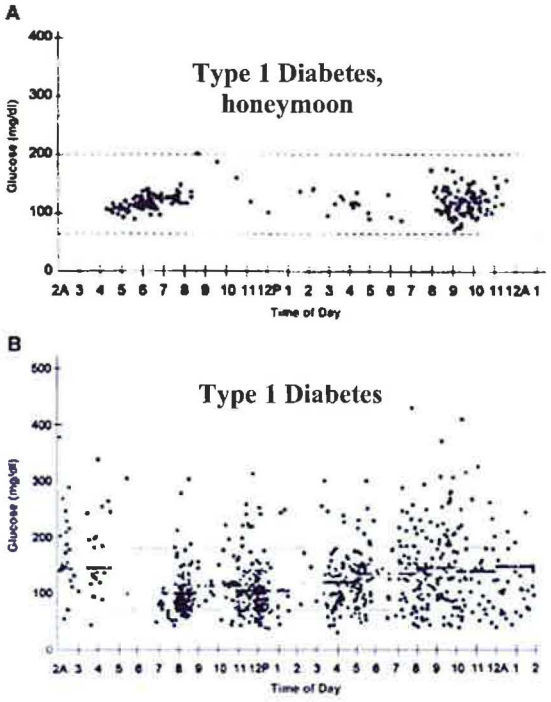


Figure 8. *Two individual self-monitoring of blood glucose (SMBG) meter downloads illustrating similar means with widely varying SDs. Each dot represents a single blood glucose determination over a 4 week period and is plotted according to blood glucose in mg/dl (y-axis) and time of day obtained (x-axis). A: Mean BG 119 mg/dl, SD 20 mg/dl. B: Mean BG 121 mg/dl, SD 61 mg/dl. Both patients are insulin treated; the patient with type 1 diabetes in the honeymoon has remaining endogenous insulin production whereas the other patient with type 1 diabetes does not. Endogenous insulin, which can be suppressed or released as blood glucose dictates, fine tunes glucose control by alleviating the extremes of glycemia, both high and low.*

This high glucose variability may be the result of the enormous anatomical disadvantage of peripherally injected insulin, which cannot meet the high insulin requirements of proximal targets such as alpha cells of the islets of Langerhans and hepatocytes without far exceeding the insulin requirements of distal targets such as muscle and fat **(Figure 9A, B)**. The dynamic variability of glucagon hypersecretion and increased hepatic glucose output contributes to the high glucose variability that characterizes type 1 diabetes. High glucose variability, itself, has emerged as an HbA1c independent risk factor for the development of complications [21-23]

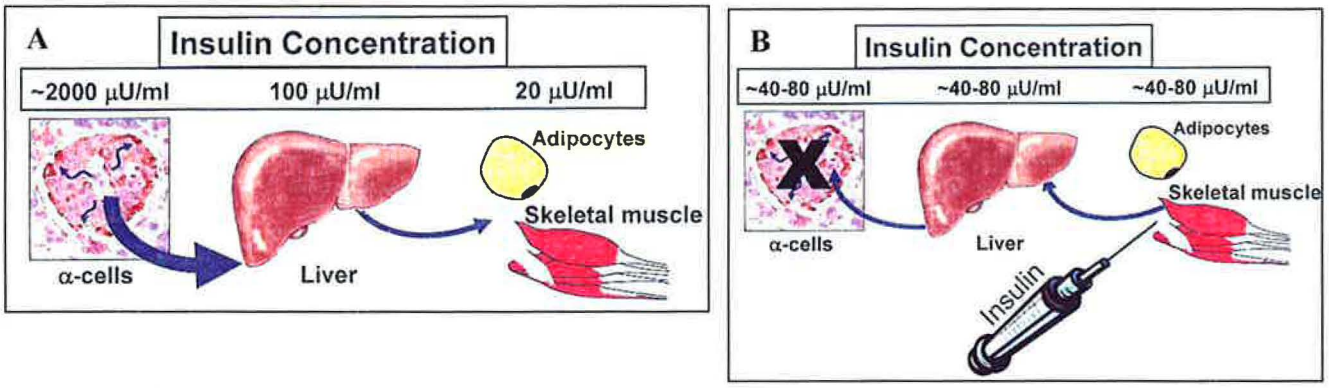


Figure 9*- Comparison of the concentration gradient of* ***(A)*** *endogenous insulin reaching the target tissues in a normal individual with levels achieved by* ***(B)*** *peripheral insulin treatment of a person with T1DM. Peripheral insulin therapy creates a single insulin concentration that cannot meet the widely varying demands of different target tissues for insulin. This disparity in demand may contribute to the lability of glycemia reported in even the best controlled T1DM patients.*

Lipolytic Surges: A second important contributing factor to glucose lability is lipolytic lability, which intermittently floods the target tissues of insulin with fatty acids that impair their sensitivity to insulin action on glucose metabolism [24-25]. This contributes to instability of glucose levels, which can fluctuate from dangerously low levels of hypoglycemia to undesirably high hyperglycemia, making frequent blood glucose determination and multiple insulin injections mandatory, thereby significantly lowering the quality of life for patients **(Figure 10)**.

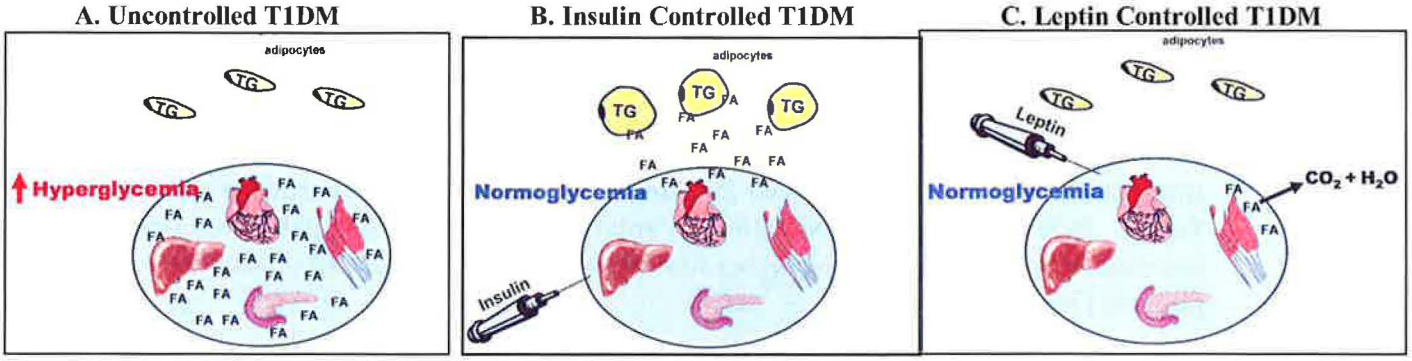


Figure 10- ***A.*** *Uncontrolled diabetes. Insulin deficiency empties the adipocytes through unopposed lipolysis. Fatty acids flood the nonadipose tissues* ***B.*** *Insulin replacement. This reverses the metabolic decompensation by reesterifying the fatty acids. The adipocyte triglyceride content is thereby restored. However, this means that the same fatty acid flood will recur whenever insulin levels drop below the antilipolytic concentration.* ***C.*** *Leptin treatment of uncontrolled diabetes, discussed below, also reverses the metabolic consequences of total insulin deficiency but it does so by increased oxidation of the ectopic fatty acids, rather than by reesterification. Thus, the fatty acids leave the body as CO_2 and H_2O, and adipocytes remain fat-depleted. This eliminates the back-and-forth fatty acid shuttling between adipocytes and nonadipocytes that may contribute to the lability of glycemia in T1DM.*

Long-term Insulin monotherapy Issues

Insulin resistance is a well characterized component of type 1 diabetes [26-28] and, as in T2DM, the degree of insulin resistance is closely associated with risk of cardiovascular disease [9]. The high prevalence of coronary artery disease among patients with T1DM [17-18] is traditionally ascribed to the disease rather than to life-long insulin monotherapy. The role of insulin in the macrovascular complications of T1DM deserves to be examined, given the relationship between the diet-driven endogenous hyperinsulinemia of obesity and the metabolic syndrome, particularly in insulin-resistant patients treated with U-500 insulin [29]. Insulin is a powerful lipogenic force [30]; a life-time of exogenous hyperinsulinemia in T1DM could also cause a form of metabolic syndrome, with obesity, insulin resistance [15, 27], hyperlipidemia, hypercholesterolemia [31] coronary artery disease and lipotoxic cardiomyopathy [32]. We now understand that intensive treatment of type 1 diabetes results in greater weight gain than conventional treatment. In the DCCT the prevalence of obesity reached 33.1% in the intensively treated subjects compared with 19.1% in the conventionally treated subjects[33].

Additionally, excessive weight gain in the DCCT was associated with lipid profile and blood pressure changes that are characterized by the metabolic syndrome [34].

Attempts at a cure

Pancreas transplantation

Pancreas transplantation can restore beta cell function lost to autoimmune destruction and has provided proof that transplantation therapies can be beneficial. Studies of pancreas transplantation uniformly show improvement in quality of life and offer compelling evidence of improved vascular outcomes [35]. The number of pancreas transplants done per year has increased about 10- fold over the last 20 years (IPTR/UNOS data) However, there are significant risks of major surgery and immunosuppression so pancreas transplantation is typically reserved for those who require kidney transplantation for renal failure. In fact, recently the indication for solitary pancreas transplantation has been questioned because of reported higher mortality rates for transplanted versus for wait-list patients [36].

Islet transplantation

Islet transplantation entails less procedural risk than whole organ pancreas transplantation and success using novel immunosuppressive regimens [38] validates strong research efforts in this field as well[37]. However, limits of islet transplantation, including scarcity of cadaveric islets, side effects of immunosuppression, and loss of function over time, support investigation of alternative therapies [39]. Unfortunately five year follow-up data of patients treated with the Edmonton protocol reveals insulin independence rates of only about 10% [40]. There are several reasons for the decline in islet transplant function including lipotoxic apoptosis of islets releasing high concentrations of lipogenic insulin into surrounding hepatocytes [41].

Stem cell based therapies

The year 2000 marks the beginning of an era of high hopes for a cellular transplantation therapy as a cure for type 1 diabetes [42]. It began when the group from Edmonton published results from an islet transplantation study testing a novel immunosuppressive regimen devoid of glucocorticoids [38]. With this steroid free regimen all 7 subjects treated achieved insulin independence one year after cadaveric islet transplantation. The magnitude of this accomplishment is realized when one learns that regimens using prednisone as an immunosuppressive achieve one year insulin independence rates of less than 10 percent.

Quickly apparent was the idea that there would be a shortage of islets for transplantation. In the year 1998 Jamie Thompson and John Gearhart both reported the ability to culture, for the first time, human embryonic stem cells [43-44], and to derive from these stem cells all of the tissue types of an adult human [45]. Motivated by the success of the Edmonton Protocol, scientists have eagerly pursued developing a paradigm for inducing differentiation of stem cells, both embryonic and adult, to a glucose-responsive insulin-producing phenotype [46-47]. T1DM is a good candidate disease to benefit from stem

cell based therapy because it is a monocellular deficiency disease, lacking only insulin producing beta cells.

The focus of my work as a fellow and then junior faculty member at Johns Hopkins was to induce differentiation of stem cells to a glucose-responsive insulin producing phenotype [48]. Although we were able to develop a paradigm for inducing differentiation of embryonic germ cell derivatives to a glucose-responsive insulin-producing phenotype, the kinetics of insulin release were far from physiologic and the percentage of cells in a mixed cell culture that produced insulin was on the order of only 1%. Stem cells offer the promise of unlimited restorative tissue. Much, however, remains to be learned about coaxing normal development of undifferentiated cells into tissues with very complex and specific function.

Regeneration

The notion of restoring endogenous beta cell function in patients with T1DM through regeneration has renewed hope for a cure for T1DM. There is accumulating evidence that humans, like mice, are capable of beta cell regeneration [49-50].
With support from Philipp Scherer I have investigated mechanisms of beta cell regeneration using the PANIC ATTAC mouse model of diabetes [51].

The goal of regeneration is to restore the body's ability to produce insulin and cure type 1 diabetes by regenerating the functional beta cells. Beta cell mass is influenced both by the formation of new beta cells and the loss of pre-existing beta cells.

Replication/Formation  Beta Cell Mass  Apoptosis

I will present unpublished data demonstrating that estrogen plays a role in protecting beta cells from apoptosis and in the restoration of beta cell function/mass even after disease onset.

My patient Chris would tell you that the idea of inducing beta cell regeneration with high dose estrogen is "titillating" but that he would prefer to continue treating his diabetes medically.

Short of a cure

It is clear that attempts to find a cure for T1DM are limited mostly by the risks associated with the cure. In fact, sometimes the cure is worse than the disease itself. Short of a cure, we must do all that we can to improve the situation for those living with T1DM.

Optimizing medical management/transition to adult care

Type 1 diabetes is usually diagnosed during childhood and after years of intensive management by the family supported by a pediatric health care team, patients are asked to shift to adult care when they turn 18; this transition to adult care is frequently accompanied by a failure to schedule or attend regular clinic appointments, problems with diabetes self-management, and a decrease in blood glucose control. I have recently

completed a pilot project assessing a coordination of care program for patients with T1DM transitioning from pediatric to adult care. The purpose of our pilot study was to examine diabetes self-management and psychosocial concomitants before, during, and after the transition to adult care. The studies include: 1) A survey of 30 youth who are more than one year from turning 18 to determine their expectations regarding the transition; 2) Assessment of the experiences of 110 youth ages 18 to 22 who provide their current diabetes management status and retrospective views on the transition period; and 3) Comparison of the experiences of 30 youth in a pediatric clinic which involves an adult care physician (myself) in the transition to adult care with that of 20 youth in a matched clinic that provides the standard transition experience. It is expected that parents and children who have not been provided with a coordinated transition will have a poorer psychosocial profile and that there will be a decline in diabetes management and glucose control relative to those youth that have a coordinated transition. This pilot study is the first step in a plan to design and evaluate programmatic changes in pediatric and adult clinics that will facilitate the transition of care.

Artificial Pancreas

Continuous blood glucose monitors are the latest in blood glucose monitoring technology. With this new technology patients have been able to improve glucose control. Most remarkable is the ability to reduce average blood glucose values while at the same time reducing the occurrence of hypoglycemia [52-53]. An artificial pancreas, consisting of a closed loop system of a continuous blood glucose monitor coupled to an insulin pump might relieve patients from much of the daily burden of disease management and achieve better glucose control than is currently achievable but challenging and unresolved problems limiting implementation remain [54].

Leptin therapy

I will conclude my talk today with a discussion of research that I have had the good fortune to be a part of with Roger Unger and his group related to the treatment of T1DM with the adipocyte derived hormone leptin.

Eighty-seven years after its discovery in 1922, insulin monotherapy remains the only therapeutic option for virtually all type 1 diabetes mellitus (T1DM) patients. Unfortunately, this therapy has at least 4 serious liabilities: **1) A heavy life-style burden**, requiring multiple blood glucose determinations and multiple insulin injections each day to achieve optimal glucoregulation: **2) Anatomical issues create intrinsic therapeutic limitations on peripherally injected insulin monotherapy**, which travels opposite to the normal direction of insulin flow from islets to periphery and thus deprives the upstream target tissues, such as pancreatic alpha cells and liver, of the much higher physiologic insulin concentrations they require; **3) Lipolytic surges** contribute to glycemic hyperlability by fluctuations of ectopic fatty acids that alter sensitivity of insulin target tissues, such that even meticulously controlled patients with optimal hemoglobin A1c levels experience episodes of hyperglycemia and hypoglycemia [20]; **4) High prevalence of coronary artery disease** despite optimal control with insulin [17], perhaps even enhancing atherogenesis via chronic exogenous hyperinsulinemia.

Dr Unger's group has previously demonstrated remarkable efficacy of adenovirally induced hyperleptinemia in restoring insulin-deficient diabetic rodents to full clinical and metabolic health without any insulin [55]. This raises the possibility that leptin therapy might eliminate some of the liabilities of insulin monotherapy. For example, the fact that leptin can suppress glucagon secretion[56] and can block glucagon's hepatic action on glucose and ketone production would eliminate the need for the hyperinsulinemic doses now required to suppress the alpha cells in T1DM; further, by reducing insulinemia to levels required by peripheral targets, the lability of glucose and free fatty acids (FFA) and, in the long-term, the high incidence of atherogenic complications might be lowered. The putative advantages of leptin therapy are shown in **Table 1**.

Table 1-Theoretical advantages of adding leptin to insulin therapy for T1DM

1. ***Normalization of glucagon hypersecretion without hyperinsulinemia***
2. ***Normalization of hepatic glucose overproduction without hyperinsulinemia***
3. ***Lowering of free fatty acid excess by oxidation, rather than re-esterification***
4. ***Normalization of insulin resistance***
5. ***Reduce glucose variability (less extreme hyperglycemia and hypoglycemia)***
6. ***Reduce HbA1c***
7. ***Reduce daily burden of T1DM disease management***
8. ***Facilitation of compliance with dietary restriction and prevention of weight gain***
9. ***Reduce lipogenesis and may lower incidence of coronary artery disease***

An Alternative Therapy

The foregoing concerns about insulin monotherapy warrant consideration of an alternative therapeutic strategy. It is now clear that leptin can do many of the anabolic chores currently assigned to insulin [55]. Like insulin, it suppresses diabetic hyperglucagonemia [56], thereby reversing the increased gluconeogenesis and glucose overproduction. Like insulin, it dramatically stops ketogenesis and protein loss. But most remarkably, leptin reverses as effectively as insulin the striking overaccumulation of fatty acyl carnitines and organic acids that characterize the metabolomic pattern of the insulin-deficient liver (see below). But whereas insulin normalizes by esterifying fatty acids, storing them in adipocytes from which they can again be released whenever insulin levels decline; leptin normalizes by oxidizing them to CO_2 and water that is eliminated from the body. The adipocyte triacylglycerol (TG) content is thereby reduced [57] and plasma and tissue FFAs are lowered. The lipolytic response to a decline in plasma insulin is attenuated and glycemic variability thereby reduced. By using leptin instead of insulin to target alpha cells and liver, insulin's only responsibility is to regulate peripheral glucose uptake in muscle and fat, which is done at relatively low levels. The hyperinsulinemia otherwise required will become unnecessary.

It is hoped that combination therapy with leptin and insulin will stabilize glycemia, reducing the need for multiple insulin injections and glucose determinations, and improve

markers of protein glycation and atherogenesis. Not the least of the predicted benefits of leptin/insulin therapy will be far better dietary compliance than with insulin alone.

PRELIMINARY DATA

Comparison of Leptin, Insulin and Leptin-Insulin Therapy

The initial results of recombinant leptin (Amylin) alone and the combination of recombinant leptin with low dose insulin delivered to insulin insufficient NOD mice are summarized in **Figures 11 and 12,** respectively. Leptin delivery by Alzet pump (10 mcg/hr) maintained plasma leptin levels above 25 ng/ml for most of the first week (**Figure 11**). Glucose levels in some of the mice fell to normal in one day, while in others glucose exceeded 100 mg/dl until the ninth day. The groups have therefore been separated into fast and slow responders. The insulin levels were higher and the FFA and glucagon levels lower in the fast responders, although hyperglucagonemia was well-suppressed in both groups at the end of the 14-day infusion of leptin. These mice have not been treated with any insulin. Although only 11 T1DM mice have thus far been treated, everyone has responded to recombinant leptin monotherapy. Preliminary findings include a profound lowering of plasma TG to 16±6 mg/dl in leptin-treated mice, compared to 42±14 mg/dl in insulin-treated mice and 99±16 mg/dl in untreated diabetic NOD mice. Normal TG levels in nondiabetic mouse liver averaged 34±7 mg/dl. This is in keeping with differences in expression of PPARα, which was much higher in leptinized compared to insulinized mice, and FOX01, which was lower.

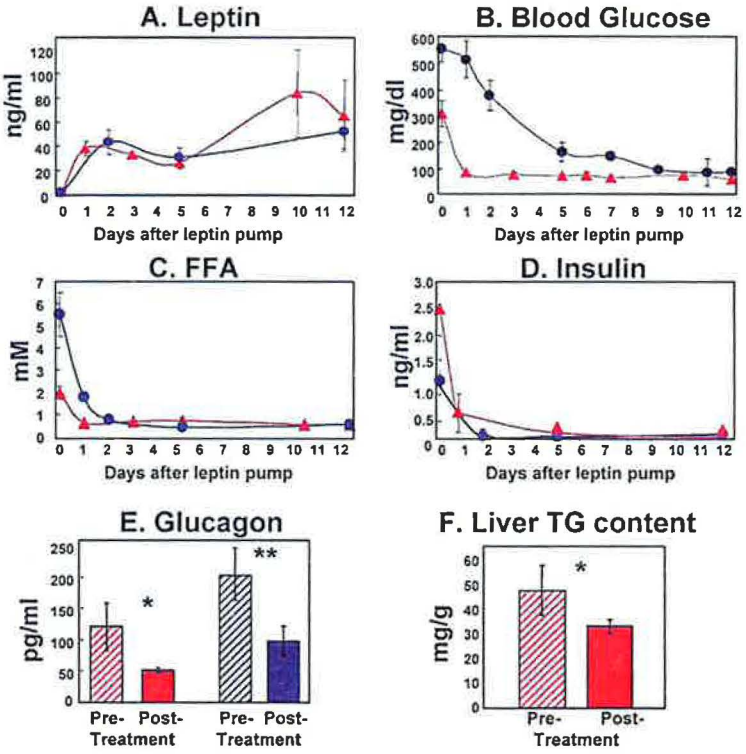


Figure 11*- The effects of recombinant leptin administered by Alzet pump to diabetic NOD mice on A. Plasma leptin, B. Glucose, C. Free fatty acids (FFA), D. Plasma insulin, E. Plasma glucagon, and F, Liver triacylglycerol (TG) content. The mice have been separated into fast responders to leptin therapy (red) and slow responders (blue).*

The results of the first 3 mice treated with combination leptin and insulin are summarized in **Figure 12**. In these studies a low dose of insulin (0.01 units of long acting insulin

levemir [Novo Nordisk] given subcutaneously twice daily) had minimal impact on blood glucose which remains persistently elevated to the 500 to 600 mg/dl range. This same low dose of insulin combined with leptin 2.4 mg twice daily not only normalized blood glucose but also reduced glucose variability (standard error of mean blood glucose) which was assessed 3 times daily in each mouse. An attempt was made to mimic this degree of glycemic control using a ten fold higher dose of insulin monotherapy (0.1 unit of levemir given twice daily). However this dose led to an average glucose significantly above the average glucose achieved with combination leptin and low dose insulin and the mice treated with this higher dose insulin monotherapy experienced extremes of both hyperglycemia and hypoglycemia. We have previously excluded the anorexic effects of leptin as the method of improvement in blood glucose by pair feeding experiments [55].

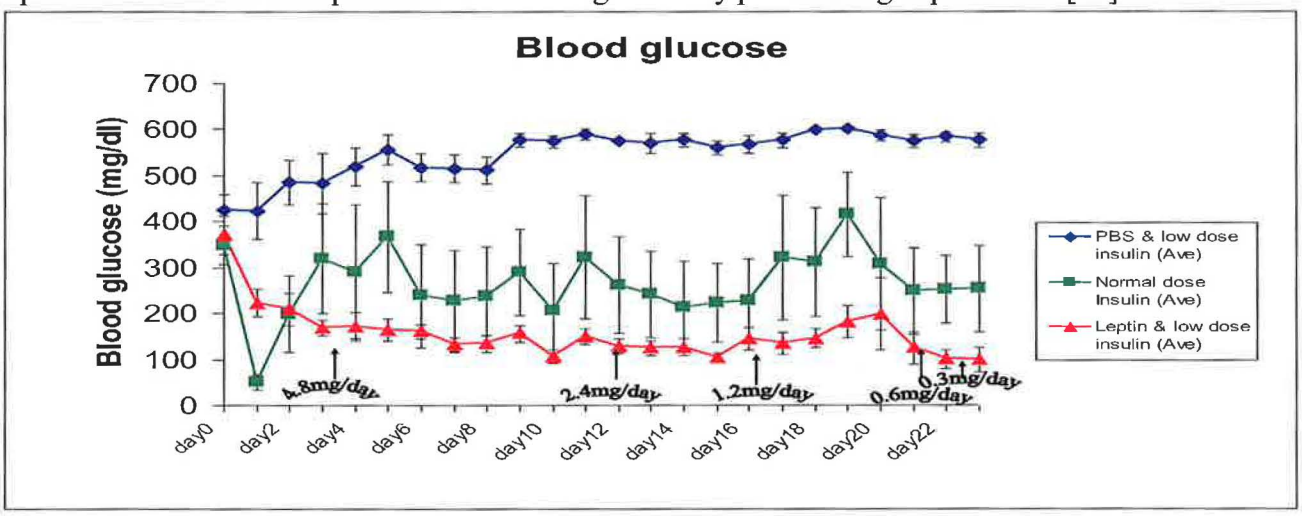


***Figure 12**. Comparison of glycemic patterns in NOD mice treated with twice daily injections of 0.1 U of long-acting insulin levemir (green, "high dose insulin"), or of 0.01 U of long-acting insulin levemir plus 2.4 mg of leptin (red, "combo therapy"), or of 0.01 U of long-acting insulin in phosphate buffered saline (black, "low dose insulin") (mean ± SEM). Note the small SEM in the leptin/insulin group compared to high dose insulin monotherapy. It is hoped that avoidance of hyperinsulinemia as a consequence of the 90% reduction in insulin dose can be translated to human T1DM patients by adding leptin therapy to provide greater glycemic stability and to reduce atherogenic complications.*

Preliminary Metabolomic Analysis of Leptin Therapy in Mice:

The first comparison of leptin monotherapy vs. insulin monotherapy has already been completed in groups of 4 mice **(Figure 13)**. The leptin-treated and insulin-treated groups of NOD mice exhibit remarkably parallel effects on a wide array of liver metabolites. Preliminary metabolomic profiling indicates that insulin-deficient NOD mice have profound abnormalities with clear increases in short chain acylcarnitines, including C2, C3, C5, and C4-OH (beta-hydroxybutyryl) species, in long-chain acylcarnitine species and in multiple TCA cycle intermediates **(Figure 13)**. All of these changes are consistent with a strong increase in catabolism of glucose, fatty acids and amino acids. Remarkably, insulin and leptin were almost equally effective at normalizing the levels of all of these diverse metabolites. The difference is that insulin apparently accomplished this by driving anabolic storage of fuels, including lipids, whereas leptin may mainly be acting

by causing oxidation of fatty acids in adipose tissue, thus lowering the supply of fatty acids at the liver. This, coupled with its effects to suppress glucagon secretion, allowed leptin to behave like an anabolic hormone, but without the undesired insulin-mediated lipid re-esterification and storage.

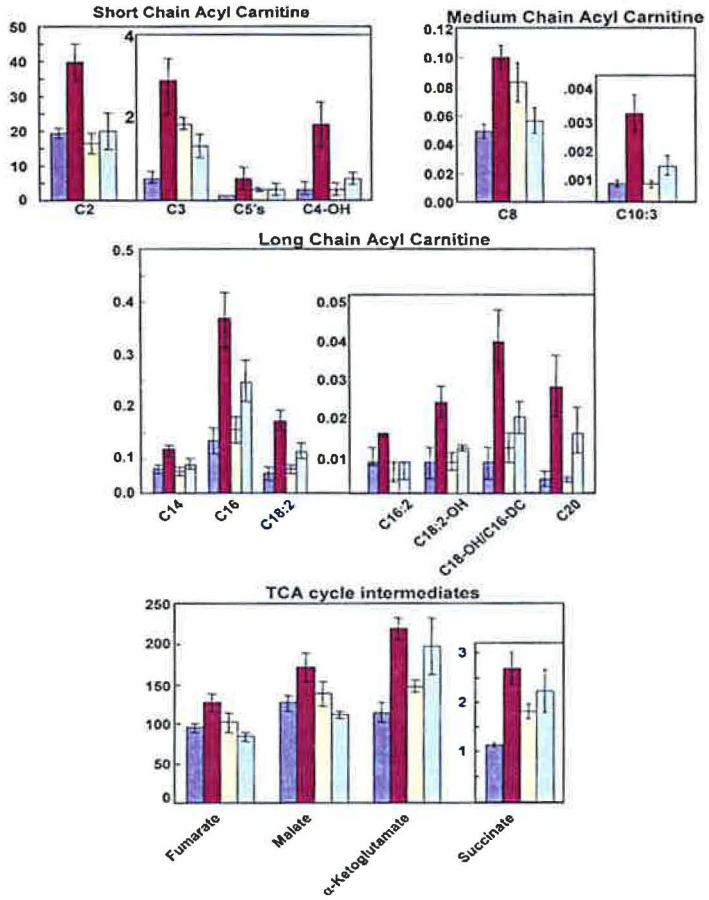


Figure 13- *Metabolomic patterns in the livers of nondiabetic (blue), untreatreated diabetic (magenta), insulin-pellet (LinShin, Canada) treated diabetic (yellow), and leptin pump-treated diabetic (aqua) NOD mice. Only acyl carnitine and TCA cycle intermediates are shown.*

After 87 years of insulin monotherapy the introduction of a novel agent to treat T1DM would seem unnecessarily risky, were it not for the 5-year experience with leptin therapy of congenital generalized lipodystrophy (CGL), a disorder with certain similarities to T1DM. Lipodystrophies are clinically heterogeneous acquired or inherited disorders characterized by selective loss of adipose tissue [58] . Affected patients have extreme insulin resistance, whereas it is mild in T1DM. Markedly reduced levels of serum leptin (severe hypoleptinemia) are frequently observed in patients with partial or generalized lipodystrophies and may play an important role in the pathogenesis of the metabolic complications. Hypoleptinemia is present in some T1DM, particularly when poorly controlled. We are fortunate to have the expertise of Abhimanyu Garg as we transition into a pilot clinical trial to test the effectiveness of leptin combined with insulin in T1DM.

Limitations of insulin monotherapy in type 1 diabetes

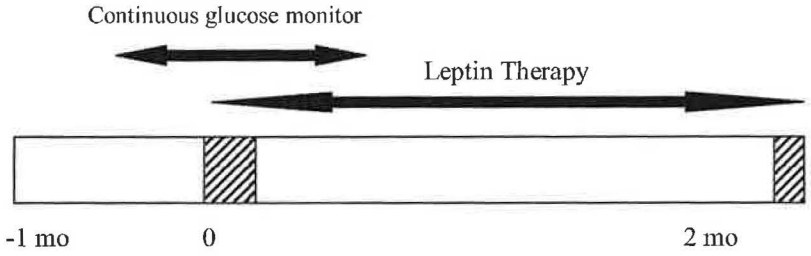
Those living with T1DM today are, on average, not well controlled. Even after the impressive results of the Diabetes Control and Complications Trial, most large clinical trials involving patients with T1DM have A1c values in the range of 8 to 10 percent [59]. This puts patients at significant risk for the development of complications. In addition,

the high glucose variability that characterizes even "well controlled" patients, has emerged as an A1c-independent risk factor for diabetic complications [21].

We believe that leptin will help to stabilize glycemia in T1DM by eliminating the need for high dose insulin to suppress glucagon and inhibit hepatic glucose production. As in the low dose insulin plus high dose leptin therapy in NOD mice (**Figure 12**) we hope to help patients to achieve target glucose control without greatly increasing the risk of hyperinsulinemia and hypoglycemia. Patients will be treated with twice daily subcutaneous leptin 0.08 mg/kg or 0.16 mg/kg b.i.d. (cf. below) with insulin doses adjusted based on fingerstick blood glucose monitoring and continuous glucose monitoring in an effort to maintain stable euglycemia.

The specific hypothesis to be tested and the aim of the study is to determine if metreleptin and insulin combined therapy will significantly ameliorate the metabolic profile and quality of life for patients with T1DM compared to insulin monotherapy alone.

Planned pilot human clinical trial



Subjects: Ten patients with T1DM.

Inclusion criteria:
1. T1DM for at least 1 year. Diagnosis of T1DM will be based on clinical criteria including: Age of onset of diabetes (16 years or younger) with insulin-dependence within 6 months of the onset, history of prior episode of ketoacidosis, or previous documentation of positive serum islet cell autoantibodies.
2. Age 21-50 years
3. Gender, male and female
4. HbA1c 8 to 10%
5. Plasma leptin levels less than the 20th percentile of normal levels in the US population (2.5 ng/ml in males and 7 ng/ml in females).

Exclusion criteria:
1. Obesity or overweight, BMI >25 kg/m^2
2. Hypoglycemia unawareness
3. HbA1c >10%
4. Current substance abuse.
5. Current infection
6. Subjects who have a known hypersensitivity to *E. Coli* derived proteins.
7. Pregnant or lactating women.

8. History of weight loss (>10%) in the last 3 months.

Study Design: The study will be conducted as an open-label observational study to assess the efficacy of leptin in T1DM. Following a screening evaluation, eligible patients will be followed for a 4-week pre-baseline period without changing their insulin regime in order to establish a baseline state. Patients will be given a standard glucose meter and daily fasting, preprandial and 2 hour postprandial self-monitored blood glucose (SMBG) values will be obtained throughout the study. Glucose meter data will be downloaded at specified intervals to calculate mean and standard deviation as a measure of glucose exposure and variability, respectively. Patients will also be outfitted with continuous glucose monitors for two weeks before and two weeks after initiation of leptin therapy so that average glycemia and glucose variability can be compared to SMBG data and to maximize patient safety at the initiation of leptin therapy.

Eligible patients will be treated with their usual regime of diet and insulin for 1 month. After a complete stable baseline has been obtained, metreleptin (Amylin Inc.) will be administered at a dose of 0.16 mg/kg body weight/day (in two divided doses) in the female subjects and at a dose of 0.08 mg/kg body weight/day (in two divided doses) in the male subjects. [In Dr. Garg's previous studies of CGL patients, this dose resulted in twice the normal physiological plasma levels of leptin in both females and in males (data not shown)]. If glucose levels decline (<80 mg/dL), insulin will be adjusted downward in decrements of 5-20% based on blood glucose levels, as described below. The goal will be to maintain good glycemic control with minimal dose of insulin. Patients will receive metreleptin by subcutaneous injections. Blood glucose response to standardized meals and insulin doses both before and after the initiation of metreleptin will guide the adjustment of insulin dosing, see details below. Continuous glucose monitoring will be required until stabilization of glucose, insulin and leptin doses have been reached. Body composition by DEXA and intramyocellular and intrahepatic lipid concentration by MRS will be assessed before and after 2 months of metreleptin therapy [60].

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