Cellular Activation Therapy
Published Reports

The following publications are those which have been peer reviewed and published in some of the more prestigious medical journals in America and elsewhere in the World. These publications show many of the results of Cellular Activation Therapy (CAT), but do not represent all of the treatment benefits as seen in patients who are not part of formal studies. After approximately 100,000 treatments there has never been a reported significant adverse event, making CAT one of the most safe and uniformly effective treatments in medicine known today.
I. A Research Compendium:

Note: Pulsatile Insulin Therapy (PIT), i.e. the use of a series of pulsed boluses of insulin in the treatment of diabetes, has been referred to in the medical literature by a number of names including:

- Metabolic Activation Therapy (MAT).
- Chronic Intermittent Intravenous Insulin Therapy (CIIT).
- Pulsatile Intravenous Insulin Therapy (PIVIT).
- For consistency in this document, Pulsatile Insulin Therapy (PIT) is used throughout.

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1. **Introduction**: Pulsatile Insulin Therapy (PIT) is a unique therapy for individuals with advanced diabetes. PIT has its roots in the work of the former Head of Metabolism Research at Joslin Diabetes Center in Boston, Massachusetts, and a current Professor of Medicine at the University of California, Davis.

The work focused on the critical role of liver dysfunction in diabetic metabolism. He theorized that end organ damage in diabetes is caused by abnormal hepatic glucose metabolism, inadequate insulin delivery, and insulin resistance. At present, a number of investigators, universities, and foundations, are involved in multiple studies to further delineate the clinical benefits of PIT and to further the understanding of its mechanisms of action.

2. **History and Biochemistry of Pulsatile Insulin Therapy**: Normally, insulin is secreted in a pulsatile fashion and in variable amounts, closely related to the intake of meals. Evidence suggests a more potent hypoglycemic effect of pulsatile insulin in comparison to continuous insulin infusion. Continuous exposure to insulin and glucagon is known to decrease the hormones’ metabolic effectiveness on splanchnic glucose production in humans. Down-regulation at the cellular level may partially explain the decreased action of steady-state levels, while pulsatile hormone secretion may allow recovery of receptor affinity or receptor numbers. Intermittent intravenous insulin administration with peaks of insulin concentrations may enhance suppression of gluconeogenesis and reduce hepatic glucose production (HGP).

For induction and maintenance of insulin-dependent enzymes essential for glucose metabolism (e.g., hepatic glucokinase, phosphofructokinase, and pyruvate kinase), the hepatocytes require a defined insulin level (200-500 µU/ml in the portal vein) concomitant with high glucose levels (bimolecular signal). In non-diabetic subjects, portal insulin concentrations are twofold to threefold greater than those in the peripheral circulation. During the first pass through the liver, 50% of the insulin is removed, strongly insinuating that the liver is the principal metabolic target organ of the gastrointestinal tract and the pancreas. The insulin retained by the hepatocytes may itself be essential for the long-term effects of insulin on hepatic glucose metabolism as well as growth and de novo enzyme synthesis. Following oral glucose intake, the liver accounts for an equal or greater portion of total net glucose uptake compared to the periphery. Insulin exerts pivotal control of glucose levels through its ability to regulate HGP directly or indirectly. The traditional subcutaneous (S.C.) insulin administration regimens used by diabetic patients a) fails to capture the pulsatile nature of natural insulin secretion and b) does not reach high enough insulin concentrations at the hepatocyte level (e.g., 10 U regular insulin injected S.C. produce a peak systemic circulation concentration of 30-40 µU/ml and an even lower portal vein concentration of 15-20 µU/ml). A relative deficiency of insulin at the hepatocyte level leads to an impaired capacity to process incoming dietary glucose. With the liver functioning as the target organ of the pancreas, it can be concluded that the primary purpose of giving insulin to the diabetic patient should not be to control blood glucose level (“control theory”) but rather...
the normalization of hepatic metabolism. Furthermore, these same hepatic enzymes are found in all glucose-utilizing bodily systems, suggesting a synchronous effect by insulin and glucose.

It has been shown that the diabetic patient’s capacity to oxidize and store exogenous carbohydrates is markedly impaired. In the resting, post-absorptive, non-diabetic subject, the energy requirement is met primarily through fat oxidation reflected by indirect calorimetry in the form of a respiratory quotient (RQ), (volume CO$_2$/volume O$_2$, of 0.7-0.8). After glucose administration, CO$_2$ production and consequently the RQ increase (to a range of 0.9-1.0), indicating that glucose has become the primary source of energy. Conversely, in the patient with diabetes mellitus on conventional insulin therapy, no such increase in RQ or CO$_2$ production is observed after glucose administration.

The possible fates of ingested glucose are a) oxidation (liver, brain, muscle), b) conversion to fat (liver, muscle, adipose tissue), c) storage as glycogen (liver, muscle), or d) transamination of intermediary metabolites to form amino acids (e.g. alanine). Only the first two processes generate the CO$_2$ requisite for an increase in the RQ. Liver and muscle appear to be the most active tissues for glucose oxidation. In 1985, Meistas, et al, showed in non-diabetic post-absorptive men that resting muscle is not the source of the increased CO$_2$ production after ingestion of a 100-gram glucose meal. An increase in the RQ to greater than 0.9 is used as the index of therapeutic efficacy of PIT. It was postulated that if hepatic treatment was achieved and maintained in patients with diabetes through this treatment, the glycohemoglobin A1c (HbA1c) blood levels and the frequency of hypoglycemic reactions should decrease.

3. Pulsatile Insulin Therapy Protocol: Pulsatile Insulin Therapy (PIT) is a process which promotes the normalization of carbohydrate metabolism in diabetic patients. PIT affects multiple organs, especially muscle, retina, liver, kidney, and nerve endings. The process involves the administration of high-dose insulin pulses similar to those found in the portal circulation of normal humans. The process is monitored by frequent glucose levels and respiratory quotients (RQ). RQ is measured by a metabolic cart which determines the ratio VCO$_2$/ VO$_2$. This ratio is specific for the fuel used at any one time by the body. The glucose levels are monitored to keep glucose levels appropriate, and the RQ determines the need to readjust the infusion. PIT is done over 1-hour periods with a 1-hour rest period between each session for three courses each day of treatment. Typically, PIT is performed on a weekly basis following the first week of two back-to-back daily sessions. The following is a typical treatment session:

The patients report to the PIT center between 7:30am and 8:00am.

- Patient clinical assessment is performed prior to treatment:
  - Vital signs.
  - Initial glucose level.
  - Review of medications.
  - Overview of patient’s overall condition.
  - Following the initial assessment, an intravenous line is established and PIT commences:

PIT treatment session:

- U/kg of Insulin, pulsed at 10 pulses/hour over 1 hour is administered by a specialty pump programmed for concentration, frequency and duration of pulses, and rest interval.
The respiratory quotient (RQ) or metabolic measurement is performed at the beginning and at the end of the hour to measure success of treatment.

Glucose levels are taken every 30 minutes or more frequently as medically indicated in patients with a tendency for hypoglycemia.

Oral carbohydrates are given to keep blood glucose over 100 mg/dl and to increase RQ.

There is a rest period of one hour between treatments in order to stabilize blood glucose levels.

This 2-hour cycle is repeated twice more in a single treatment day.

Frequent monitoring of respiratory quotient is essential in order to verify patient response to PIT treatment. When RQ is low (0.7-0.8), fat is the primary fuel and at RQ’s of 0.9-1.0, glucose is the primary fuel. Protein and mixed fuel utilization have intermediate RQ’s of 0.8-0.9.

PIT increases the respiratory quotient from levels around 0.7 to levels greater than 0.9. This reflects the underlying physiologic changes of the treatment, confirming the conversion from fat metabolism, typical in the diabetic patient, to a normal metabolic state utilizing carbohydrate as the primary fuel consumed. Both the total amount of insulin contained within each pulse as well as the total amount of consumed glucose is altered in order to maximize treatment results.

4. Summary of Published Studies on Pulsatile Insulin Therapy:


This study examined 20 “brittle” diabetics (defined as patients with wide glucose swings and frequent hypoglycemic episodes) treated with the PIT protocol. This was a prospective study with patients serving as their own historic controls. All patients had been on intensive insulin therapy (four shots daily) for at least one year prior to entrance into the study. The results of this study were as follows:

A significant decline in HbA1c from the baseline of 8.5% to 7.0% at the end of the observation period (p < 0.0003): decline in the frequency of major hypoglycemic events from 3.0 to 0.1 per month (p < 0.001).

A decline in the frequency of minor hypoglycemic events from 13.0 to 2.4 per month (p < 0.001).


This is a prospective, randomized, crossover study, examining antihypertensive medication requirements in 26 hypertensive insulin dependent diabetic patients. These patients were randomized into treatment and control groups. All patients were stabilized prior to the study on four shots of insulin daily and antihypertensive medications (ACE inhibitors, calcium channel blockers, loop diuretics, and alpha two agonists). Following three months in either the treatment or control group, the patients were crossed over into the other group. Total antihypertensive medication requirements were then tabulated.
Antihypertensive dosage requirements decreased significantly (46%, p<0.0001) and linearly over time during the treatment phase, while remaining stable in the control group. Following the crossover, the previously treated patients (now controls) returned to their baseline antihypertensive needs within the subsequent three months.


This is a multicenter, retrospective, longitudinal study, involving 31 patients with type 1 diabetes mellitus and overt diabetic nephropathy. All patients were on intensive (four daily shots) insulin therapy and weekly PIT. All patients were on ACE therapy and aggressive antihypertensive regimens. All patients were followed with creatinine clearance measurements.

Patients were followed for an average of 37 months. Creatinine clearance remained essentially unchanged during this period. These observations suggested that PIT could successfully stabilize renal function in patients with diabetic nephropathy.

d. Effects of pulsatile intravenous insulin therapy (PIVIT) on the progression of diabetic nephropathy: Dailey, et al. *Metabolism (2000) 49: 1491-1495.* The Dailey study is a multi-institutional prospective, randomized, controlled study evaluating the effect of PIT in patients with diabetic nephropathy. This study included diabetic centers at Mayo Clinic, Scripps Clinic, Joslin Diabetes Center, University of Maryland, University of Arizona, and Temple. 49 patients with type 1 diabetes and chronic kidney disease were randomized into treatment and control groups in an 18 month study. Treatment group (PIT) patients had a statistically significant improvement in renal function as compared to the control group.

e. PIT Effect on Diabetic Autonomic Neuropathy: Aoki et al. Effect of intensive insulin therapy on abnormal circadian blood pressure pattern in patients with type 1 diabetes mellitus. *Online J Curr Clin Trials (1995) 199.* This is a randomized, controlled clinical study evaluating the abnormal circadian blood pressure pattern in insulin dependent diabetic (IDDM) patients and its response to PIT. 74 IDDM patients were randomized to a treatment (PIT) group or a control group. 24 hour blood pressure monitoring was performed monthly along with HbA1c levels. All study patients were evaluated weekly by investigators and all were on four shots of insulin daily prior to and during the study.

Following three months of PIT, the night/day systolic BP ratios decreased from 0.97 to 0.94 and increased from 0.95 to 0.98 in the control group (p = 0.0224). The night/day diastolic BP ratio decreased from 0.93 to 0.90 in the treatment group and increased from 0.91 to 0.94 in the control group (p=0.0037. This improvement of an autonomic neural pathway is consistent with a small series reported separately of IDDM patients with severe uncontrollable postural hypotension improving after two months of PIT (Aoki et al., Am J Med (1995) 99: 683-684.

f. Background of Expanded Clinical Studies: Following the publication in 2000 of the independent multi-institutional study (Mayo Clinic, Scripps Clinic, Harvard’s Joslin Diabetes Center, University of Maryland, University of Arizona, and Temple) demonstrating PIT’s effectiveness in stabilizing renal disease (see above), many related questions naturally followed:

- Could these findings with PIT be expanded to other diabetic complications?
- Is the effect of PIT on patients with type 2 diabetes analogous to its demonstrated effect on patients with type 1 diabetes?
• Could this treatment be reproduced in a consistent fashion in multiple clinical settings in order
to develop a powerful tool for the treatment of patients with severe diabetic complications?

At present, Florida Atlantic University, the Bascom Palmer Eye Institute at the University of Miami
(retinopathy study), East Virginia Medical School (neuropathy study), and Johns Hopkins’ Wilmer
Ophthalmological Institute (retinopathy study) are participating in the study and evaluation of PIT.

5. Study Designs: All human trials involving PIT are approved by a federally sanctioned institutional
review board. All studies are performed in a prospective, controlled fashion. Endpoint parameters
are scored in a coded blind fashion. Patients in the treatment group undergo PIT treatments
according to the protocol as outlined above. Two treatment sessions (consisting of three treatments
per session) are performed initially, followed by weekly treatments for the duration of the study. The
control group undergoes weekly evaluations by the clinic nurse, including blood pressure checks, and
interim history checks. Any adjustments to their daily medical regimens as dictated by glucose levels
or blood pressure are done during these weekly visits. Testing specific to any particular study is
performed prior to the initiation of PIT therapy and at intervals as specified in the study protocol. All
patients must meet entrance criteria for PIT prior to qualifying for any clinical study.

6. Current Studies: Current Clinical Studies of the Center for Complex Systems and Brain
Sciences, Florida Atlantic University.

   a. Entry Criteria for PIT: Based on the above clinical studies and independent clinical
observations, the following criteria have been developed for patients to qualify for Pulsatile Insulin
Therapy. All patients must be under the care of an endocrinologist, or other physician competent in
the care and management of diabetes, be compliant with their medical regimens, and be willing to
undergo weekly PIT program. One or more of the following criteria must be met:

   • Patient has HgbA1c >8.0 in spite of 2 or more injections of short acting and/or intermediate
     acting insulin daily.

   • Patient has hypoglycemia unawareness. This is a major and unique indication for PIT.

   • Patient has labile diabetes (sugars ranging from <60 to >300 more than 2 days a week) in
     spite of multiple insulin injections (>2) or the use of an insulin pump.

   • Patient has significant proteinuria (>300 mg/24 hrs) in spite of ACE inhibitors and/or ARB’s.

   • Patient’s creatinine clearance is less than 60ml/min and declining at greater than 1 ml/month
     on ACE/ARB’s and insulin therapy.

   • Patient’s blood pressure is uncontrolled (>130/80) on more than 3 BP drugs with a HgbA1c
     greater than 8.0% on more than 2 insulin shots daily.

   • Patient has progressive, severe diabetic peripheral neuropathy.

   • Patient has orthostatic hypotension due to autonomic neuropathy of advanced diabetes.

   • Patient has advanced gut neuropathy with gastroparesis or diabetic diarrhea.

   • Patient has a non-healing diabetic foot ulcer (no or minimal evidence of healing over 2 months)
in the absence of surgically remediable LE ischemia, gangrene and/or osteomyelitis.

- Patient has progressive retinopathy.

**Compliance Review:**

- Patient is willing and able to devote 6 hours a week over a minimum of 4 months to PIT.
- Patient is currently compliant with his/her diabetes treatment regimen.
- IF PATIENT HAS ANY OF ABOVE INDICATIONS AND COMPLIANCE REVIEW IS FAVORABLE, PATIENT IS A LIKELY CANDIDATE FOR INCLUSION IN A PIT STUDY.

b. Clinical Effects of Pulsatile Insulin Therapy on Cognitive Function in Patients with Diabetes Mellitus.

**Introduction:** Cognitive impairment is common in patients with diabetes mellitus, especially for memory and executive function (see [1] for review). Moreover, patients with type 2 diabetes, especially women over age 65, not only show lower levels of cognitive function but also increased rates of cognitive decline relative to age- and sex-matched cohorts [2]. The growing awareness of cognitive impairments in diabetes is especially intriguing given the well documented presence of insulin receptors in brain tissue that are selectively expressed in areas of the brain associated with memory functions and deteriorate in Alzheimer's disease [3]. Furthermore, Watson and Craft [4] demonstrated temporary improvement of memory functions following the administration of intravenous insulin in a subtype of Alzheimer’s patients with insulin resistance in these receptors.

**Protocol:** This is a prospective, controlled, single-blind study examining cognitive function in patients undergoing PIT. Patients receive cognitive testing before beginning PIT and at three month intervals thereafter. Cognitive testing includes several modified Wechsler III memory tasks (immediate and delayed recall of stories and word lists). In addition, serial laboratory studies include CBC, chemistry profile, TSH, B12, folate, HbA1c, and urine protein. These results are compared with an age, sex, and disease matched control group who also undergo the cognitive testing every three months, and the laboratory studies. Entry criteria include diabetic patients, aged 21 – 80, without other primary causes of cognitive impairment (brain tumor, previous neurosurgery, memory impairing medications). These patients must meet one or more criteria for PIT (see Criteria for PIT above). Patients undergo PIT as noted in previous protocols weekly over a period of 6-12 months with renewals for successive 6-month periods. Control group patients undergo weekly visits with the clinic nurse with vital sign and glucose checks as described above. All testing is recorded for later off-line analysis by an independent researcher blind to the patient’s grouping.

**Endpoints:** Endpoints include measurements of changes in short and long term memory abilities as indicated by the cognitive tests.

**Statistics:** Statistical evaluation uses repeated measures ANOVA with one grouping factor (PIT vs. control group) and multiple regressions (the latter to evaluate effects of age, sex, and duration of diabetes).

**References:**


c. Pulsatile Insulin Therapy in the Treatment of Diabetic Patients with Nephropathy.

**Introduction:** Diabetic nephropathy is a progressive complication leading to end stage kidney disease with anemia, hypertension and eventually dialysis. A multi-center trial of Pulsatile Insulin Therapy (PIT) in type 1 diabetics showed a slowing of the progression over a control group using maximal conventional therapy (1). Diabetic nephropathy develops in 20-50% of type 2 diabetics and is one of the most common causes of end stage renal disease (2). Proteinuria occurs early in the disease and continues despite maximal treatment although the rate may slow. This study is to determine the effect of PIT on both types of diabetes in patients with nephropathy.

**Protocol:** Entry criteria are patients (age 21-80) who have diabetes with serum creatinine over 1.2 mg/dl, minimal to severe proteinuria, and no evidence of other kidney disease (renal obstruction, renal tumors). This is a prospective, randomized, single blinded control study. Treatment groups will undergo PIT weekly for 12 months with self-renewing 12 month periods. Laboratory studies to be done before PIT will include CBC, chemistry profile, HbA1c, TSH, lipid profile, C-peptide, and urine protein studies. Research labs to be assessed every 6 months are fructosamine, aldosterone, brain natriuretic protein, TGF-beta, endothelin 1, fibrinogen, creatinine clearance and urine protein excretion. The study will be evaluated every 6 months statistically and compared with the control group by the study parameters noted.

**Endpoints:** serum creatinine, creatinine clearance, degree of proteinuria, blood pressure.

**Statistics:** ANOVA.

**Blood:** 1 purple, 2 red tops: Urine: 24 hour overnight collection for protein and Creatinine.

**References:**


d. Pulsatile Insulin Therapy in the Treatment of Diabetic Patients with Heart Disease.

**Introduction:** Altered glucose metabolism in the heart makes fatty acids a primary energy source (1). Fatty acids require increased oxygen utilization for energy production while producing decreased contractility (2). Improved glucose metabolism has been shown to improve intimal thickness (3).
Glucose metabolism is preferred by the heart muscle but impaired in diabetic patients by insulin resistance and decreased post-prandial glucose uptake. This study is designed to test the effect of PIT on cardiac function in diabetic patients with significant cardiac disease (4).

**Protocol:** Individuals selected (ages 21 and older) will have diabetes with impaired cardiac function. For inclusion into the study, the patient must exhibit one of the following:

- Impaired cardiac function as noted by NYHA (class 2 or class 3).
- Abnormal ejection fraction as determined by echocardiography (30-50% of predicted normal).
- Documented coronary artery disease.
- Diffuse cardiomyopathy.
- Exclusion criteria include significant renal disease, right sided heart failure, valvular heart disease, or significant pulmonary disease.

Patients selected will undergo PIT for 12 months with carotid ultrasound to determine intimal thickness, echocardiography, and questionnaires before treatment and every 3 months. The study has renewals of successive 6-month periods. Laboratory studies include CBC, chemistry profile, TSH, HbA1c, aldosterone, fructosamine, endothelin 1, homocysteine, free fatty acids, and lipids.

**Endpoints:** intimal thickness, ejection fraction, wall motion, and cardiac questionnaire. Laboratory levels: aldosterone, endothelin 1, homocysteine, free fatty acids.

**Statistics:** ANOVA.

**Blood:** two redtops.

**References:**


e. Effects of Pulsatile Insulin Therapy (PIT) on Diabetic Neuropathy (Cooperative study of Florida Atlantic University and Strelitz Diabetes Institute of Eastern Virginia Medical School).

**Introduction:** Diabetic neuropathy (DN) is a progressive complication causing serious problems in 25%-40% of diabetics. Significant complications produce painful peripheral dysthesias, loss of sensation, and gastroparesis. DN may affect the peripheral motor and sensory nerves in addition to the autonomic nervous system (1-3). Treatment strategies for patients with DN have generally
concentrated on pain relief, without addressing the underlying pathophysiology of the disease (4). Anecdotal reports from patients treated with PIT for other complications suggest that this treatment may show efficacy in patients with DN. This study is designed to compare patients with DN who receive PIT treatment with a control population.

Protocol: Patients to be selected may have type 1 or 2 diabetes on oral agents or insulin and neuropathy primarily related to diabetes, preferably age 21-80, and the study will be 6-12 months in duration with renewals of successive 6 month periods. The studies to be done initially are CBC, chemistry profile, TSH, HbA1c and urine protein. Other research blood markers to be determined will be drawn baseline and every 3 months thereafter. Questionnaires on the neuropathy will be completed every 3 months as well.

Endpoints: modified Michigan neuropathy questionnaire or Norfolk Quality of Life Neuropathy questionnaire.

Statistics: ANOVA.

Blood: 2 red tops.

References:


f. Diabetic Impact Management Scale (DIMS) in Patients with Diabetes Mellitus Treated with Pulsatile Insulin Therapy.

Introduction: Diabetes is a metabolic disorder with many possible complications such as heart disease, kidney disease, neuropathy, and retinopathy. As such, diabetes can have a profound effect upon an individual's Quality of Life (QOL). In the present study, we ask whether the QOL in diabetes is improved after PIT relative to conventional insulin therapy. One well-established measure of QOL in diabetes is the Diabetes Impact Measurement Scale (DIMS). The DIMS is a 44-item questionnaire devised by Hammond and Aoki [1] specifically to evaluate quality of life in diabetes. The test consists of five subscales that evaluate different facets of living with diabetes: symptoms relatively specific for diabetes, symptoms less specific for diabetes, diabetes-related morale, social-role fulfillment, and overall well being. The DIMS has been shown to be a reliable and valid measure of subjective quality of life in a variety of diabetic populations [2].

Protocol: This is a prospective, controlled; single-blind study examining quality of life in patients undergoing PIT. Entry criteria include diabetic patients, aged 21 – 80. These patients must meet one or more criteria for PIT (see Criteria for PIT above). Patients in the treatment group receive PIT weekly as per physician's orders for the appropriate complication requiring therapy. PIT is performed weekly.
over a period of 6-12 months with renewals for successive 6-month periods. As for previous protocols, routine laboratory studies done before therapy include CBC, chemistry profile, TSH, HbA1C, B12 and folate and urine protein studies. An age, sex, and disease matched control group is included with participants undergoing weekly visits with the clinic nurse with vital signs and glucose checks as described above.

The DIMS is a paper-and-pencil questionnaire that is scored by an independent researcher blind to the patient’s grouping. Patients complete the DIMS questionnaire before starting PIT and at three month intervals thereafter. Changes in subjective QOL as indexed by the DIMS score are compared with QOL reported by the control group, who also complete the DIMS questionnaire every 3 months.

**Endpoints:** Changes in total score on the DIMS questionnaire and its subscales.

**Statistical evaluation:** Repeated measures ANOVA with one grouping factor (PIT vs. control group) and multiple regressions (to evaluate effects of age, sex, and duration of diabetes on subjective QOL).

**Preliminary results:** The subjective evaluation of quality of life and well-being improved in only 3 months for a group of 55 PIT patients, but not for a group of 30 control patients [3].

**References:**


g. Effects of Pulsatile Insulin Therapy on Progressive Retinopathy with Diabetes Mellitus (Protocol developed in conjunction with Scott Cousins, MD, former Professor of Ophthalmology, Bascom Palmer Eye Institute, current Professor of Ophthalmology, Duke University Medical Center and Ingrid Zimmer-Galler, MD, Department of Ophthalmology, the Johns Hopkins Hospital).

Diabetic retinopathy is one of the leading causes of blindness in the world. Signs of retinopathy are detected in almost 100% of type 1 diabetic patients who have had their disease for at least 20 years and almost 100% of type 2 diabetic patients with the similar duration of disease (1). Histopathologic findings range from microaneurysms and cotton wool spots to more ominous neovascularization. The latter process, known as proliferative diabetic retinopathy, can progress to total blindness if untreated. The biochemical mechanisms responsible for PDR have been extensively studied, and appear to be multi-factorial. Associated findings include abnormalities of vasoactive peptides such as vascular endothelial growth factor (VEGF), pigment epithelium derived factor (PEDF), and insulin-like growth factor (ILF-1), lipids, oxidative pathways, enzymatic pathways, such as protein kinase, and carbohydrate metabolism (1-4). Whether these (and other) factors are interrelated or have a common underlying defect is unknown. The common endpoint, however, is vascular leakage with neovascularization. Current therapeutic regimens based on these biochemical abnormalities have to date been unsuccessful in stemming the progression of proliferative diabetic retinopathy. Current treatment strategies emphasize glycemic and blood pressure control, with laser photocoagulation and vitrectomy for advanced cases (5).
Early retinal disease in diabetic patients may take the form of diabetic macular edema (DME). This is observed in 20% to 25% of both type 1 and type 2 diabetic patients. The pathophysiology of DME involves the leakage of plasma from small vessels in the macula. Resorption of this fluid followed by hard exudate formation can lead to severe impairment of central vision (6).

Anecdotal evidence from ophthalmologic institutions (Houston Eye Institute, Shands at University of Florida, Bascom Palmer Eye Institute) suggests that this treatment arrests the progression of retinal disease in patients with proliferative diabetic retinopathy. The mechanism of this effect is unknown, but may be related to reversal of retinal ischemia or down regulation of vasoactive peptides by restoration of hepatic metabolism.

**Protocol:** This study is designed as a prospective, controlled, single blinded evaluation of PIT in the role of diabetic retinopathy. The patients entered into the study will be from two distinct sources. First, in conjunction with a national eye imaging company, patients with known type 1 or type 2 diabetes will be evaluated for retinal disease. This evaluation will consist of mydriatic fundus photography in diabetic patients not having had recent ophthalmologic evaluation (period greater than 12 months). The fundus photographs will be read by an observer under the auspices of the Wilmer Ophthalmologic Institute at Johns Hopkins Hospital. Three classifications of patients will be evaluated in this study:

- Patients with non high risk proliferative diabetic retinopathy.
- Patients with severe non proliferative diabetic retinopathy.
- Patients with clinically non-significant diabetic macular edema.

Patients who are diagnosed as one of these three classifications will be offered entrance into the study. Study patients will be matched for age, sex, and disease severity into a treatment and control group. All study patients will be evaluated in conjunction with an ophthalmologist and have thorough ophthalmologic evaluation prior to entrance. This evaluation will include clinical examination, fluorescein angiography, and optical coherence tomography. Treatment group patients will undergo weekly PIT sessions as per protocol above. Control group patients will have weekly clinic visits to maximize glycemic and hypertensive control. All patients will repeat their fundus photography at three month intervals, with ophthalmologic evaluation as above every six months, or more often if requested by the ophthalmologist.

Additional entry criteria in this study includes patients aged 21 to 80 years of age who have no other serious form of eye disease. Patients must be under good glycemic and hypertensive control. Exclusion criteria include patients with severe high risk proliferative retinopathy.

**Endpoints:** Serial fundus photography will be interpreted by treatment blinded grader and scored as to stabilization, progression, or improvement of appearance. In addition, episodes of bleeding and necessity for intervention will be evaluated in all groups. Changes in lab values, including VEGF, C peptide, Endothelin-I, and hsCRP will be analyzed.

**Statistics:** ANOVA of laboratory tests; blinded analysis of treatment success by retina specialist.

**Blood:** 2 red tops.

**References:**

h. Pulsatile Insulin Therapy in Patients with Non-healing Wounds.

**Introduction:** Diabetic vascular disease and neuropathy produce the leading cause of lower limb amputations but the incidence has decreased significantly due to improved wound care (1). This study evaluates in an uncontrolled manner patients who have non-healing wounds which have been resistant to known therapies. Each individual is studied as compared to his previous treatment to evaluate if PIT can heal resistant wounds.

**Protocol:** Individual diabetic patients ages 21-80 who are on oral agents and/or insulin are evaluated with photographs of their wounds. Conventional therapy is continued during the PIT course. Initial laboratory studies include CBC, chemistry profile, TSH, HbA1c and urine protein studies as well as any vascular evaluation examinations done previously and records of the previous treatments. PIT is done weekly and photographs of the wounds are done at each PIT session.

**Endpoint:** wound status.

**Statistics:** none.

**Blood:** none.

**References:**


**Introduction:** Insulin produces vasodilatory, anti inflammatory and anti thrombotic effects (1-4). However the effects of PIT on circulating risk factors for vascular and metabolic disease is unknown. This study is used to evaluate circulating risk markers of vascular and metabolic disease compared to a matched control group.

**Protocol:** Patients selected have diabetes mellitus, 21 years of age and older, and are treated with
oral agents and/or insulin. The study is for a minimum of 6 months and may continue for 1-2 years if a significant difference is shown following the initial 6 months. Blood markers will be determined every 3 months for the first year, and every 6 months after that. They include the following: BNP, fructosamine, PAI-1, fibrinogen, homocysteine, endothelin 1, aldosterone, VCAM, ICAM, IGF-1, TGF-beta, TNF-alpha, hs-CRP, and IL-6). The results are compared to an age and fructosamine matched control group.

**Endpoint:** Changes in markers.

**Statistics:** ANOVA.

**Blood:** 2 purple, 2 red, 2 blue tops.

**References:**


Elias AN, Eng S, Homocysteine Concentrations in Patients with Diabetes Mellitus-Relationship to Microvascular and Macrovascular Disease, Diabetes, Obesity and Metabolism 7:117-21, 2005.


### j. Nitric Oxide Levels in Diabetic Patients Undergoing Pulsatile Insulin Therapy.

**Introduction:** Preliminary studies on 15 patients treated with PIT suggested a decrease in homocysteine, endothelin 1 and aldosterone over 6 months. These three factors are all interrelated by inhibiting nitric oxide formation. Theoretically the lowering of their levels should allow it to rise with beneficial effects on vascular tone and metabolism. (1-3). This study is a controlled study comparing controls to PIT treated patients, and measuring the chronic effects of PIT on nitric oxide formation over 6 months as well as short term effects measured in the hours of a single PIT session.

**Protocol:** Inclusion criteria include patients 21 years or older with type 1 or 2 diabetes. They must be on insulin or oral diabetes agents. Serum nitric oxide levels will be measured prior to treatment and at 3 month intervals. They will be compared to age and fructosamine matched controls. Serum is measured by spectrophotometric assay at the Florida Atlantic University biomedical laboratory.

**Endpoint:** change in nitric oxide level.

**Statistics:** ANOVA.

**Blood:** 1 red, blue or purple top tube.

**Bibliography:**
Introduction: PIT depends crucially on exploiting the normal pulsatile secretion of insulin in non-diabetic individuals. By intravenously delivering insulin in a pulsed fashion, PIT increases the respiratory quotient from levels around 0.7 to levels greater than 0.9. This reflects the underlying physiology of the treatment, confirming the conversion from fat metabolism, typical in the diabetic patient, to a normal metabolic state utilizing carbohydrate as the primary fuel consumed. Nevertheless, we do not yet have definitive evidence that peripheral administration of pulsed insulin maintains its pulsatile form at the portal vein. In this work, we use an animal model, diabetic (Zucker) rats, to explore directly whether the form of insulin delivery in a peripheral vein is maintained at the entry to the liver. In future work, we will sacrifice the rat and analyze tissue from the liver to assay the amount of insulin uptake and the level of liver enzymes. In addition, levels of compounds (IRS-1, IRS-2, and AKT) that are integral components of insulin signaling pathways [1] and indicate the status of arterial function [2] will be analyzed from several types of tissue from the diabetic rats.

Protocol: The first phase of the proposed work investigates whether pulsed insulin in a peripheral vein results in pulsated levels of insulin at the portal vein.

A diabetic rat model (Zucker rats) will be used to investigate both the timing and amplitude of insulin measured at the portal vein. When initially administered via a peripheral vein in the hind limb. To this end, a cannula will be inserted in a hind limb of the rat and another in the portal vein. Baseline levels of insulin will be recorded and, if necessary, maintained by low-dosage subcutaneous insulin drip. A bolus of insulin will be injected into the peripheral vein and a blood sample taken at the portal vein, every 15 sec for a period of 3 minutes. The bolus will be varied in both amplitude and duration of administration so that the appropriate delivery parameters for the rat can be discovered. In essence, this protocol will 1) establish a dose response curve for pulsed insulin delivery; 2) determine whether the insulin that gets to the liver maintains its pulsatile character; and 3) determine the amount of insulin that is preserved at the level of the portal vein.

Endpoints and Statistics: Amplitudes and durations of measurable insulin levels above baseline will be measured at the portal vein. Standard dose response curves will be determined for insulin levels and durations administered in the periphery and measured at the portal vein. Uptake in the liver will be assessed using 125 I labeled insulin and using radiomicrography on homogenized liver tissue, in order to determine percent total insulin uptake. ANOVA will be used to evaluate percent total insulin uptake relative to baseline and to the bolus injected at the periphery.

References:


Okon EB, Chung AW, Rauniyar P, Padilla E, Tejerina T, McManus BM, Luo H, van Breman C:
I. Phase II Cognitive Study – Correlation of Enhanced Memory Function in PIT Treated Diabetic Patients with Functional Magnetic Resonance Imaging.

Introduction: The study is based on an earlier controlled study demonstrating that PIT enhances short term memory function (also called working memory) in patients with diabetes. Briefly, working memory allows humans to maintain a limited amount of information in an active state for a brief period of time and to manipulate that information [1]. The next step is to correlate the improvement in memory function with an objective assessment of brain activity patterns. Functional MRI has demonstrated efficacy in localizing active brain regions during working memory tasks [21]. This study will examine active brain areas in PIT patients involved in working memory processes in an attempt to relate the enhanced memory abilities observed in our earlier cognitive study with specific changes in neural activity.

Protocol: This is a prospective, controlled, single blind study. Patients with types 1 or 2 diabetes and who demonstrate mild or moderate cognitive impairment on the modified Wechsler test III, will undergo functional MRI prior to initiation of therapy and every three months. Patients in the treatment group will undergo weekly PIT sessions according to protocol. Patients in the control group will be evaluated weekly for optimizing blood pressure and glycemic control.

The task used will be an “N-back” task, which explores working memory. In the N-back task, subjects see or hear a series of one to four target letters, followed by a probe letter. The subject’s task is to respond “yes” or “no” (by pressing one of two buttons) indicating whether the probe was identical to one of the targets. The distance from the target to the probe is varied so that subjects respond whether the probe matches the target that was 0-, 1-, 2- or 3-back from the target item [3] Thus, the N-back task varies the memory load incrementally throughout the task.

Endpoints and Statistics: Changes in activity levels in working memory areas, and changes in which areas are activated, will be correlated with behavioral abilities of the individual subject. Especially interesting are whether increases in working memory ability after PIT are correlated with activity patterns observed with functional MRI, and the changes in fMRI that occur with changes in memory load.

References:


Patients undergoing PIT with Quantitative Neurologic Measurements.

**Introduction:** Initial studies done (part D) showed a 51% improvement in pain relief and a 42% improvement in sensation. The study of this significant effect of PIT has been amplified by adding quantitative and qualitative testing of peripheral nerve function. Additionally, a more inclusive questionnaire has been substituted to better elucidate the areas of improvement and autonomic testing has been added to evaluate the effect of PIT on this form of neuropathy, a risk factor for sudden death (1, 2).

**Protocol:** Patients with diabetic neuropathy, ages 21 or older, who are on oral agents and/or insulin are eligible for this study. Nerve conduction studies (Neurometrix Corp) are done initially to evaluate for diabetic neuropathy (3) and to exclude those with other forms of neuropathy. Those that are included in the study are then tested with quantitative testing for hot/cold and vibratory sensation (MEDOC Corp) (4) and autonomic dysfunction using cardiac beat-to-beat variation (5) (Ansar Corp). The Norfolk neuropathy quality of life questionnaire is used and continued monthly while the objective nerve testing is done every 3 months. Treatment group patients will be compared with an age, sex, and disease matched control group.

**Endpoints:** 1) Changes in nerve conduction, sensory testing and/or autonomic testing. 2) Changes on questionnaire.

**Statistics:** ANOVA.

**Bibliography:**


**n. Effectiveness of PIT on “Brittle” Diabetes and Glucose Control.**

**Introduction:** Diabetes can produce wide swings in blood glucose with erratic control even under optimal conditions. Reasons for this include insulin resistance, erratic insulin secretion, and counter-regulatory hormones.(1,2) This can occur despite minimal changes in overall diabetic control as evidenced by hemoglobin A1c levels. This study was instituted to evaluate the effect of PIT on improving diabetic control in these circumstances.

**Protocol:** Diabetics over age 21 referred for erratic control and elevated hemoglobin A1c despite optimal therapy are placed on PIT weekly. Weekly glucose diaries and glucose monitor recorded glucoses are reviewed after a 30 day baseline before treatment to determine if PIT improves control.
The number of glucoses above 300 mg/dl and below 70 mg/dl as well as mean glucose level are determined. Hemoglobin A1c and/or fructosamine levels are determined every 3 months. Treatment continues for 6 months and then continues if improvement is noted.

**Endpoints:** Hemoglobin A1c, fructosamine, mean and SD of glucose levels, number of glucoses above and below specific levels.

**Statistics:** ANOVA.

**Blood:** none.

**References:**


Parrish R, Petersen KF, Mitochondrial Dysfunction and Type 2 Diabetes, Current Diabetes Reports 5:177-183,2005.

**Bibliography:**


II. Supporting Extracts Listed by Secondary Complications of Diabetes.

1. Renal (kidney) Disease: In patients with advanced diabetic kidney disease, the gradual deterioration of kidney function (decrease of creatinine clearance [CrCl] by 8-10 ml/min/year) cannot be arrested with "routine" insulin therapy. This study reports the treatment outcome of an average of 37 months (range 1-7 years) of CIIIT in 31 patients with Type I diabetes and advanced diabetic renal disease. The CrCl at the end of the treatment period was essentially unchanged, suggesting that adding weekly CIIIT to daily intensive insulin therapy could arrest or markedly delay progression to the end stage renal disease, at which time dialysis or transplantation would be required.


2. Renal Disease: A nine month clinical trial conducted at research centers in Boston, the Scripps Institute, Mayo Clinic, Temple University and University of Arizona demonstrated the ability of chronic intermittent intravenous insulin therapy to slow the progression of diabetic nephropathy in 70 acutely ill patients. While patients in the control group experienced an average decline in creatinine clearance during the study period of 8.15 ml/min/year, the treatment group slowed only experienced a decline of 0.89 ml/min/year. The researchers found the stabilizing effects on renal function to be independent of
improved glucose control or blood pressure and independent of differences in office visit attendance between groups.


3. Hypoglycemia – (loss of consciousness due to low blood sugar): A study of 20 diabetic patients over 42 months showed that CAT (CIIIT) resulted in a 98 percent decrease in major hypoglycemic reactions. Patients with “brittle” diabetes who previously were unable to recognize when they were in danger of losing consciousness due to hypoglycemia became aware of perilously low drops in their blood glucose levels. The patients went from an average of three severe hypoglycemic reactions (requiring outside intervention) per month to an average of 0.1 episodes per month. The average frequency of hypoglycemic reactions returned to three per month when CAT (CIIIT) was stopped.


4. Hypertension - high blood pressure: Chronic intermittent intravenous insulin therapy for patients with high blood pressure led to a 46% decrease in the amount of medication required to control the patient's blood pressure.


5. Hypertension (evening blood pressure): Patients with severe diabetes often have increased nighttime blood pressure, a condition that may worsen the complications of diabetes. Patients in randomized, controlled clinical trials comparing two treatments 1) four subcutaneous insulin injections daily, vs. 2) weekly CIIIT added to the four subcutaneous injections daily had monthly measures of 24-hour ambulatory blood pressure. The group on weekly CIIIT in addition to four subcutaneous insulin injections daily had a 3% decline in the night/day blood pressure ratio. In contrast, those on only four subcutaneous injections daily had a 3% increase in night/day blood pressure ratio. In addition, the group on CIIIT had a significant improvement in the average HbA1c levels.


6. Hypotension - low blood pressure: On CIIIT therapy, patients reported complete relief from dizziness and fainting when they stood up and blood pressure no longer dropped precipitously with upright posture.

7. **Obstetrics – pregnancy**: A group of 3 insulin-dependent diabetic pregnant patients received CIIIT in addition to the usual regimen of 3 insulin shots per day and home glucose monitoring. Compared to 15 matched controls, the CIIIT group all had normal hemoglobin A1c levels at delivery, none developed hypertensive complications requiring early delivery, and none required extra antepartum hospital days. Infants of the CIIIT group were not hypoglycemic, and 2 of the 3 were discharged at the same time as their mothers.


8. **Quality of Life**: The overall quality of life and energy is improved as shown by measurement of health status in diabetic patients: diabetes impact measurement scales.


9. **Physiology – biochemistry**: Acute insulin effects on plasma homocysteine levels in patients with diabetes mellitus.

Aoki TT, Grecu EO, Medina M, Goodman M.


10. **Physiology – biochemistry**: IGF-1 and IGFBP-1 blood levels in Type 1 diabetes mellitus on intensive intravenous insulin therapy.

Aoki TT, Grecu EO.

**Journal**: *J Invest Med*, 1999; 47(2) 78 A.


Foss MC, Vlachokosta FV, Cunningham LN, Aoki TT.

**Journal**: *Diabetes* 1982; 31: 46-52.

12. **Physiology – biochemistry**: Role of muscle in CO2 production after oral glucose administration in man.

Meistas MT, Vlachokosta FV, Gleason RE, Arcangeli M, Aoki TT.


13. **Osteoporosis**: Is lateral spine dual energy X-ray absorptiometry of value in diagnosing osteoporosis in women.
Aoki TT, Grecu EO, Srinivas PR, Arcangeli MA.


Aoki TT, Grecu EO, Srinivas PR, Arcangeli MA.


15. Osteoporosis: Prevalence of osteoporosis in women varies with the skeletal site where bone mineral density is measured.
Aoki TT, Grecu EO, Srinivas PR, Prescott P, Benbarka MM, Arcangeli MA.

Journal: Endocrine Practice (in press).

III. Papers Supporting Pulsatile Insulin Infusion.


Effects of Fasting on Physiologically Pulsatile Insulin Release in Healthy Humans.

Juhl C, Grofte T, Butler PC, Veldhuis JD, Schmitz O, Porksen N.

Department of Endocrinology and Metabolism M, Aarhus University Hospital, Aarhus, Denmark. Department of Endocrinology and Metabolism and Diabetes, University of Southern California, Los Angeles, CA. U.S. Department of Medicine and National Science Foundation Center for Biological Timing, Charlottesville, VA.

Insulin is released as secretory bursts superimposed on basal release. The overall contribution of secretory bursts was recently quantified as at least 75%, and the main regulation of insulin secretion is through perturbation of the amount of insulin released and the frequency of these secretory bursts. The mode of delivery of insulin into the circulation seems important for insulin action, and therefore physiological conditions that alter the pattern of insulin release may affect insulin action through this mechanism. To assess the mechanisms by which fasting changes the amount of insulin released and the frequency, amplitude, and overall contribution of pulsatile insulin secretion, we used a validated deconvolution model to examine pulsatile insulin secretion during 10 and 58 h of fasting in seven healthy subjects. The subjects were studied for 75 min before (0--75 min) and 75 min during (115--190 min) a glucose infusion (2.5 mg center dot kg(-1) center dot min(-1)). We found that the pulsatile insulin release pattern was preserved and that, at fasting, overall insulin release is adjusted to needs by a reduced amount of insulin released (10.1 plus minus 1.7 vs. 16.0 plus minus 3.2 pmol/l/pulse, P < 0.05) but similar frequency (6.3 plus minus 0.4 vs. 6.1 plus minus 0.4 min/pulse) of the insulin
secretory bursts. In both states, glucose infusion caused an increase (P < 0.05) in amount (100−200%) and frequency (similar20%). The impact of increased glucose concentration on pulse frequency seems distinct for in vivo versus in vitro pulsatile insulin secretion and may indicate the presence of a glucose-sensitive pacemaker, which initiates the coordinated secretory bursts. Increased insulin/C-peptide ratio at long-term fasting (6.0 vs. 9.1%, P < 0.01) indicates that the changes in insulin release patterns may be accompanied by changes in hepatic insulin extraction.

PMID: 11815488 [PubMed - as supplied by publisher].


Pulsatile insulin secretion by human pancreatic islets.

Song SH, Kjems L, Ritzel R, McIntyre SM, Johnson ML, Veldhuis JD, Butler PC.

Department of Pathology, University of Edinburgh, Edinburgh EH8 9YL, Scotland, UK.

Insulin is secreted in discrete bursts. These pulses are also present when individual or groups of islets are perfused. Interpretation of the measured frequency and magnitude of pulsatile hormone secretion requires an examination of the sensitivity and specificity of the methods for pulse detection and validation of these for the experimental apparatus and hormone assay in which they are applied. In the present study we achieve these aims for a perfusion method for measurement of pulsatile insulin release by human islets. A deconvolution technique previously developed for measurement of pulsatile hormone secretion in vivo was specifically validated for in vitro pulse detection in the present study. Deconvolution analysis reliably (>90%) detected insulin pulses with an amplitude 20% or more above baseline and recovered quantitatively the insulin secretion profile, insulin secretion rate, and insulin pulse mass from single as well as multiple perfused islets. Cluster analysis was less sensitive, but was able to detect most (>80%) pulses with an amplitude of 40% or more above baseline. With this limitation, cluster analysis is potentially useful for groups, but not single perfused human islets. Analysis of single human islets showed that enhanced insulin secretion by increased glucose concentrations in the perfusate is achieved by enhancing insulin pulse mass with no change in pulse frequency. Perfused single or groups of human islets exhibited an interpulse interval (approximately 6-8 min) comparable to that observed in humans in vivo. Dynamic in vitro perfusion should facilitate studies of the mechanisms driving pulsatile insulin secretion.

PMID: 11788649 [PubMed - indexed for MEDLINE].


Decrease in beta-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig.


Diabetes Research Unit and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland.

Most insulin is secreted in discrete pulses at an interval of approximately 6 min. Increased insulin
secretion after meal ingestion is achieved through the mechanism of amplification of the burst mass. Conversely, in Type 2 diabetes, insulin secretion is impaired as a consequence of decreased insulin pulse mass. Beta-cell mass is reported to be deficient in Type 2 diabetes. We tested the hypothesis that decreased beta-cell mass leads to decreased insulin pulse mass. Insulin secretion was examined before and after an approximately 60% decrease in beta-cell mass achieved by a single injection of alloxan in a porcine model. Alloxan injection resulted in stable diabetes (fasting plasma glucose 7.4 +/- 1.1 vs. 4.4 +/- 0.1 mmol/l; P < 0.01) with impaired insulin secretion in the fasting and fed states and during a hyperglycemic clamp (decreased by 54, 80, and 90%, respectively). Deconvolution analysis revealed a selective decrease in insulin pulse mass (by 54, 60, and 90%) with no change in pulse frequency. Rhythm analysis revealed no change in the periodicity of regular oscillations after alloxan administration in the fasting state but was unable to detect stable rhythms reliably after enteric or intravenous glucose stimulation. After alloxan administration, insulin secretion and insulin pulse mass (but not insulin pulse interval) decreased in relation to beta-cell mass. However, the decreased pulse mass (and pulse amplitude delivered to the liver) was associated with a decrease in hepatic insulin clearance, which partially offset the decreased insulin secretion. Despite hyperglycemia, postprandial glucagon concentrations were increased after alloxan administration (103.4 +/- 6.3 vs. 92.2 +/- 2.5 pg/ml; P < 0.01). We conclude that an alloxan-induced selective decrease in beta-cell mass leads to deficient insulin secretion by attenuating insulin pulse mass, and that the latter is associated with decreased hepatic insulin clearance and relative hyperglucagonemia, thereby emulating the pattern of islet dysfunction observed in Type 2 diabetes.

PMID: 11522665 [PubMed - indexed for MEDLINE].


Direct measurement of pulsatile insulin secretion from the portal vein in human subjects.

Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC.

Liver Research Unit, Royal Infirmary of Edinburgh, University of Edinburgh, Edinburgh, Scotland.

Insulin is secreted in a high frequency pulsatile manner. These pulses are delivered directly into the portal vein and then undergo extraction and dilution before delivery into the systemic circulation. The reported frequency of these insulin pulses estimated in peripheral blood varies from an interpulse interval of 4-20 min. We postulated that this discrepancy is due to the attenuation of the pulse signal in the systemic circulation vs. the portal circulation. In the present study we measured pulsatile insulin release directly in the portal circulation of human subjects who had indwelling transjugular intrahepatic portasystemic stent shunts (TIPSS) to decompress portal hypertension. We quantitated pulsatile insulin secretion in both the overnight fasted state (fasting) and during a hyperglycemic clamp (8 mmol/L). Direct portal vein sampling established that pulsatile insulin secretion in humans has an interval (periodicity) of approximately 5 min. The amplitude (and mass) of the insulin concentration oscillations observed in the portal vein was approximately 5-fold greater than that observed in the arterialized vein and was similar to that observed in the dog. Increased insulin release during hyperglycemia was achieved through amplification of the insulin pulse mass. In conclusion, direct portal vein sampling in humans revealed that the interpulse interval of insulin pulses in humans is about 5 min, and this frequency is also observed when sampling from the systemic circulation using a highly specific insulin assay and 1-min sampling, but is about 4-fold greater than the frequency observed at this site using single site RIAs. We confirm that enhanced insulin release in response to hyperglycemia is achieved by amplification of these high frequency pulses.

Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in Type 2 diabetes.

Laedtke T, Kjems L, Porksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC.

Division of Endocrinology and Diabetes, Keck School of Medicine, University of Southern California, Los Angeles 90089, USA.

Impaired insulin secretion in Type 2 diabetes is characterized by decreased first-phase insulin secretion, an increased proinsulin-to-insulin molar ratio in plasma, abnormal pulsatile insulin release, and heightened disorderliness of insulin concentration profiles. In the present study, we tested the hypothesis that these abnormalities are at least partly reversed by a period of overnight suspension of beta-cell secretory activity achieved by somatostatin infusion. Eleven patients with Type 2 diabetes were studied twice after a randomly ordered overnight infusion of either somatostatin or saline with the plasma glucose concentration clamped at approximately 8 mmol/l. Controls were studied twice after overnight saline infusions and then at a plasma glucose concentration of either 4 or 8 mmol/l. We report that in patients with Type 2 diabetes, 1) as in nondiabetic humans, insulin is secreted in discrete insulin secretory bursts; 2) the frequency of pulsatile insulin secretion is normal; 3) the insulin pulse mass is diminished, leading to decreased insulin secretion, but this defect can be overcome acutely by beta-cell rest with somatostatin; 4) the reported loss of orderliness of insulin secretion, attenuated first-phase insulin secretion, and elevated proinsulin-to-insulin molar ratio also respond favorably to overnight inhibition by somatostatin. The results of these clinical experiments suggest the conclusion that multiple parameters of abnormal insulin secretion in patients with Type 2 diabetes mechanistically reflect cellular depletion of immediately secretable insulin that can be overcome by beta-cell rest.

PMID: 10950818 [PubMed - indexed for MEDLINE].


In humans at least 75% of insulin secretion arises from punctuated insulin secretory bursts.

Porksen N, Nyholm B, Veldhuis JD, Butler PC, Schmitz O.

Department of Endocrinology and Metabolism M, Aarhus University Hospital, Denmark.

Detection of insulin secretory bursts in peripheral blood is hampered by hepatic insulin extraction, dilution in the systemic insulin pool, and time-delayed damping of secretory burst amplitude. Previous studies in dogs in vivo and other experiments in vitro have shown that approximately 70% of all insulin is released within distinct insulin secretory bursts. To establish a method for detection and quantification of pulsatile insulin release in humans on the basis of peripheral insulin concentration measurements, we used a high-sensitivity, -specificity, and -precision insulin enzyme-linked immunosorbent assay (ELISA) and optimized an established deconvolution methodology to quantify the frequency, mass, and amplitude of insulin secretory bursts as well as to estimate the relative contribution of pulsatile insulin release to overall insulin secretion. By use of minutely sampled serum insulin concentrations measured by a highly sensitive insulin ELISA and insulin kinetics of 2.8 min
(first half-life), 5.0 min (second half-life), and a fractional slow component of 0.28, the deconvolved insulin secretion rates in 20 healthy subjects during glucose infusion (4.5 mg.kg⁻¹.min⁻¹) could be resolved into a series (4.7 +/- 0.1 min/pulse) of approximately symmetric insulin secretory bursts with a mean mass of 87 +/- 12 pmol.l⁻¹ pulse⁻¹ and a mean amplitude (maximal release rate) of 35 +/- 4.7 pmol.l⁻¹.min⁻¹. The relative contribution of pulsatile to overall insulin secretion was 75 +/- 1.6% (range 59-85%). We conclude that in vivo insulin secretion in humans during nominal glucose stimulation consists of a series of punctuated insulin secretory bursts accounting for > or = 75% of total insulin secretion.

PMID: 9374676 [PubMed - indexed for MEDLINE].


IGF-I inhibits burst mass of pulsatile insulin secretion at supraphysiological and low IGF infusion rates.

Porksen N, Hussain MA, Bianda TL, Nyholm B, Christiansen JS, Butler PC, Veldhuis JD, Froesch ER, Schmitz O.

Department of Endocrinology and Metabolism M, Aarhus University Hospital, Denmark.

Insulin-like growth factor I (IGF-I) shares structural and functional features with insulin, affects carbohydrate metabolism, and inhibits insulin secretion. Insulin secretion is pulsatile, and it is regulated by changing frequency and/or mass of secretory bursts. To examine the mechanism of IGF-I's inhibition of insulin secretion, eight healthy volunteers were studied three times. During glucose infusion (2.5 mg x kg⁻¹ x min⁻¹) blood was sampled minutely at time 75-200 min for triplicate insulin concentration measurements by enzyme-linked immunosorbent assay (ELISA; coefficient of variation 2.1%). Time 125 min infusion of saline, low-dose IGF-I (0.025 microg x kg⁻¹ x min⁻¹) or high-dose IGF-I (0.15 microg x kg⁻¹ x min⁻¹) was commenced and continued until 200 min. Data were compared before (75-125 min) vs. during infusion (150-200 min). Insulin concentration time series were deconvolved, using validated pulse-detection criteria, to assess insulin secretory burst mass and frequency. During saline infusion no time effect occurred. After IGF-I infusion, serum C-peptide decreased (582 +/- 85 vs. 481 +/- 82 pM, low-dose IGF-I, P < 0.05; 539 +/- 84 vs. 427 +/- 69 pM, high-dose IGF-I, P < 0.01). Total insulin secretion rates decreased by 17 and 21%, respectively, via specific inhibition of the insulin secretory burst mass (31 +/- 8 vs. 20 +/- 4 pmol/ml, low-dose IGF-I, P = 0.06; 22 +/- 4 vs. 17 +/- 3 pmol/ml, high-dose IGF-I, P < 0.05), whereas the frequency was not affected (10.5 +/- 1.3 vs. 10.7 +/- 1.3 pulses/h, low-dose IGF-I, P = 0.85; 8.7 +/- 1.0 vs. 11.1 +/- 1.2 min/pulse, high-dose IGF-I, P = 0.15). We conclude that IGF-I inhibits pulsatile insulin secretion by specific inhibition of mass but not frequency of secretory bursts.

PMID: 9124538 [PubMed - indexed for MEDLINE].


Effects of glucose ingestion versus infusion on pulsatile insulin secretion. The incretin effect is achieved by amplification of insulin secretory burst mass.

Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC.
In the present studies, we used a recently validated canine model to determine 1) if glucose ingestion stimulates insulin secretion by amplifying the pulsatile component of insulin release, and if so, 2) whether this effect is achieved preferentially through burst mass or frequency modulation, and 3) if the mechanism of incretin effect of insulin secretion is mediated via the pulsatile mode of secretion. We report that 30 g of glucose ingestion stimulates an approximately 550% increase in the overall rate of insulin secretion (1.8 +/- 0.2 to 11.6 +/- 1.5 pmol.kg-1.min-1), which is achieved via an approximately 400% increase in the mass of insulin secreted per burst (202 +/- 38 to 1,003 +/- 147 pmol/pulse, P < 0.001) and a approximately 40% increase in burst frequency (8.7 +/- 0.5 to 12.3 +/- 0.6 pulse/h, P < 0.001). Of the insulin secreted after glucose ingestion, 68% (+/-4) was released in discrete secretory bursts. Further analyses showed that the incretin effect of ingested (GPO) versus infused glucose (GIV) is achieved through regulation of pulsatile insulin secretion. Glucose ingestion led to an approximately 70% greater rate of insulin secretion than intravenous glucose delivery (10.0 +/- 1.6 vs. 5.9 +/- 0.9 pmol.kg-1.min-1, P < 0.005, GPO vs. GIV). This incretin effect was achieved by the specific mechanism of an approximately 70% greater pulse mass (930 +/- 196 vs. 558 +/- 97 pmol/pulse, P < 0.02, GPO vs. GIV) but with a comparable pulse frequency (13.1 +/- 0.9 vs. 12.0 +/- 0.5 pulses/h, P = 0.14, n = 9 dogs, GPO vs. GIV). We conclude that in vivo glucose regulates overall insulin secretion almost exclusively by amplification of the pulsatile mode of insulin secretion, and that the incretin effect is achieved by preferential enhancement of insulin secretory burst mass.

PMID: 8826965 [PubMed - indexed for MEDLINE].


Effects of somatostatin on pulsatile insulin secretion: elective inhibition of insulin burst mass.

Porksen N, Munn SR, Steers JL, Veldhuis JD, Butler PC.

Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota 55905, USA.

Although it is well known that somatostatin inhibits net insulin secretion, it is unknown whether this is achieved by regulation of the basal or pulsatile components of insulin secretion and, if the latter, whether this is through modulation of pulse mass or frequency. We addressed these questions with a canine model. Portal vein blood was sampled at 1-min intervals in five dogs for 60 min before (basal) and 90 min after ingestion of 30 g glucose on two different occasions, during a saline (SAL) or a somatostatin (SMS, 175 ng/min) infusion. Plasma glucose concentrations were similar during SAL and SMS. SMS had no effect on pulse frequency before (8.4 +/- 0.7 vs. 9.2 +/- 1.0 pulses/h, SMS vs. SAL, P = 0.54) or after glucose (13.3 +/- 1.1 vs. 11.6 +/- 0.9 pulses/h, SMS vs. SAL, P = 0.22). In contrast, SMS decreased insulin pulse mass in the postabsorptive (84 +/- 28 vs. 214 +/- 73 pmol/pulse, SMS vs. SAL, P < 0.05) and fed states (676 +/- 143 vs. 913 +/- 183 pmol/pulse, SMS vs. SAL, P < 0.05). In the postabsorptive state, SMS decreased insulin clearance by approximately 50% (0.32 +/- 0.04 vs. 0.60 +/- 0.09 l/min, P < 0.05), but after glucose ingestion, insulin clearance was comparable during SMS or SAL (0.72 +/- 0.04 vs. 0.80 +/- 0.08 l/min, P = 0.4). SMS appeared to alter insulin clearance through modulation of insulin pulse amplitude, because in the postabsorptive state clearance was closely correlated to the pulse amplitude (r = + 0.87, P < 0.0001). In conclusion, somatostatin regulates the rate of insulin secretion by selective inhibition of pulsatile insulin secretion. Regulation of secretory burst mass (and amplitude) may secondarily influence transhepatic and thus total body clearance of endogenously secreted insulin and thereby serve as a novel mechanism to dictate the systemic insulin concentration.
Little is known about the optimal experimental conditions for assessing pulsatile insulin secretion in vivo. To address this, we employed a recently validated canine model (n = 12) to determine the consequences of 1) sampling from the systemic circulation (SC) vs. the portal vein (PV), 2) sampling intensity and duration, and 3) deconvolution vs. cluster analysis on assessing pulsatile insulin secretion. PV vs. SC sampling resulted in approximately 40% higher pulse frequency by deconvolution (9.0 +/- 0.5 vs. 6.6 +/- 0.9 pulses/h, P < 0.02) and cluster analysis (7.5 +/- 0.3 vs. 5.6 +/- 0.6 pulses/h, P < 0.01) due to a higher signal-to-noise ratio (19 +/- 4.8 PV vs. 12 +/- 1.8 SC). PV sampling also disclosed a higher calculated contribution of the pulsatile vs. nonpulsatile mode of delivery to total insulin secretion (57 +/- 4 vs. 28 +/- 5%, P < 0.001). Analysis of the relevance of sampling intensity revealed that 1-min data yielded a markedly higher estimate of pulse frequency with PV sampling than 2-min data (9.0 +/- 0.5 vs. 5.4 +/- 0.5, P < 0.02, deconvolution; 7.5 +/- 0.3 vs. 4.3 +/- 0.6 pulses/h, P < 0.001, cluster). Optimal sampling duration was shown to be 40 min or more. We conclude that the resolving power of the analytical tool, the anatomic site of blood withdrawal, the frequency of blood sampling, and the duration of the total observation interval all significantly influence estimated insulin secretory pulse frequency and the fraction of insulin secreted in pulses. With the assumption that PV 1-min insulin data constitute the "gold standard," our in vivo inferences of 7.5-9.0 insulin pulses/h closely recapitulate in vitro islet secretory activity.

The present study was designed to examine the effect of pulsatile versus continuous insulin delivery on glucose and lipid metabolism in insulin-resistant subjects. Six obese women (body mass index, 40.0 +/- 2.8 kg/m2) underwent a euglycemic glucose clamp (plasma glucose, 90 mg/dL) twice. In random order, insulin was infused intravenously for 375 minutes either at a constant rate (0.4 mU/kg/min) or in a pulsatile manner (2.4 mU/kg/min for 2 minutes followed by an off interval of 10 minutes). Endogenous insulin release was suppressed by infusion of somatostatin (250 micrograms/h). Mean circulating insulin concentrations were similar during the two protocols (pulsatile v continuous infusion, 60 +/- 10 v 56 +/- 9 mU/L), but pulsatile infusion was accompanied by
oscillations with an amplitude of 120 mU/L. After 6 hours of pulsatile versus continuous insulin, isotopically determined total glucose disposal (3-3H-glucose) and hepatic glucose production (HGP) were comparable (pulsatile v continuous, 2.80 +/- 0.56 v 2.82 +/- 0.51 and 0.37 +/- 0.14 v 0.32 +/- 0.17 mg/kg/min). However, the rate of glucose oxidation (indirect calorimetry) was augmented (P < .05), whereas lipid oxidation tended to be diminished (.10 > P > .05) following pulsatile infusion. In addition, blood glycerol was more suppressed with pulsatile (31 +/- 9 nmol/L) than with continuous infusion (36 +/- 10 nmol/L, P < .05), whereas blood lactate, alanine, and 3-hydroxybutyrate were similar in the two infusion protocols.