Cellular Activation Therapy
Studies and Reports

The following publications and reports include 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 and reports 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.

Research Compendium:

Note: Cellular Activation Therapy (Pulsatile Insulin Therapy), i.e. the providing of pulsed boluses of insulin using the Bionica Microdose for 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 (CIIIT).*
- *Pulsatile Intravenous Insulin Therapy (PIVIT).*
- *Pulsatile Insulin Therapy (PIT)*
- *For consistency in this document, Cellular Activation Therapy (CAT) is used throughout.*

All of the clinical trials use the FDA certified Bionica MicroDose which is labeled for CAT. The Bionica MicroDose also holds the CE certificate and is ISO 9003 compliant.

We would like to thank the tireless efforts of our many research Associates and the founder of CAT therapy, Bionica Inc manufacturer of the Bionica MicroDose for Cellular Activation Therapy and G Ford Gilbert, JD, CEO of Bionica Inc.

This work is dedicated to all of the diabetic patients and their families who have suffered all too long.

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1. Introduction: Cellular Activation Therapy (CAT) also is an effective therapy for individuals with advanced diabetes. CAT has its roots in the work of George Cahill, MD of the Joslin Diabetes Center in Boston, Massachusetts, and the Bionica Inc infusion device developers.

The early work focused on the critical role of liver dysfunction in diabetic metabolism. The hypothesis was that organ damage in diabetes is caused by abnormal hepatic glucose metabolism, inadequate carbohydrate and lipid metabolism, and insulin resistance, all of which are effectively treated by CAT.

Dr. Cahill recognized the fact that diabetic patients present much like patients who have undergone a prolonged fasting. This was reportedly the first time that focus was shifted from insulin as a cellular hormone to insulin as an organ stimulation hormone.

As we now know once a diabetic patient's liver receives the stimulation that is normally provided by a non-diabetic pancreas, the liver of the diabetic patient nevertheless responds and provides the enzymatic pathways necessary for proper metabolism. This inducible phenomenon of reestablishing normal hormone fueled metabolism by a diabetic person is the key to the effective treatment of diabetes and all of its complications.

The obvious fear in treating diabetes would be that after a prolonged failure of the pancreas to properly stimulate the liver in a pulsatile fashion, the liver might effectively "forget" how to produce and activate various enzymes necessary for carbohydrate metabolism.

However studies have shown that CAT is effective no matter how long in duration the patient has been diabetic. Because the liver of the diabetic patient can reestablish proper enzymatic activities when properly stimulated by CAT, It is therefore clear that the DNA of the liver is sufficiently encoded to perform these functions so long as the hepatocytes are not damaged by other disease states.

2. History and Biochemistry of Pulsatile Insulin Therapy: Normally, insulin is secreted almost exclusively in a pulsatile fashion, in amounts closely related to the intake of meals. The pulses of insulin coupled with the presence of glucose, provides the necessary two signals to the liver for proper carbohydrate metabolism, whereas in comparison, continuous insulin infusion does not.

In fact, continuous exposure to insulin and glucagon in a non-pulsed fashion is known to decrease the hormones' metabolic effectiveness on splanchnic glucose production in humans. Down-regulation at the cellular level partially explains the decreased action of steady-state levels, while pulsatile hormone (insulin) secretion allows recovery of receptor affinity or receptor numbers. CAT insulin administration with its tailored peaks of insulin, enhances the suppression of gluconeogenesis and reduce endogenous hepatic glucose production (EHGP).

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 pulsed insulin levels of 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 tenfold 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. CAT provides this bimolecular signal using the bionic eye infusion device to provide tailored pulses of insulin delivered at specific pressures.

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, including Continuous Subcutaneous Insulin Infusion pumps (CSII); a) fail to mimic the pulsatile nature of natural insulin secretion and b) do 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).

The relative deficiency of insulin at the hepatocyte level leads to an impaired capacity to process incoming dietary glucose by the diabetic. Since the liver is obviously the target organ of the pancreas, it must 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. Cellular Activation Therapy alone corrects this deficiency.

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 CO2 /volume O2, of 0.7-0.8). After glucose administration, CO2 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 CO2 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 CO2 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 CO2 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 CAT. 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, and this is what is uniformly seen in patients.

3. Pulsatile Insulin Therapy Protocol, CAT: Cellular Activation Therapy (CAT) is a process which promotes the normalization of carbohydrate metabolism in diabetic patients. CAT affects multiple organs, especially muscle, retina, liver, kidney, and nerve endings. The process involves the administration of insulin pulses similar to those found in the portal circulation of normal humans using the Bionica MicroDose which is FDA labeled for this treatment. The process is monitored by frequent glucose levels and respiratory quotients (RQ). RQ is measured by a metabolic cart which determines the ratio of VCO2/ VO2. 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. CAT is done over 1-hour periods with a ¾ to 1-hour rest period between each session for three courses each day of treatment. Typically, CAT 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 CAT center between 7:00am and 8:30am.

- 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 CAT commences:

CAT treatment session:

- U / kg of Insulin, pulsed at 10 pulses/hour over 1 hour is administered by the Bionica MicroDose specialty pump programmed for the concentration, frequency and duration of pulses, and rest intervals.

- The respiratory quotient (RQ) or metabolic measurement is performed at the beginning and end of each one hour treatment to measure success of treatment and adjust the amount of insulin and glucose.

- 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. Approximately 100 g of glucose is given, (400 cal.)

- There is a rest period of 40 minutes to one hour between treatments in order to stabilize blood glucose levels.

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

- Patient is evaluated after the session and discharged when stable.

- Frequent monitoring of respiratory quotient is essential in order to verify patient response to CAT 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.

- CAT increases the respiratory quotient in diabetic patients 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 Cellular Activation Therapy:

a. CAT Effect on Glycemic Control: Long-term intermittent intravenous insulin therapy and type 1 diabetes mellitus**. Lancet. 1993, 342: 515-517.**

This study examined 20 "brittle" diabetics (defined as patients with wide glucose swings and frequent hypoglycemic episodes) treated with the CAT 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 ($p < 0.0003$): was achieved with CAT. With a reduced HbA1c increases in hypoglycemia would be anticipated however, with proper hepatic glycogen storage and release there was actually a market decline in the frequency of both and major hypoglycemic events from 3.0 to 0.1 per month ($p < 0.001$).

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

It is often said that diabetes is a disease of improper metabolism not a disease of improper blood sugar levels. However, when the patient is able to achieve proper metabolism a natural result of that state is automatically a better storing and releasing of glucose based upon blood sugar levels. Reestablishing this natural buffering of glucose leads to better glycemic control as well as a renewed ability by the diabetic person to sense sugar levels.

b. CAT Effect on Hypertension: Effect of chronic intravenous insulin therapy on antihypertensive medication requirements in IDDM subjects with hypertension and nephropathy**. Diabetes Care (1995): 1260-1265.**

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.

Accordingly this study demonstrates that CAT has an ameliorative effect on hypertension in the diabetic patient. While the pathophysiology of hypertension is not well known, it is generally understood that hypertension is a comorbidity factor with other diabetic complications including kidney disease. Thus it might be said that the effects of CAT on kidney disease may well be associated with hypertension.

It may well be that hypertension is a subset of diabetes which would provide insight into why the mechanisms of action for hypertension have never been satisfactorily described.

c. CAT Effect on Diabetic Nephropathy: Effect of intensive insulin therapy on progression of overt diabetic nephropathy in patients with IDDM. **Endocrine Practice (1999) 5: 174-178**.

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 CAT. 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 CAT could successfully stabilize renal function in patients with diabetic nephropathy.

Needless to say, diabetic nephropathy has not been shown to be stabilized by any treatment other than CAT. In addition to the study, anecdotal data continues to build as patients with nephropathy receive treatment.

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 CAT 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 (CAT) patients had a statistically significant improvement in renal function as compared to the control group.

The purpose of the study was to obtain independent collaboration of the extraordinary results obtained in the first nephropathy study. This pivotal study did not include the original CAT sites.

e. CAT 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 CAT. 74 IDDM patients were randomized to a treatment (CAT) 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 CAT, 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 CAT (Aoki et al., Am J Med (1995) 99: 683-684.

The correlation between blood pressure, the reestablishment of the circadian rhythm and nephropathy provide special insight into the very broad remedial effects of CAT. And, when the outcomes are coupled with unpublished anecdotal data regarding cardiac myopathy, endothelial cells may well have an important role in the reestablishment of cardiovascular and kidney health.

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 CAT's effectiveness in stabilizing renal disease (see above), many related questions naturally followed:

- How do these findings with CAT relate to other diabetic complications?

- How should this treatment be reproduced in multiple clinical settings as a powerful tool for the treatment of patients with severe diabetic complications?

5. Medicare & Studies: For a short while, Medicare was able to pay for continued studies to demonstrate the broad application of CAT to many of the other diabetes complications. For reasons unrelated to CAT Medicare funding has been curtailed.

a. Entry Criteria for CAT: Based on the above clinical studies work and independent clinical observations, the following criteria have been developed for patients to qualify for Cellular Activation Therapy where Medicare payment is to be sought. However, patients who wish to avoid the progression of complications can be treated, the only disadvantage being that Medicare and Insurance will not likely pay until overt secondary complications begin. One or more of the following criteria must be met for Medicare or insurance payment:

- 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 with resulting loss of consciousness. This is a major and unique indication for CAT.

- 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 ulcer (no or minimal evidence of healing over 4 months) in the absence of surgically remediable LE ischemia, gangrene and/or osteomyelitis.

- Patient has progressive retinopathy.

b. Clinical Effects of Cellular Activation 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.

Treatment Protocol: All patients are treated with the standard metabolic improvement protocol for CAT. Patients receive cognitive testing before beginning CAT 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 can be compared with their prior condition, as well as an age, sex, and disease matched group. 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 CAT (see Criteria for CAT above). Patients undergo CAT as noted in previous protocols weekly over a period of 6-12 months with renewals for successive 6-month periods. Depending upon the patient's individual ability to maintain normalize carbohydrate metabolism for longer than one week, patients may be extended to treatment once every two weeks. All testing is recorded for potential off-line analysis by an independent researcher blind to the patient's grouping.

Endpoints: Endpoints include improved glucose uptake of the brains as shown by PET scan, and measurements of changes in short and long term memory abilities as indicated by the cognitive tests.

References:

Stewart R, Liolitsa D: Type 2 diabetes mellitus, cognitive impairment and dementia. Diabetic Medicine 16: 93-112 (1999). PMID 10229302.

Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan V, Cummings SR: Is diabetes associated with cognitive impairment and cognitive decline among older women? Arch Intern Med 160: 174-180 (2000). PMID 10647755.

Steen E, Terry BM, Rivera EJ, Cannon JL, Nelly TR, Tavares R, Xu XJ, Wands JR, dela Monte SM. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? J. Alzheimers Dis 7: 63-80 (2005). PMID 15750215.

Watson GS, Craft S. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. Eur. J Pharmacol 490: 97-113 (2004). PMID 15094077.

c. Cellular Activation 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 Cellular Activation

Therapy (CAT) 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. The effect of CAT on theses patients is significant. Each patient should experience a cessation of the normal progression of nephropathy. In patients with more than 30% of their kidney function remaining, patients should see an improvement in creatinine clearance.

Treatment Protocol: Treatment criteria for patients seeking reimbursement 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). Patients will undergo CAT weekly for 12 months with self-renewing 12 month periods. Laboratory studies to be done before CAT will include CBC, chemistry profile, HbA1c, TSH, lipid profile, C- peptide, creatinine clearance and urine protein excretion studies. Labs to be assessed every 6 months are fructosamine, aldosterone, brain natiuretic protein, TGF-beta, endothelin 1, fibrinogen, creatinine clearance and urine protein excretion. The patient will be evaluated every 6 months. It is not suggested that patients with significant nephropathy be allowed to extend treatment duration to once every two weeks in absence of extremely long maintenance of metabolic normality as demonstrated by both lab values and metabolic measurement.

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

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

References:

Daily GE, Boden GH, Creech RH, Johnson DG, Gleason RE, Kennedy FP, Weinrauch LA, Weir M, D'Lia JA, Effects of Pulsatile Intravenous Insulin Therapy on the Progression of Diabetic Nephropathy, Metabolism 49:1491-95, 2000.

American diabetes Association, Position Statement: Nephropathy, Diabetes Care, 26:S94-98 as amended.

d. Cellular Activation 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. The effect of CAT on cardiac function in diabetic patients with significant cardiac disease should result in complete recovery.

Treatment Protocol: Individuals who have diabetes with impaired cardiac function including congestive heart failure and cardiac myopathy. For reimbursed treatment 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 (20-50% of predicted normal).

- Diffuse cardiomyopathy.

- Exclusion criteria include right sided heart failure, valvular heart disease, or significant pulmonary disease.

Patients selected will undergo CAT 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. Once the patient has stabilized for a period of three months and has regained cardiac health, the duration between treatments can be extended to once every two weeks provided the patient continues to maintain carbohydrate metabolism as shown by the standard treatment protocols.

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

Blood: two redtops.

References:

Parley D and Pepin EC, Ischemic Heart Disease: Metabolic Approaches to Management, Clin Cardiology 27:439-41, 2004.

Hutter JD, Piper HM, Spieckerman PG, Effects of Fatty Acid Oxidation on Efficiency of Energy Production in Rat Heart, Am J Physio 24: H723-28, 1985.

Esposito K, Giugliano D, Nappo F, Marfellen R Regression of Carotid Atherosclerosis By Control of Postprandial Hyperglycemia in type 2 Diabetes, Circulation 110:214-19, 2004.

Shah A, Shannon R, Insulin Resistance in Dilated Cardiomyopathy, Rev Cardiovasc Med 4 (supp 6) S50-57, 2003.

e. Cellular Activation Therapy (CAT) on Diabetic Neuropathy and Gastroparesis.

Introduction: Diabetic neuropathy (DN) is a progressive complication causing serious problems in 25%-40% of patients with diabetes. 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 and one study at the University of Puerto Rico shows that patients with DN can expect increased nerve conduction velocities and a reversal of DN to a point in time approximately 5 years prior to the starting of CAT. The University of Puerto Rico study is pending publication.

Treatment Protocol: Patients to be treated may have either type 1 or 2 diabetes on oral agents or insulin and neuropathy primarily related to diabetes, preferably age 21-80, treatment will be from 6-12 months in duration with renewals of successive 6 month periods. The labs are CBC, chemistry profile, TSH, HbA1c, urine protein and other blood markers in particular to that patient will be drawn baseline and every 3 months thereafter. Sensation testes and questionnaires on the neuropathy will be completed every 3 months as well.

Endpoints: modified Michigan neuropathy questionnaire or Norfolk Quality of Life Neuropathy questionnaire, sensation tests, physician notations of walking changes and any available PET scan reports..

Blood: 2 red tops.

References:

Tesfaye S, Chaturvedi N, Eaton SEM, Ward JD, Manes C, Ionescu-Tirgoviste C, witte DR, Fuller JH, Vascular Risk factors and Diabetic Neuropathy N Engl J Med 352:341-50, 2005.

Neuropathy Trust, Diabetic Neuropathy: Prevalence, www.neurocentre.com.

Potter PJ, Maryniak O, Yamorski R, Jones IC, Incidence of Peripheral Neuropathy in the Contralateral Limb of Persons with Unilateral Amputation due to Diabetes, Journal of Rehabilitation Research and Development 35:335-39, 1998.

Goldstein DJ, Lu Y, Detke MJ, Lee TC, Iyengan , Duloxetine versus Placebo in Patients with Painful Diabetic Neuropathy, Pain 116:109-18, 2005.

Martinez, Jose Hernan, U. Puerto Rico, in submission, Neuropathy Improvement with CAT, 2010.

f. Diabetic Impact Management Scale (DIMS) in Patients with Diabetes Mellitus Treated with Cellular Activation 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). The QOL in diabetes has uniformly significantly improved after CAT 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 [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].

Treatment: This is a quantification of the quality of life in diabetic patients undergoing CAT. Entry criteria include diabetic patients, aged 21 – 80. These patients must meet one or more criteria for CAT (see Criteria for CAT above). Patients in the treatment group receive CAT weekly as per physician's orders for the appropriate complication requiring therapy. CAT 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. Once a patient is stabilized and has achieved significant improvement in the QOL, the patient may be considered for extending the duration of time between treatments to two weeks provided a carbohydrate metabolism remains within normal ranges.

The DIMS is a paper-and-pencil questionnaire. Patients complete the DIMS questionnaire before starting CAT and at three month intervals thereafter. Changes in subjective QOL as indexed by the DIMS score are compared with all prior QOL scores of the patient.

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

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

References:

Hammond GS, Aoki TT: Measurement of health status in diabetic patients: diabetes impact measurement scales. Diabetes Care 15:469-77, 1992 PMID 1499460.

Watkins K, Connell CM: Measurement of health-related QOL in diabetes mellitus. Pharmacoeconomics 22: 1109-1126, (2004). PMID 15612830.

Winchester J, Tuller B, and Zeller S, Valk T: Effects of Pulsatile Insulin therapy on quality of life in diabetes, awaiting publication.

g. Effects of Cellular Activation Therapy on Progressive Retinopathy with Diabetes Mellitus.

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 CAT 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 and body wide metabolism.

Treatment: The patient is treated to determine the effect of CAT in the role of halting and reversing diabetic retinopathy. The patients will be from 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 should consist of mydriatic fundus photography in diabetic patients not

having had recent ophthalmologic evaluation (period greater than 3 months). The fundus photographs will be read by an observer. Two classifications of patients will be maintained:

- 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 should be treated. All patients will be evaluated in conjunction with an ophthalmologist and have thorough ophthalmologic evaluation prior to entrance. This evaluation will include clinical examination, fluoroscein angiography, and optical coherence tomography. Patients will undergo weekly CAT sessions as per protocol above. 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.

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. Changes in lab values, including VEGF, C peptide, Endothelin-I, and hsCRP will be analyzed.

Blood: 2 red tops.

References:

Frank, R.N. Diabetic retinopathy. NEJM 350 (1) 48-58 (2004).

Caldwell, RB et al. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Diabetes Metab Res Rev. 19(6): 442-55 (2003).

Singleton, JR et al. Microvascular complications of impaired glucose tolerance. Diabetes 52(12): 2867-73 (2003).

Kumar, MA et al. The role of lipids in the development of diabetic microvascular complications: implications for therapy. Am J cardiovasc Drugs 3(5): 325-338 (2003).

Sarraf, JA and Fong, D. Preventing diabetic retinopathy through control of systemic factors. Curr Opin Ophthalmol 14(6) 389-94 (2003).

Fong DS, Aeillo L, Gardner N, King GL, Blankenship G, Cavellerado J, Ferris F Klein R, Diabetic Retinopathy:Position Statement, Diabetes Care, 26:S99-102, 2003.

h. Cellular Activation 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). Treatment is appropriate for patients who have non-healing wounds which have been resistant to known therapies. Each individual will be compared to his previous treatment to evaluate the progression of CAT in healing 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 CAT course. Initial laboratory studies include CBC, chemistry profile, TSH, HbA1c and urine protein studies as well as any vascular evaluation examinations performed previously and records of the previous treatments. CAT is administered weekly and photographs of the wounds are taken at each CAT session.

Endpoint: wound status completely healed with no residual infection noted.

References:

Smith CL, Pharmocotherapy of Diabetic Foot Ulcers, Journal of Pharmacy Practice 17:66-74, 2004.

i. Biochemistry of Cellular Activation Therapy - Effect of CAT on Circulating Risk Markers of Vascular and Metabolic Complications.

Introduction: Insulin produces vasodilatory, anti inflammatory and anti thrombotic effects (1-4). . However the effects of CAT on circulating risk factors for vascular and metabolic disease is somewhat unknown. Patients who seek to achieve better circulation and lowered risk markers for vascular disease should be treated with the standard CAT protocol:

Treatment: Patients selected have diabetes mellitus, 21 years of age and older, and are treated with oral agents and/or insulin. Treatment should be for a minimum of 6 months and continue 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).

Endpoint: Changes in markers.

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

References:

Katakam PVG, Tulbert CD, Snipes JA, Erdos B, Miller AW, Busija DW, Impaired Insulin-induced Vasodilation in Small Coronary Arteries of Zucker Obese rats is Mediated by Reactive Oxygen Species, AJP-Heart 288:854-60, 2005.

Chakraborty K Sinha AK, The Role of Insulin as an Antithrombotic Humoral Factor, BioEssays 26:91-98, 2003.

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.

Patiag D, Qu X, Gray S, Idris I, Wilkes M, Seale JP, Donnely R, Possible Interactions between Angiotensin II and Insulin:Effects on Glucose and Lipid Metabolism in vivi and in vitro, Journal of Endocrinology 167: 525-31, 2000.

j. Nitric Oxide Levels in Diabetic Patients Undergoing Cellular Activation Therapy.

Introduction: Preliminary studies on 15 patients treated with CAT 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). Patients may be treated and measure the chronic effects of CAT on nitric oxide formation over 6 months as well as short term effects measured in the hours of a single CAT session.

Treatment: 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.

Endpoint: change in nitric oxide level.

Blood: 1 red, blue or purple top tube.

Bibliography:

Hanke CJ, Drewett JG, Myers CR, Campbell WB, Nitric Oxide Inhibits Aldosterone Synthesis by a Guanylyl Cyclase-independent Effect, Endocrinology 139: 4053-60, 1998.

Mather KJ,Ltief A, Steinberg HO, Baron AD, Interactions between Endothelin and Nitric Oxide in Regulation of Vascular Tone in Obesity and Diabetes, Diabetes 53: 2060-66, 2004.

Ellis G, Adatia I, Yazdanpanah M, Makela SK, Nitrate and Nitrite Analysis: A Clinical Biochemistry Perspective, Clinical Biochemistry 31: 195-220, 1998.

K. Correlation of Enhanced Memory Function in CAT Treated Diabetic Patients with Functional Magnetic Resonance Imaging.

Introduction: Based on an earlier controlled study demonstrating that CAT enhances short term memory function (also called working memory) in patients with diabetes for patients where the physician deems a benefit, an MRI may be warranted. 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]. Patients may wish to examine active brain areas in association with CAT as working memory processes in an attempt to relate the enhanced memory abilities observed in earlier cognitive studies with specific changes in neural activity.

Treatment: 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 will undergo weekly CAT sessions according to protocol.

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” 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 CAT are correlated with activity patterns observed with functional MRI, and the changes in fMRI that occur with changes in memory load.

References:

Baddeley AD: Working memory. Science 225, 556-559 (1992).

Smith EE, Jonides J: Neuroimaging analyses of human working memory. Proc. Natl. Acad. Sci. USA 95, 12061-12068 (1998).

Smith EE, Jonides J, Marshuetz C, Koeppe RA: Components of verbal working memory: Evidence from neuroimaging. Proc. Natl. Acad. Sci. USA 95: 876-882 (1998).

Cohen JD, Forman SD, Braver TS, Casey BJ, Servan-Schreiber D, Noll DC: Treatment of prefrontal cortex in a non-spatial working memory task with functional MRI. Hum. Brain Mapping 1, 293-304 (1994).

L. Correlation of Neuropathy Improvement in Diabetic Patients undergoing CAT with Quantitative Neurologic Measurements.

Introduction: Initial studies showed a 51% improvement in pain relief and a 42% improvement in sensation. The physician may wish to track the effect of CAT by adding quantitative and qualitative testing of peripheral nerve function. The physician can evaluate the effect of CAT on this form of neuropathy, a risk factor for sudden death (1, 2).

Treatment: Patients with diabetic neuropathy, ages 21 or older, who are on oral agents and/or insulin are eligible for treatment. 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 3-4 months.

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

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Koury CB, ADA Releases Diabetic Neuropathies Statement, Diabetic Microvascular Complications Today, 2:18-20, 2005.

Vinik AI, Emley MS, Megerian JT, Gozani SN, Median and Ulnar Nerve Conduction Measurements

with the Symptoms of Diabetic Peripheral Neuropathy Using the NC-Stat System, Diabetes Technology and Therapeuticss, 6; 816-24, 2004.

Zinman LH, Bril V, Perkins BA, Cooling Detection Thresholds in the Assessment of Diabetic Sensory Polyneuropathy, Diabetes Care 27:1674-79, 2004.

Curtis BM, O Keefe JH, Autonomic Tone as a Cardiovascular Risk Factor: The Dangers of Chronic Fight or Flight, Mayo Clin Proc 77: 45-54, 2002.

n. CAT for "Brittle" Diabetes and Glucose Control.

Introduction: Diabetes can produce wide swings in blood glucoses 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 CAT on improving diabetic control in these circumstances.

Treatment: Diabetics over age 21 referred for erratic control and elevated hemoglobin A1c despite optimal therapy are placed on CAT weekly. Weekly glucose diaries and glucose monitor recorded glucoses are reviewed after a 30 day baseline before treatment to determine the extent to which CAT 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 as improvement is noted.

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

References:

Quinones MJ, Nicholas SB, Lyon CJ, Insulin Resistance and The Endothelium, Current Diabetes Reports 5:246-53,2005.

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

Bibliography:

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Polonsky KS, Givens BD, Canter EV: Twenty-four-hour profiles and patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988; 81:442-448.

Vinik AI, Holland MT, Le Beau JM, Luissi FJ: Diabetic Neuropathies. Diabetes Care 1192; 15:1926-1975.

Weinhouse S Regulation of glucokinase in liver. In: Horecker B.L., Stadtman E.R. (eds). Current topics in cell research. New York: Academic Press 1976, 1-5.

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 CAT 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.

Journal: Effect of intensive insulin therapy on progression of overt nephropathy in patients with Type I diabetes mellitus. Aoki TT, Grecu EO, Gollapudi GM, Barber RA, Arcangeli MA, Benbarka MM, Prescott P, Meisenheimer R. **Endocrine Practice 1999; 5: 174-8.**

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.

Journal: Weekly pulsatile IV insulin treatments appear to slow progression of diabetic nephropathy. Dailey G, Boden G, Creech R, D'Elia J, Johnson D, Kennedy F, Weir M. **Diabetes 1995; 44: 24A(abstract).**

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.

Journal: Long-term intermittent intravenous insulin therapy and Type I diabetes mellitus. Aoki TT, Benbarka MM, Okimura MC, Arcangeli MA, Walter RM Jr., Wilson LD, Truong MP, Barber AR, Kumagai. **Lancet 1993; 342: 515-8.**

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.

Journal: Effect of chronic intermittent intravenous insulin therapy on anti-hypertensive medication requirements in IDDM subjects with hypertension and nephropathy. Aoki TT, Grecu EO, Prendergast JJ, Arcangeli MA, Meisenheimer R. **Diabetes Care 1995; 18: 1260-5.**

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.

Journal: Effect of intensive insulin therapy on abnormal circadian blood pressure pattern in patients with Type 1 diabetes mellitus. Aoki, TT, Grecu EO, Arcangeli MA, Meisenheimer R. **The Online Journal of Current Clinical Trials, 1995; Dec 13: Doc. No.199.**

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.

Journal: Chronic intermittent intravenous insulin therapy corrects orthostatic hypotension of diabetes. Aoki TT, Grecu EO, Arcangeli MA. **Amer. J. Med. 1995; 99: 683-4.**

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.

Journal: The effect of chronic intermittent intravenous insulin therapy of pregnancy outcome in insulin dependent diabetes mellitus. Field N, Boe N, Gilbert W, Benbarka M, Aoki T. **Journal Soc. Gynecol. Invest. 1997; 4(1, supplement): 196A.**

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.

Journal: Hammond GS, Aoki TT. **Diabetes Care 1992; 15: 469-477.**

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

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

Journal: J Invest Med (in Press).

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.

11. Physiology – biochemistry: Restoration of glucose homeostasis in insulin-dependent diabetic subjects. An inducible process.

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.

Journal: Diabetes 1985; 34: 960-63.

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.

Journal: J Invest Med 1999; 47(2): 94A (abstract).

14. Osteoporosis: Measuring bone density in men. Does the site matter?

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

Journal: J Invest Med 1999; 47(2): 98A (abstract).

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 Cellular Activation Therapy

1. Diabetes 2002 Feb; 51 Suppl 1:S255-S257.

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 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].

2. J Clin Endocrinol Metab 2002 Jan;87(1):213-21.

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 perifused. 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 perifused 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].

3. Diabetes 2001 Sep;50(9):2001-12.

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

Kjems LL, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefebvre P, Butler PC.

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].

4. J Clin Endocrinol Metab 2000 Dec; 85(12):4491-9.

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.

PMID: 11134098 [PubMed - indexed for MEDLINE].

5. Am J Physiol Endocrinol Metab 2000 Sep;279(3):E520-8.

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.

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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].

6. Am J Physiol 1997 Nov;273(5 Pt 1):E908-14.

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

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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.

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7. Am J Physiol 1997 Mar;272(3 Pt 1):E352-8.

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

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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.

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8. Diabetes 1996 Oct;45(10):1317-23.

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

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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.

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9. Am J Physiol 1996 Jun;270(6 Pt 1):E1043-9.

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

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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.

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10. Am J Physiol 1995 Dec;269(6 Pt 1):E1106-14.

Impact of sampling technique on appraisal of pulsatile insulin secretion by deconvolution and cluster analysis.

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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 a 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.

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11. Metabolism 1994 Jul;43(7):842-6.

Augmented effect of short-term pulsatile versus continuous insulin delivery on lipid metabolism but similar effect on whole-body glucose metabolism in obese subjects.

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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.