The Artificial Pancreas System: A Medical Review of the Literature and Unpublished Data

Diabetes Mellitus and the Role of the Artificial Pancreas System

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Dr. Kevin Buckman, MD
INTRODUCTION

This is a review of published studies and unpublished data about the role of pulsing insulin and Metabolic Correction for diabetics with the Artificial Pancreas System.

This is also a review of many of the 100,000 treatments that have been given in the last 16 years. The Artificial Pancreas System consists of metabolic monitors, Pulsing Intravenous Insulin Dosing, and a Metabolic Protocol for those with Diabetes. A special pump is used to administer insulin in pulses to mimic that of normal pancreas activity. The therapy is given once a week, usually in the setting of a doctor’s office, clinic or Artificial Pancreas System center.

The Artificial Pancreas System is also known as Activation Therapy or Cellular Activation Therapy for its benefits for proper cellular activity and function.

The various studies indicate that non-diabetic, normal people respond to a cascade of metabolic enzymes and pathways when food is ingested. In a normal person this pulsing insulin triggers the liver to release and regulate a cascade of hormones and enzymes that are needed for proper metabolism. This does not occur in those with diabetes.

Our current use of subcutaneous administration of insulin is not physiological; that is, it is not being pulsed from the pancreas, and therefore proper metabolism cannot be achieved. Studies indicate that insulin needs to be pulsed in a very specific manner for the dose, frequency and protocol to simulate a physiological pathway that imitates what happens in healthy non-diabetics. Although insulin is frequently given IV in the hospital by pumps, this is not the same as the Artificial Pancreas System simply because it is not pulsing, nor is there monitoring and regulating dosing by the Artificial Pancreas System Metabolic Chart.

CLINICAL STUDIES

In a recent publication in Diabetes Metabolism Research and Review, Peter Bergsten, Department of Medical Cell Biology, University of Uppsala, Sweden, found that normally plasma insulin displays 5 to 10 minute oscillations.

In Type 2 diabetes the regularity of these pulsing oscillations disappears. This can cause insulin receptor down regulation and glucose intolerance (blood sugars and metabolism are out of control). Like others doing this research, he states that this is “why pulsatile delivery of the hormone (insulin) has a greater hypoglycemic effect than continuous delivery.”

There is much more to diabetes that chasing blood sugar levels. There are billions of chemical reactions every day and countless metabolic pathways that involve every organ in the body.

Studies go back over 20 years showing the same information. This includes studies by Matthews et al published in Diabetes in 1983 on the role of pulsatile insulin for improved glucose control. Other authors (Paolisso et al) published studies in the 1980’s about benefits of pulsatile insulin in even non-insulin-dependent diabetics.
Why does pulsing make a difference? We know that oscillations in the cytoplasmic Ca+ (calcium) concentration play a role in the synchronization of the secretory activities of the beta cells of the pancreas. When these have been measured in non-diabetics, there is perfect synchronization of the oscillatory Ca+ patterns. These are ions with biochemical-electrical activity. There are gap junctions between the beta cells of the pancreas which allow the passage of the ions and small molecules (Westerlund et al J Clin Invest 1996: 97: 1860 1863). ATP and cyclic AMP play a role as do potassium controls that are regulated by glucose levels. These are some of the ionic-electric pathways where pulsing (oscillations) of insulin help to adjust the secretory activities of beta cells in the islet of the pancreas, which also affects hepatocyte activity (liver function). Receptor expression was significantly higher in liver cells exposed to oscillatory insulin concentration (Goodner et al Diabetes 1988; 37 1316-1323).

In diabetics these normal patterns and pathways do not happen, including the coordination of the release, control, and use of insulin and related metabolic pathways. There are many metabolic pathways and enzymes that are linked to this whole process, of which pulsatile synchronization is the key.

Interesting enough is how the liver and pancreas work together; intrahepatic transplanted healthy islet cells in humans secrete insulin in a coordinated pulsatile manner directly into the liver (Meier et al Diabetes, August 2006). Just as there is electrical pulsing from the sinus node of the heart to coordinate a series of events to fill the heart, empty one chamber, then another, and the synchronous contractions of the 4 different chambers of the heart, the cells of the pancreas operate in a similar manner.

According to a popular hypothesis, intrapancreatic ganglia serve as a pacemaker to generate these oscillations. Normally insulin is pulsed in response to oral glucose load. Porksens et al have found that pulsatile insulin secretion accounts for 70 percent of total insulin secretion during fasting (Porksens et al American J. of Physiology, 269; E478-E488).

Insulin in the basal state and after stimulation is secreted in a pulsatile manner in normal subjects; that response does not occur properly in subjects with diabetes. Activation therapy provides pulsatile insulin release into the vein of a patient, with a proprietary pump, in order to assist those with Type 2 diabetes with an altered plasma insulin pattern. Studies show that tight control of glucose alone is not enough to prevent insulin resistance and complications of diabetes in general. No oral agents or use of insulin can imitate the pulsing benefits of the Artificial Pancreas System.

New studies show the importance of proper normal hepatic metabolism by use of the Artificial Pancreas System, which has also been called Hepatic Activation Therapy. More than eight critically needed enzymes are released from the liver in normal diabetes and those with diabetes who are “activated” with the Artificial Pancreas System. These enzymes are needed for control of metabolism that is more involved than just a blood glucose level.

Experimental clinical islet transplantation may accomplish insulin independence just for short periods of time with the problems associated with transplantation and immunosuppression.
Many complications of diabetes remain hidden for many years, also known as the silent killers. The best examples of these are heart disease, renal disease, and arteriosclerosis leading to strokes and sudden death syndromes. All diabetics, no matter how healthy they may seem, are developing some level of life limiting complications. Some develop the complications more quickly than others. Poor metabolism attacks the diabetic at his weakest points. There are two different types of diabetic people. Type 1, "juvenile onset" diabetics (which also occurs in adults) fail to produce any or much insulin. Type 2, “adult onset” diabetics do produce insulin but are slow to release insulin and are often resistant to insulin. Both types of diabetes are not just conditions of insufficient insulin or insulin resistance; they are diseases of improper body metabolism. That is the technical definition of diabetes: a “disease of metabolism”.

The Artificial Pancreas System Treats Both Type 1 and Type 2 Diabetic Conditions. Even though the two types of diabetes have very different causes, both types of diabetics suffer from the same complications due to the common failure to properly process food fuels (metabolize). The Artificial Pancreas System treats both Type 1 and Type 2 diabetics equally well by addressing the core problem at the cellular level and by making each cell more normal in its carbohydrate and lipid metabolism. Approximately 90% of diabetic people have Type 2 (adult onset) diabetes and 10% have Type 1 diabetes.

Insulin therapy was introduced in 1923. Other than Pulse Insulin Therapy, there have been few significant changes in treatment since 1923. Before the Artificial Pancreas System, physicians could only try to control blood sugar levels; with the Artificial Pancreas System they can correct the metabolic dysfunction, body wide. Insulin therapy alone technically saves half of all diabetic patients from early death, only to have them experience the fearful complications of diabetes: blindness, renal failure, accelerated coronary disease, cerebral vascular disease, disabling neuropathy and severe peripheral vascular disease which occasionally leads to the amputation of extremities. Thus, insulin is needed; but so is the Artificial Pancreas System to halt the complications of diabetes.

Swings in blood glucose for the non-activated patient affect the diabetic much the same way as a "normal" person gets mean and/or hungry, or when full, tired and sort of sick feeling. With wide swings these sensations are felt by a diabetic person, only more drastically and there is never rest from the struggle of trying to balance sugars with insulin. The liver and body of a normal person perform this balancing function automatically when they metabolize normally.

In other acute diseases, physicians take over many decisions regarding treatment and prescribe most actions, thereby taking over some of the responsibility of care. The diabetic patient must self-diagnose and treat every minute of every day.

Review of the Literature on Carbohydrate and Lipid Metabolism. Because diabetes is a disease of improper metabolism, there are whole body widespread severe basic biochemical abnormalities. The fundamental defect is the reduced ability of glucose to be used as the fuel for body tissues and a corresponding increase in the release of glucose from the liver into general circulation. Diabetic people fail to metabolize (burn) the carbohydrates (sugars or glucose) and instead metabolize lipids (fats) at a much higher rate than a normal person. Thus, diabetics have been referred to as "butter burners." Unlike the control theory of conventional treatments where
the patient is starved of usual carbohydrates, the Artificial Pancreas System allows the patient to metabolize carbohydrates as do non-diabetic people. This in turn prevents the accumulation of harmful metabolites such as free fatty acids, ketones and other acids. The reduction of these substances has shown a decrease in the complications of diabetes.

An Artificial Pancreas System study was done to determine the wound healing effects of the Artificial Pancreas System on the stated ethnicities of patients who have chronic non-healing ulcers of six weeks duration with wounds having borders no smaller than one centimeter by one centimeter in size, and no larger than six centimeter by six centimeter in size, with progressive breakdown of tissue, notwithstanding conventional therapy by others for their wounds. Patients with non-healing ulcers may be associated with peripheral neuropathy, trauma due to non-recognition of inadvertent injury, impaired vascularization and simple/complex trauma both with and without infection.

The study objective is to a) examine, b) quantify and c) record the wound healing effects of the Artificial Pancreas System, reporting the results of the study in a means able to be compared with the natural course of healing.

The Artificial Pancreas System is Additive to Existing Therapies. The Artificial Pancreas System therapy is additive and only augments existing therapies replacing the patient’s insulin only on the day of treatment. Accordingly, the Artificial Pancreas System is designed not to change any existing treatments or medicines used for either the care of diabetes or other medical conditions. The patients will not be given care other than the Artificial Pancreas System and the results of their tests for use by their primary care physicians and specialists. The patients will be followed as to the care given by the primary provider and specialists, and any questions or observations about that care will be provided by the Investigator to the primary care provider(s).

Wound Care. People with diabetes who have non-healing ulcers (defined as ulcers which have positive breakdown of tissue after six weeks or longer of conventional care by others, and with the size of the wound size a minimum of 1cm by 1cm, and no larger than 6cm by 6cm) have metabolic deficiencies such that their wound healing ability is significantly impaired, reducing their chances of healing by over 50%. Diabetes is a disease of improper metabolism, caused by enzymatic deficiencies resulting in body-wide complications including wound healing impairment. By addressing the core problem of the disease, metabolism, the resulting manifestations of diabetes may be slowed, stopped and in some ways reversed. People who are so impaired by diabetes that their skin will not recover from wounds and ulcers have been anecdotally seen to have resolved old and progressive wounds while under the Artificial Pancreas System therapy. By providing more proper metabolism, hyperglycemia is reduced and the natural healing process is more likely to result in healing. The Artificial Pancreas System addresses the basic metabolic insufficiency of diabetes.

Measurement of Resting Metabolism. In order to adjust the amounts of insulin and glucose to stimulate improved body-wide resting metabolism, a means for accurate measurement of metabolism is required. Metabolism is accurately measured and quantified by indirect calorimetric measurement through the determination of a Respiratory Quotient (RQ).
Respiratory Quotient (RQ) Measurement. All animal and human cells obtain energy in the form of ATP by oxidizing food molecules through the process of respiration. The hydrolysis of ATP supplies energy needed for cellular processes, such as the transport of molecules or cellular movement. Carbohydrates and fatty acids are the most important fuels for generating ATP in human cells. Respiration in human cells is dependent upon available oxygen. Electrons from the chemical bonds of the fuel source combine with oxygen and hydrogen ions to form water and carbon dioxide. Cells couple this reaction to the production of ATP. Much knowledge is gained about body-wide metabolism by comparing the volume of oxygen consumed by an organism to the volume of carbon dioxide produced. These volumes will change depending on the energy source the patient is using. The method of indirect metabolic measurement by the determination of this ratio has been proven accurate by comparison of direct calorimetric measurement, (directly measuring energy from each of lipid, protein and carbohydrate), and correlating those direct measurements to the indirect calorimetric measurement of the ratio of oxygen consumed to carbon dioxide produced.

Variables for Measurement. The following are the known variables for determination of body-wide resting metabolism ratios and thus the determination and recordation of resting carbohydrate and lipid metabolism:

<table>
<thead>
<tr>
<th>RQ</th>
<th>Respiratory quotient</th>
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<tr>
<td>$V_c$</td>
<td>volume of carbon dioxide released</td>
</tr>
<tr>
<td>$V_o$</td>
<td>volume of oxygen consumed</td>
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The most useful method for understanding metabolism is the respiratory quotient which can be measured a) during exercise or b) while at rest. The respiratory quotient (“RQ”) measures the ratio of the volume of carbon dioxide ($V_c$) produced by a patient, to the volume of oxygen consumed ($V_o$) by that patient. This ratio is represented by the following equation:

$$RQ = \frac{V_c}{V_o}$$

Range of Possible RQ’s: The total possible range of RQ ratios is from .7 to 1.0.

An RQ of .7 to .8 represents lipid metabolism.
An RQ of .8 to .9 represents protein metabolism.
An RQ of .9 to 1.0 represents carbohydrate (glycolytic) metabolism.

Therapeutic Changes to Effectuate Cellular Activation. The therapeutic approach of the Artificial Pancreas System is to normalize resting metabolism for patients with diabetes by giving pulses of insulin and concomitantly providing a glucose load with oral glucose (the two signals given to the liver in patients without diabetes.) Diabetic patients are well known in literature to suffer from carbohydrate metabolism deficiencies. As shown in the UKPDS study, the progression of diabetes is mostly degenerative, with little or no reversal. The Artificial
Pancreas System provides a mimicking of these two signals received by the liver of a non-diabetic person, to produce the required enzymes for proper metabolism. The livers of diabetic patients do not receive these signals, and thus do not achieve normal resting carbohydrate metabolism. The average resting RQ for a diabetic patient with a glucose rich meal is below .8, and for a non-diabetic people, the RQ is above .9. The Artificial Pancreas System effectively shifts the resting RQ from below .8 (which represents mostly lipid metabolism) to over .9 representing normal resting carbohydrate metabolism.

**Physiological Results at Cellular Levels.** The result of increasing the resting metabolism (RQ) to a level of over .9 is to cause the mitochondria of the treated patient’s cells to accept and process glucose. At these .9+ RQ’s, cellular metabolism is shifted from diabetic like, to nearly normal, thereby addressing the very core problem of both Type 1 and Type 2 diabetes. Type 1 patients have little or no insulin production which is the genesis of improper metabolism, and the genesis of improper metabolism for Type 2 patients is an insulin secretion which is blunted, without first phase insulin release, coupled with insulin resistance. The change from impaired glucose processing to body-wide cellular “activation” of glucose processing should result, and has been seen to result in improved wound healing. Demonstrating and quantifying the improved wound healing is the purpose of this Study.

**Conventional Therapy.** Conventional treatment of diabetes attempts to control blood glucose by limiting glucose in food and injecting insulin in amounts tailored to anticipate meals, exercise, blood sugar, emotional levels and a host of other variables. Trying to keep the blood glucose levels within a relatively normal range is sometimes called "tight control". The best type of control is to use multiple injections or the “insulin pump.” The Artificial Pancreas System is not related in any way to the currently widely used “insulin pumps”, as these pumps replace shots to help control glucose, but cannot address the basic problem of metabolism. The Artificial Pancreas System is needed because even with extremely tight control, many diabetics will develop complications in just ten years; and while there is no study, eventually all diabetic people succumb to the disease.

**Tight Blood Glucose Control Does Not Stop the Complications.** Associated with “tight control”, that is, keeping blood sugars down, was the hope that this would prevent all of the many micro vascular, atherosclerotic and neuropathic complications of diabetes. However, while poor glucose control will result in the earlier onset of complications, tight control has not proven to avoid complications. Tight control often leads to blood sugars which are too low, which has been shown to adversely affect neurological processes and cause loss of consciousness.

**The DCCT study itself shows that in an average of 25 to 40 percent of all patients on tight control go on to develop complications within 10 years. (Obviously after that, the incidence increases).**

Most physicians do not know or state that these patients developed complications within just ten years. Thus, these patients began to suffer from the usual problems, no matter how well controlled.
There was a 40%+ failure rate for “tight control” by intensive therapy in trying to slow the progression of secondary complications even in young healthy patients free from secondary complications. Intensive therapy causes a threefold increase in hypoglycemia. “Tight control” causes weight gain. “Tight control” carries risks of hypertension. In patients with already “advancing complications,” there is no basis for concluding “tight control” was effective. Quality of clinical support given in DCCT cannot be practically reproduced in “real world” settings.

This is due to release of stress hormones and chemicals as a result of low glucose. These hormones and chemicals include epinephrine, cortisone, dopamine, norepinephrine and others. These cause the glucose to bounce back up and other chemicals that cause sudden heart attack, arrhythmias and death.

**Benefits of Treatment.** Attached is a list of the benefits of the treatment. Diabetic patients uniformly feel better, are able to live more normal, longer lives and continue to improve physically. In general, diabetics who are not on the Artificial Pancreas System treatment are metabolically impaired and their cells do not metabolize carbohydrates and lipids properly. They suffer from increased medical problems including heart disease (cardiovascular), nerve disease, eye disease, kidney disease and many other disease states.

**University Studies.** Studies at University of California, Harvard (Joslin Diabetes Center), Mayo Clinic, Scripps Clinic, University of Arizona, Temple University, University of Maryland, Donnellson (HCA Tennessee) and other sites have documented the treatment benefits. Independent investigators have published their peer reviewed articles documenting the treatment’s effects using several different names of their own, including CIIT (Chronic intermittent intravenous insulin therapy), PIVIT (Pulsatile intravenous insulin therapy), Hepatic Activation, Cellular Activation Therapy (CAT) and the Artificial Pancreas System depending upon the reviewer’s terminology. All of these terms are related solely to the company’s Artificial Pancreas System. (Copies of the peer reviewed articles and information are part of this business plan.)

The treatment works in a physiologically natural and normal way, stimulating the liver to do its normal job of providing a host of enzymes necessary for proper metabolism. This treatment merely gives the liver and other tissues what they need to respond in a non-diabetic way and as a result leads to wellness. It stimulates tissue throughout the body while delivering the treatment benefits to the whole person.

1. **All Type 1 and Type 2 Diabetic Patients of All Ages and Conditions Respond.** There are no known individuals who have not responded to the treatment and no known side effects resulting from the treatment. The treatment addresses basic metabolism by natural hormone stimulation, just as a normal person’s metabolism is stimulated, with pulses of insulin. With over 100,000 patient treatments, the treatment has never failed to reestablish more normal metabolism, and has never harmed any person.

The treatment has also been successfully used on diabetics who are undergoing
surgery or are seeking to become pregnant. Because the treatment is safe, it is even appropriate for expectant mothers and those trying to become pregnant. The youngest patient to ever be treated, a five year old girl, at age 23 has two healthy boys, and has not been hospitalized since her treatments began, 18 years ago (except for the birth of her children).

2. **No Drug or Treatment Incompatibilities.** Because the treatment normalizes metabolism, there is no drug or treatment, which should not be used in combination with the Artificial Pancreas System. Even patients with pancreas transplants, islet cell transplants and gene therapy, if it is ever available, will logically benefit from the treatment (as none of these therapies except gene therapy is designed to restore proper metabolism.)

3. **Factors Leading to the Discovery.** Work began with a realization that “starvation” is much like “diabetes.” Diabetic cells are starving in a sea of glucose because they cannot metabolize carbohydrates. It became clear that the high blood glucose levels of diabetes are primarily attributable to the loss of proper insulin and glucose signals to the liver (metabolic integrity of the liver). The liver is the organ into which the pancreas secretes insulin. All diabetics (Type 1 and Type 2) fail to stimulate their livers properly, which causes body-wide improper glucose and lipid use (metabolism).

Realizing that metabolic dysfunction is the core of the problem helped to develop a solution. The method of intravenous administration of insulin, coupled with carbohydrate loading, was developed to restore proper liver stimulation and thus body-wide metabolic functions. This proprietary treatment uses the Bionica pump with its special treatment software program (algorithm) and the oral administration of carbohydrates. Using this treatment administered by the Bionica Inc. device, reestablishes the patient’s ability to process food, and has been demonstrated to improve metabolism in every one of the diabetic patients treated to date.

4. **Provides Immediate Improvement.** The Artificial Pancreas System offers immediate improvement in disease management for diabetic people who are brittle or otherwise require acute care, are often hospitalized and do not respond to conventional insulin therapy. As a result of the treatment, the body begins to metabolize more glucose molecules and less fatty substance, shifting from an unhealthy means of staying alive, (fat use) to a proper metabolic profile as shown by conventional metabolic measurements.

In addition, there are a host of other enzymes which are depressed in the diabetic person and which the Artificial Pancreas System also stimulates. These enzymes are set forth in the “Artificial Pancreas System PowerPoint Presentation”. The results of the treatment have led physicians to realize that more than just carbohydrate and lipid metabolism is being beneficially affected. For example, the blood-brain barrier does not stop insulin, and indeed neurological aspects are beneficially affected by the treatment; so much so that part of the Company’s plan...
is to begin treating for non-diabetic conditions relating to heart, healing and neurological treatments for senility.

5. **Multiple Benefits.** Because the therapy works at the cellular level to restore metabolic integrity and proper energy production in cells body-wide, the treatment has been proven to arrest the fearful complications of diabetes. In certain types of complications the treatment can even cause reversal as evidence to this effect can be found in the ongoing research programs.

Another uniform benefit of the treatment is the greatly improved overall sense of well-being in patients. This sense of being normal and feeling well is of primary importance to diabetic people and their families. Most of these people have forgotten what it feels like to feel well, as they have slowly deteriorated in their energy and wellness. After treatment the results are profound on their energy and health states and they often state that they just did not realize how sickly they felt.

Another important benefit of the treatment is the reduction or elimination of diabetic loss of consciousness due to low blood sugar or hypoglycemic coma. Not only is low blood sugar a condition which can cause a diabetic to have traffic and other accidents, it is a condition which has been shown to cause brain impact. With a blood glucose of only 53 mg/dl (normal is 80 to 120), neurological symptoms appear. Conventionally treated diabetics regularly fall below this number. Hypoglycemic reactions are especially common to diabetics trying to achieve tight control of their blood sugars and of course are common to those diabetics who are brittle.

Another benefit of the treatment is that it dramatically helps to reduce hypertension (high blood pressure) in patients with this problem. Hypertension is directly linked to all forms of complications. In patients who have hypertension, the randomized cross-over studies have clearly demonstrated highly significant improvement in blood pressure, which is most encouraging to diabetics as high blood pressure is associated with heart disease and kidney loss, always resulting in early death.

**Diabetic Complications.** All of the long-term studies have shown, to a greater or lesser degree, that the Artificial Pancreas System has the ability to stop, limit or avoid complications of diabetes. In addition, depending on the status of the patient on entry, the reversal of complications can also be seen.

Because both Type 1 and Type 2 diabetes are diseases of faulty metabolism, the technology is designed for, and has been proven effective for all diabetic people, and indeed all diseases caused by diabetes. The treatment is effective for those on oral medications as well as those on insulin. There is no treatment which is known to be inconsistent with the Artificial Pancreas System’s normalization of metabolism.

**Long Development Cycles.** In chronic diseases it takes many years of studies to show that a treatment is effective and then continues to stay effective instead of losing effect or causing
harm. The Artificial Pancreas System has been in development for 18 years as to Type 1, and 13 years as to Type 2 diabetes. Because of this, the Artificial Pancreas System should have actually taken much longer than average to become available. However, the hard work and dedication to helping people has made this a uniquely important help to mankind.

**No Negative Physician Reports.** Not one single physician who worked with the Artificial Pancreas System has reported negative outcomes as to the treatment or has given the opinion that it does not work. They all have seen the “logic” of doing what a normal body does to stimulate proper metabolism.

**Acute Care Brittle Diabetics.** Type 1 diabetic patients who are "brittle" have greater difficulty in controlling their diabetes, such that they experience episodes of wide swings of blood glucose from lows of 30 to highs in excess of 600 mg/dl. (Normal is 80 to 140 mg/dl). Conventional therapy provides poor results, frequent hospitalization and improper metabolism for these diabetic patients. This initial market also includes pregnant women, certain preschool children, and a limited number of patients with secondary complications so that their improvement can be documented.

Major authorities and statisticians conservatively estimate that at least fifteen percent of all Type 1 diabetic patients are brittle. These brittle individuals are not amenable to the regimens that normally afford some blood glucose control to most diabetics. Conventional treatment control regimens may work for a while in these patients, but invariably and unpredictably, their blood sugar levels either soar or plummet, requiring emergency care or hospitalization. After some time in the hospital, the situation somewhat stabilizes, the patient is released and the cycle begins anew. The cycle begins again because their livers are dormant as to certain blood control functions, and their bodies cannot metabolize or properly process carbohydrates and lipids. They try to control glucose with tailored injections when the best solution is to awaken their livers to naturally control this process.

**Pregnant Diabetics.** From 4 to 15 per 1,000 pregnancies occur in diabetic women, the majority of whom are insulin dependent. (This does not include gestational diabetes or diabetes caused by pregnancy, the incidence of which is much more common). Pregnant insulin dependent women are urged to keep their blood sugar levels in a very low range of 65 to 85 mg/dl before eating and less than 120 mg/dl after eating. (Non-pregnant insulin dependent patients strive for 80 to 100 mg/dl before eating and less than 150 mg/dl after eating). This degree of tight control is extremely difficult to achieve and these women have a greatly increased incidence of three or more significant hypoglycemic reactions per day. The physical damage to these women as a result of frequent hypoglycemic reactions is unknown but reasoned to be significant. Brittle pregnant women cannot achieve any real measure of control.

There is evidence from Dr. Norbert Freinkel at Northwestern Medical School and from investigators in Japan and Europe that single episodes of hypoglycemia in pregnant guinea pigs result in significant central nervous system disorders. Thus the frequent episodes of hypoglycemia, currently considered "acceptable", and experienced by pregnant women and others striving for "tight control" are now associated with significant risk to the fetus. The concept that "going low" is a great risk to the nervous system has been the subject of many new
studies and papers, all of which suggest that hypoglycemia is a much more dangerous and damaging condition than previously thought.

In contrast, when on the Artificial Pancreas System treatment protocol there is a marked diminution in the frequency and severity of hypoglycemic reactions in diabetics and an ability to maintain a very acceptable blood glucose level, as well as more normal body-wide metabolism.

The youngest child to be treated with the Artificial Pancreas System (age 5) has at age 23 become a mother for the second time. Her pregnancies were without any hypoglycemic reactions as she continued on the Artificial Pancreas System and has healthy normal children. Her picture can be seen on www.diabetes.net.

**Diabetic Children.** Diabetes in young children is an extraordinarily difficult disease to manage. In order for the child to grow to full size and develop properly, tight control of blood sugar concentrations should be maintained. This usually means multiple injection regimens. Tight control carries with it the danger of hypoglycemic reactions and young children simply cannot recognize when they are slipping into hypoglycemia.

Although there are no statistics on incidence of diabetes in preschool age children, according to *Diabetes in America*, 1 in 600 school age children have diabetes and certain other estimates are as high as 1 in 300.

**Diabetic Patients with Common Complications (the most obvious).** As mentioned, diabetes brings on numerous secondary complications over time: blindness, kidney disease, heart, nerve damage and vascular disease and more. The organs of a diabetic person appear to be older than the chronological age of that person. Conventional insulin therapy does not stop the progression of these complications or relieve the symptoms. The Artificial Pancreas System treatment is designed to arrest the progression of secondary complications and to allow those tissues that are capable of healing to regenerate. This treatment will be shown to provide many different types of improvement for patients with existing complications where no help is currently available.

**Surgery and Acute Illness.** Patients who are to undergo surgery or have an acute episode of some common illness can benefit greatly from the treatment. Before surgery, diabetics are reviewed for their need of the treatment, because it has been found that treated patients do much better than non-treated diabetics. Healing is greatly improved and hospital stays shortened with the Artificial Pancreas System.

**Conventional Treatment.** Existing conventional insulin therapies are not viewed as competitors but collaborators. The Artificial Pancreas System works most effectively in conjunction with a tightly controlled insulin therapy regimen which is delivered by multiple injections, insulin pump, possibly in the future with inhaled insulin or any other new insulin delivery modalities. Potential partnerships or joint ventures could be possible with any or all of the above.

The unique and patented method of treatment with the Artificial Pancreas System and its benefits cannot be duplicated by other existing conventional treatment therapy.
Experimental Cell Transplant Therapies. Pancreas transplant and islet cell transplant have been in use for over 10 years with consistently poor results. Recently a Canadian group reported better results with a small number (7) of patients remaining “insulin free” for up to 15 months. This therapy does not apply to 90% of all diabetic people (Type 2) and has been criticized by leading physicians who point out that even without immune-suppressants (should that ever become possible) islet cells will not normalize metabolism. Although this represents an advancement in cell transplant therapy, two critical technicalities must be addressed before this could become a viable treatment for diabetes.

Immunosuppression. All patients undergoing transplant must receive powerful immunosuppressive drugs for the rest of their lives. This is not a simple or low risk therapy. The drugs themselves have toxic effects on heart, kidney and liver. Chronic long-term immunosuppression increases the development of certain cancers and makes the patient more susceptible to infection. The patients selected for the Canadian Study were deemed so ill with their diabetes that the serious surgical and drug risks were outweighed by possible benefits. Until the immunosuppression problem can be solved, this approach will not go beyond the experimental stage and that is not foreseen in the near future. It is assumed that there are at least ten years before such non-toxic immunosuppressive drugs will become available.

Availability of Islet Cells. Patients in the Canadian study had to receive the cells from at least two matched cadaver pancreases before being insulin free, and as many as four. Currently there are not enough donated hearts, lungs, kidneys, livers or pancreases to meet current demand. Unless a new source of islet cells can be found it will not be a practical alternative therapy.

Stem Cell Treatment. The idea of stem cell treatment is widely seen as a new frontier for all types of diseases. In the diabetic population, there is no evidence that stem cells will reestablish normal metabolism as opposed to replacing the production of insulin. For this and other technical reasons, stem cell therapy is not considered competition.

The following are abstracts of peer-reviewed journals relating to the Artificial Pancreas System. The full transcripts are available.

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 kidney transplantation would be required.

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 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.


Hypoglycemia – (loss of consciousness due to low blood sugar.)

A study of 20 diabetic patients over 42 months showed that the Artificial Pancreas System (aka CIIT) 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 the Artificial Pancreas System (CIIT) therapy was stopped.


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.


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 CIIT 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.


**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.


**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.


**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.


**Physiology - Biochemistry**

Acute insulin effects on plasma homocysteine levels in patients with diabetes mellitus. Aoki TT, Grecu EO, Medina M, Goodman M.


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.

Restoration of glucose homeostasis in insulin-dependent diabetic subjects. An inducible process. Foss MC, Vlachokosta FV, Cunningham LN, Aoki TT.

Osteoporosis


Papers Supporting Pulsatile Insulin Infusion.

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 hours 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 (similar 20%). 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]

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 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 for 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]

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,**
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]

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 trans jugular 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.

Publication Types:
- Clinical Trial

PMID: 11134098 [PubMed - indexed for MEDLINE]

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.

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.

Publication Types:
- Clinical Trial
- Controlled Clinical Trial

PMID: 10950818 [PubMed - indexed for MEDLINE]

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.

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]

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-I 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]

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.

Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC.

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

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 an 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 post absorptive (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 post absorptive 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 post absorptive 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.
Impact of sampling technique on appraisal of pulsatile insulin secretion by deconvolution and cluster analysis.

Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC.

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

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 an 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.

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

Schmitz O, Pedersen SB, Mengel A, Porksen N, Bak J, Moller N, Richelsen B, Alberti KG, Butler PC, Orskov H.

Department of Medicine M (Endocrinology and Diabetes), Aarhus Kommunehospital, Denmark.

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.

BIBLIOGRAPHY


3. Aoki, TT, Grecu, EO, Benbarka, M, Prescott, P, Jong Ho, A: Chronic Intermittent Intravenous Insulin Therapy, a New Frontier in Diabetes Therapy, Diabetes Technology & Therapeutics, Vol 3, no 1, 2001


15. Aoki, TT, Benbarka M,: Type 1 Diabetes, the “Sleeping Liver” Hypothesis and its Clinical Implications, Modern Medicine vol 60: 73-84, 1992

16. Aoki, TT, Grecu, EO, IGF-1 and IGFBP-1 blood levels in type 1 diabetes mellitus on intensive intravenous insulin therapy. *Journal of Invest Medicine*, 1999; 47(2) 78 A.


22. 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.


55. Juhl CB, Porksen N, Pincus SM, Hansen AP, Veldhuis JD, Schmitz O. Acute and Short-Term Administration of a Sulfonylurea (Gliclazide) Increases Pulsatile Insulin Secretion in Type 2 Diabetes *Diabetes* 50:1778–1784, 2001


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