THE EFFECTS OF PARTIAL PANCREATECTOMY AND ACUTE STAPHYLOCOCCAL ALPHA-TOXIN PANCREATITIS ON THE PLASMA GLUCOSE, INSULIN AND GLUCAGON DURING A H-IVGTT IN THE DOG

by

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D.V.M., University of Georgia, 1972

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

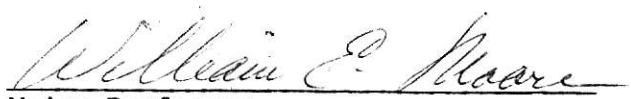
Department of Infectious Diseases

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1976

Approved by:



Major Professor

To My Parents and Wife

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ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. W. E. Moore for serving as my major professor and for his assistance in the preparation of this thesis. Special thanks are extended to Dr. N. V. Anderson for allowing me the opportunity to work in his major area of research under his guidance. I am grateful for the assistance of Dr. A. C. Strafuss in reviewing the histopathological tissue sections.

LITERATURE REVIEW

INSULIN

Von Mering and Minkowski, in 1889, showed that removal of the pancreas of dogs caused serious disturbances of glucose metabolism, with elevation of the blood glucose concentration and the clinical picture of diabetes mellitus. The assumption that this effect was due to removal of a necessary hormone was confirmed in 1921 when Banting, Best and MacLeod prepared a pancreatic extract capable of decreasing blood glucose concentration. This substance, insulin, has since been purified and its composition determined.[7]

Biosynthesis

Insulin is synthesized by the beta cells of the islets of Langerhans as a single chain precursor, proinsulin, which is later cleaved to insulin.[94] Biosynthesis of proinsulin occurs on the rough endoplasmic reticulum, is passed into the Golgi apparatus and transferred to storage granules formed by vesiculation from the Golgi periphery. Insulin is released from beta cells by extrusion (emiocytosis) of intact granules.[65] Transformation of proinsulin to insulin by proteolytic cleavage occurs either in the Golgi apparatus or during granule maturation.[45,68,76] In either case, insulin rather than proinsulin is the storage and secretory form of the hormone; however, small amounts of proinsulin do escape into the circulation. Proinsulin does cross-react to some degree with antibodies to insulin.[34]

Circulating Insulin

The earliest attempts to determine insulin in body fluids depended on bioassay in animals made sensitive by hypophysectomy and adrenalectomy. In vitro assays using tissue slices to measure the glucose uptake replaced earlier techniques. It became apparent that measurement of glucose uptake

is, by itself, nonspecific for the action of insulin. A more precise method of measuring insulin was introduced by Berson and Yalow, who used antibody against insulin to measure the concentration of immunologically reactive insulin-like material in plasma.[45,68,76] Immunoreactive insulin (IRI) rises appropriately after a glucose lead, is absent after pancreatectomy and is absent from the plasma and pancreas of juvenile diabetics. It is now generally accepted that immunoreactive insulin is the most specific and reliable measurement of biologically active insulin available.

Insulin is secreted directly into the portal vein, with 40 per cent or more removed by the liver; consequently the concentration of insulin in the portal vein is three to ten times greater than in peripheral plasma.[6,26] This portal-peripheral gradient is of physiological significance inasmuch as small increments in insulin secretion may result in alterations of hepatic glucose metabolism in the absence of changes in peripheral glucose utilization.[5,28]

CONTROL OF INSULIN SECRETION

It is not yet possible to determine directly the rate of insulin secretion, and calculations of insulin delivery rates involve a large number of assumptions and fail to take into account the portal-peripheral insulin gradient.[26,79] Studies with radioiodinated insulin reveal a direct linear correlation between insulin degradation and plasma insulin concentration over a wide range of steady state insulin levels, suggesting lack of saturability of the insulin removal mechanism.[77] It appears that changes in plasma insulin concentration reflect changes in hormone secretion rather than alterations in the rate of insulin removal.[68]

Effect of the administration of carbohydrate

Among the factors capable of stimulating insulin secretion, glucose is preeminent. However, the precise mechanism of glucose action on the beta cells to cause insulin release has not been entirely clarified.[76] In vitro studies have demonstrated that in addition to glucose, other metabolizable sugars (e.g., mannose) stimulate insulin release; whereas galactose, which is not metabolized by the islet cell, does not stimulate secretion.[46] Inhibitors of glucose metabolism such as mannoheptulose (which blocks phosphorylation of glucose) interfere with glucose-stimulated insulin secretion.[46] Apparently, it is not glucose but a metabolite formed within the beta cell which ultimately gives rise to discharge of secretory granules.[5,18] Data implying a requirement for glucose metabolism by the beta cell have been taken from in vitro incubation studies using pieces of pancreas or isolated islets. When rat pancreas was perfused, non-metabolizable sugars such as galactose and glucosamine have been effective insulin secretogoues. The theory has been proposed that a "glucoreceptor" situated on the beta cell membrane may be stimulated by glucose or by other sugars.[18,48] Further metabolism of glucose within the beta cell may increase the response generated by initial contact with the glucoreceptor.

The adenyl cyclase system is an intricate part of the secretory mechanism of insulin, but the precise manner in which cyclic AMP augments insulin secretion has not been identified.[15,18,48,55]

A characteristic feature of the insulin response to glucose is its biphasic nature.[9,68,69,76,79] An initial rapid secretory burst begins within two minutes and declines over the ensuing three to five minutes. A second phase, characterized by a more gradual increase in insulin levels, begins about five to ten minutes after the injection of a large bolus of glucose and gradually declines as the plasma glucose returns toward a

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normal level. Puromycin, an inhibitor of protein synthesis, prevents the second phase but has no effect on the early phase of insulin release.[25] These observations suggest that insulin exists within the beta cell as a two-pool system.[69] An immediate release pool of preformed insulin is rapidly discharged during the early secretory phase, while a delayed release pool, composed of newly synthesized insulin, small amounts of proinsulin and some stored, preformed insulin, is gradually discharged during the second phase.[6,69,76,79]

Gastrointestinal hormones

In addition to its direct action on the beta cell, a major stimulus to insulin secretion occurs as a result of the interaction of glucose with receptors outside the pancreas but within the gastrointestinal tract. As a result, the plasma concentration of insulin is higher after administration of a given dose of glucose into the jejunum than when it is given intravenously.

The mechanism by which glucose administered into the intestinal tract stimulates insulin release is related to hormones released by cells of the stomach or upper small intestine.[17,64] There is general agreement that intravenously administered secretin, pancreozymin, and gastrin enhances insulin release.[22] Of these hormones, secretin probably has an important physiological role in insulin response to carbohydrate ingestion. The action of secretin may involve direct effects on the beta cell, particularly on the immediate releasable pool, as well as potentiation of insulin release in response to hyperglycemia.[44]

Another factor which may contribute to carbohydrate induced insulin release is "gut glucagon" or "glucagon-like immunoreactivity" (GLI).

This polypeptide hormone of extrapancreatic origin, produced by the upper portion of the intestinal tract, cross-reacts immunologically with glucagon and stimulates insulin secretion. In contrast to pancreatic glucagon, GLI lacks glycogenolytic or gluconeogenic activity, and its secretion is stimulated rather than inhibited by glucose ingestion.[75,82,90,92]

Amino acids

Ingestion or intravenous administration of proteins or amino acids stimulate insulin secretion, although the exact means by which these substance stimulate the release of insulin is not clear. Probably more than one mechanism is involved since patients who respond to the intravenous infusion of leucine may not respond to the other amino acids, and diazoxide suppresses insulin release after leucine but not after arginine.[24,76]

Feedback control of insulin secretion

Both glucose and glucogenic amino acids have a feedback control loop in their relation to insulin secretion. Each stimulates the release of insulin when its concentration in the plasma is increased. In each instance insulin increases the entry of the stimulating substance into cells and into the formation of polymers (glycogen or protein). In the case of glucose, increased oxidation to carbon dioxide and water occurs as well. As a result of these effects of insulin, the concentration of glucose and of amino acids in the plasma is decreased, the stimulus to secretion of insulin its removed and the feedback loop is completed.

Other hormones

A variety of hormones have been demonstrated to influence insulin secretion both <u>in vivo</u> and <u>in vitro</u>. Growth hormone administration, in

normal subjects, induces an elevation in basal insulin levels which precedes a change in blood glucose, suggesting a direct beta-cytotropic effect of this hormone.[27]

A stimulatory action of glucagon on beta cell secretion has been demonstrated with the perfused pancreas,[37] and with physiologic doses of this hormone administered in vivo via the portal vein.[43,75] Interest in glucagon as a possible regulator of insulin secretion was initially evoked by the report that glucose ingestion was associated with a rise in serum glucagon levels which preceded the increment in serum insulin.[73] Subsequently, the seeming hyperglucagonemia caused by carbohydrate ingestion was demonstrated to be entirely due to an elevation in the plasma concentration of the gastrointestinal factor (GLI) which cross-reacts immunologically with pancreatic glucagon.[57,64] Inasmuch as alpha cell secretion is decreased by glucose, it is unlikely that endogenous pancreatic glucagon contributes significantly to the physiologic regulation of insulin secretion.[80]

Adrenocorticosteroids increase gluconeogenesis and impair glucose utilization by insulin requiring cells which results in hyperglycemia. Hyperinsulinemia is greater than would be expected for the hyperglycemia alone; this is a consequence of peripheral insulin resistance. An endogenous hyperaminoacidemia is also induced which, along with the insulin resistance of the alpha cells, results in hyperglucagonemia.[56,67]

Hyperinsulinemia has been observed with exogenous administration of estrogens, progestins and parathyroid hormone. Since glucose levels are not decreased in these situations, it may be inferred that resistance to the effectiveness of insulin accompanies these hormonal changes.[7]

PHYSIOLOGIC ACTION OF INSULIN

Although the most dramatic effect of insulin is its ability to reduce the concentration of glucose in plasma, insulin influences fat and protein metabolism as well. Insulin stimulates synthesis of glycogen in liver and muscle, and fat in liver and adipose tissue. Protein synthesis is stimulated and insulin is essential for growth and maturation. Plasma potassium, free fatty acid and free amino acid concentrations are decreased in association with these functions.[84] The actions of insulin on the major metabolic fuels are synergistic so that the net result is conservation of body fuel supplies.

Insulin receptor

It has been shown that insulin must become bound to a specific protein in the plasma membrane in order for it to exert its action. Insulin binding is limited almost entirely to the plasma membrane, with none attached to nuclei, microsomes, mitochondria, or other cytoplasmic components. When insulin is covalently bound to agarose beads, it is still biologically active.[11] This suggests that the insulin receptor is on the surface of the cell membrane. The receptor has a high degree of specificity for binding insulin and insulin derivatives.[18] Physiologic changes that decrease insulin action, produced by hydrocortisone or fasting, do not alter insulin binding.[13,67]

Effects on carbohydrate metabolism

Although direct action of insulin on the liver was long the subject of debate, it is currently recognized that the liver occupies a central role in the action of insulin on carbohydrate homeostasis. The membrane

of the liver cell is freely permeable to glucose. The level of insulin in portal blood is three to ten fold greater than in peripheral blood[6,26] and absorbed hexoses reach the liver via the portal vein prior to delivery to peripheral tissues.[5]

The effects of insulin on the liver differ from those on other target tissues. Insulin acts on the liver not only to promote glucose uptake but to suppress intracellular processes involved in glucose production and release and this action is mediated by altering enzyme activity rather than by directly influencing transport processes. Small increases in glucose concentration and insulin secretion result in an effect on the liver in the absence of stimulation of peripheral glucose utilization,[5,28]

In view of the permeability of the hepatocyte to glucose, uptake of glucose in the liver is not rate-limiting. A crucial step in the glycolytic pathway involves the phosphorylation of fructose-6-phosphate by phosphofructokinase. In the absence of insulin, the activity of this enzyme is diminished favoring reversal of the glycolytic scheme and the formation of glucose.[5]

Insulin activates glycogen synthetase within minutes after it is administered. Glycogen accumulation is further facilitated by insulin inhibition of phosphorylase, the enzyme catalyzing the rate-limiting step in glycogen breakdown. Insulin decreases the output of glucose from the liver not only by its action on glycogen synthesis and breakdown, but also by inhibiting gluconeogenesis.[13,28]

Unlike the situation in the hepatocyte, at physiological concentrations of plasma glucose, the rate of entry of the sugar into themuscle cell is the rate-limiting step. A major effect of insulin in this tissue is to control the transport of glucose across the cell membrane.[13]

In the fat cell, the situation is similar to muscle, in that insulin acts primarily to stimulate transport of glucose across the cell membrane.

Effect on fat metabolism

In the liver the synthesis of fatty acids is reduced when insulin is absent but is restored to normal by insulin administration. Although the insulin deprived liver does not synthesize fatty acids actively, it is capable of esterifying free fatty acids with glycerol, which can be activated in the liver by phosphorylation under the control of glycerophosphokinase. The availability of this enzyme in liver cells permits the esterification of fatty acids in the absence of glycolytic breakdown of glucose to α-glycerophosphate.

In adipose tissue the formation of fatty acids in the absence of insulin is affected much as it is in the liver. In addition, the absence of phosphorylated 3-carbon compounds derived from glycolysis, especially α-glycerophosphate, prevents esterification of the free fatty acids which are constantly being released from the triglycerides of the fat cell. The rate of triglyceride breakdown is increased in the absence of insulin. This is due to an increase in a hormonally sensitive lipase, the activity of which is normally inhibited by insulin. The administration of insulin restores the glycolytic pathway providing α-glycerophosphate, enhancing fatty acid synthesis, and reducing triglyceride breakdown, returning the metabolism of the fat cells to normal.

Effects on amino acid and protein metabolism

Insulin increases uptake of most amino acids into muscle, but may be difficult to demonstrate unless the simultaneous stimulation of protein

synthesis is blocked. Growth studies of insulin deprived animals and human beings indicate that the effect of insulin on growth is a general one, involving almost all tissues. The effects of growth hormone on somatic protein synthesis cannot be observed unless adequate amounts of insulin are available.[84]

METABOLISM OF INSULIN

Insulin is rapidly removed from the blood by the liver, which clears about 40 to 60 percent of the hormone presented to it in a single passage.[5,41] Most of the insulin taken up by the liver is destroyed by "insulinase" activity, especially glutathion-insulin transhydrogenase,[41] which reduces the disulfide bonds joining the A and B chains which, separately, are inactive.

In the kidney, insulin is apparently filtered by the glomerulus and concentrated in the proximal tubule cells, where it is absorbed and destroyed. In patients with renal insufficiency insulin uptake by the kidney may fall to 9 per cent.[30] This results in diminished insulin requirements for patients with certain chronic renal disease.

GLUCAGON

Shortly after the discovery of insulin, Kimball and Murlin described the presence of a substance in pancreatic extracts which caused an increase of blood glucose concentration when injected intravenously and which they named "glucagon".

The alpha cells of the islets of Langerhans of the pancreas are believed to be the course of glucagon based on the following evidence: (1) The concentration of glucagon is 10 to 20 times higher in the pancreaticoduodenal venous blood than in peripheral blood; (2) Extracts of the uncinate process of the dog pancreas, which lacks alpha cells, lack glucagon activity; (3) Extirpation of all of the dog pancreas except the uncinate process decreases pancreaticoduodenal veinous glucagon concentration to undetectable levels.[26,80]

GLUCAGON IN PLASMA

The determination of the concentration of pancreatic glucagon in plasma by immunoassay techniques has been fraught with difficulty.[39,82] Most anti-glucagon sera prepared by immunization of rabbits with pancreatic glucagon reacts to varying degrees with a glucagon-like material (glucagon-like immunoreactivity, GLI) derived from the gastrointestinal tract.[23] This material differs in chemical structure and biologic activity from true pancreatic glucagon.[57,80,90,93] The GLI from the gastrointestinal tract is biologically and immunologically indistinquishable from pancreatic glucagon or true glucagon. One source of true or pancreatic glucagon, as shown by histochemical techniques, is from alpha cells in the stomach and duodenum in man[85] and the stomach in the dog.[75] These alpha cells are indistinquishable from pancreatic islet alpha cells.[75,91] GLI represents over 80 per cent of the total measurable plasma glucagon in the fasting state.[57,64,86,92]

Mechanism of glucose control of glucagon secretion

It is reasonable that the secretory activity of cells designed to maintain an appropriate rate of endogenous glucose production should vary inversely with their own supply of fuel. Glucagon secretion, in vitro, can be decreased by energy-yielding fuels, such as glucose, free fatty acids, and ketones, and this suppression can be prevented by blocking ATP formation.[84] Agents that block intracellular glucose metabolism, such as 2-deoxyglucose or mannoheptulose, when given intravenously, result in paradoxical hyperglucagonemia despite the presence of extracellular hyperglycemia.[62] Insulin lack, whether produced by anti-insulin serum, alloxan, or streptozotocin, also results in paradoxical hyperglucagonemia irrespective of the magnitude of hyperglycemia. Moreover, the glucagon response to a variety of stimuli is greatly exaggerated during insulin insufficiency.[62,74,86] In experimental diabetes loss of the response to extracellular hyperglycemia is instantly corrected by insulin, even in very small amounts, suggesting that the alpha cell is an insulin-requiring cell. Severe insulin lack is, therefore, a cause of increased basal glucagon secretion, and in the absence of insulin, the alpha cell is incapable of responding appropriately to plasma glucose concentration.[20,23,86]

Glucose "need"

Cerebral glucose delivery is endangered in starvation, hypoglycemia, strenuous exercise, and during peripheral vascular collapse. In each of these situations, glucagon secretion rises to a varying degree, and insulin secretion either remains in the basal level or decreases to subbasal levels, i.e., the molar ratio of insulin to glucagon is decreased. This increases the hepatic production of glucose.[16,51]

In normal subjects starvation is accompanied within 48 hours by a rise in plasma glucagon, averaging approximately 50 per cent, and a 60 per cent decline in insulin.[1,84] The molar insulin:glucagon ratio is reduced. However, as starvation continues glucagon declines to slightly above baseline levels. The recession of the plasma glucagon level from its 48-hour peak coincides with decreased cerebral glucose requirements resulting from increased central nervous system utilization of ketones as a fuel,[14] an adaptation that reduces the need for gluconeogenesis.[12,81]

When arterial glucose concentration declines, glucagon secretion rises dramatically and insulin falls. At glucose levels less than 60 mg/dl hyperglucagonemia is invariably present.[64] This is the case in acute hypoglycemia induced by insulin,[33,62,87] and sulfonylureas,[10] and in chronic hypoglycemia caused by phloridzin administration.[87]

The enormous increase in fuel consumption generated by strenuous exercise is associated with a marked increase in hepatic glucose production. In dogs and man exercise is accompanied by a progressive increase in plasma glucagon concentrations to four to six times the normal basal level.[3,29,84] The marked hyperglucagonemia is accompanied by a rise in glucose, excluding hypoglycemia as the stimulus to glucagon secretion. In rats the response of the islets to exercise is apparently mediated by catecholamine secretion and/or sympathetic nerve discharge to the islets of Langerhans.

Impaired cerebral blood flow may be the most desperate category of glucose need, involving an immediate threat to cerebral nutrient delivery and to live itself. Glucagon levels rise in many types of shock;[21,41,71] in normal man traumatic shock elicits hyperglucagonemia that averages four times the normal basal concentration;[49] cardiogenic shock in man[84] and

hemorrhagic shock in dogs[49] are also associated with striking hyperglucagonemia, even though concomitant hyperglycemia is the rule. In each of these conditions insulin concentrations are in or near the normal basal range and are low in relation to the accompanying hyperglycemia. The decreased molar insulin:glucagon ratio increases arterial glucose concentration and maintains more adequate cerebral glucose delivery in the face of a declining cerebral blood flow.

Hyperaminoacidemia may, in a sense, be regarded as a form of glucose need. Infused amino acids or ingested protein, in a normal well-fed individual, stimulates the release of insulin,[84] without which incorporation of the ingested amino acids into protein cannot occur. The aminogenic rise in insulin allows glucose to move from extracellular to intracellular space; glucose concentration remains constant, although its turnover increases, presumably because precisely the amount of glucose required is released from the liver.[61,89] The magnitude of the glucagon response to hyperaminoacidemia varies with the need for an increase in hepatic glucose secretion. If exogenous glucose is available, as when carbohydrates are consumed with proteins, this action of glucagon is unnecessary and glucagon secretion does not occur. Hyperglycemia completely prevents aminogenic release of glucagon in normal subjects.[61,62,80,84]

It is known that the primary source of glucagon is the alpha cells from the islets of Langerhans but several investigators have reported a persistent, glucagon-like immunoreactivity in the plasma of depancreatized dogs. Some investigators[57,92] report that both fractions of gastrointestinal glucagon (true glucagon and GLI) are increased in the plasma of depancreatized dogs, while others[20] report that only true glucagon is increased and GLI remains at normal levels. The glucagon of depancreatized

dogs mimics the secretory behavior of pancreatic glucagon with respect to its response to arginine, insulin or somatostatin infusion.[20,57,58,92,93]

EFFECTS OF GLUCAGON

Although a variety of biologic effects have been attributed to glucagon, the physiologic role of this hormone has been difficult to assess. A proper experimental model for glucagon deficiency has not been developed.

Glucagon has a hyperglycemic action resulting primarily from stimulation of hepatic glycogenolysis. In addition, studies with perfused liver have demonstrated that glucagon enhances, gluconeogenesis as indicated by increased incorporation of carbon from amino acids and lactate into glucose, and increased urea formation.

Glucagon is believed to enhance glycogenolysis and glyconeogenesis by interacting with a hormone sensitive adenyl cyclase present in the plasma membrane of hepatic cells. The increase in intracellular cyclic AMP results ultimately in an elevation in phosphorylase activity and in more rapid conversion of pyruvate to glucose. The hyperglycemic effects of glucagon do not involve alterations in peripheral glucose utilization.[8]

Glucagon has also been implicated in the regulation of lipid metabolism. Administration of small doses of the hormone to anesthetized dogs results in an elevation in plasma free fatty acids.[47] In contrast, administration of large doses of glucagon to normal man results in an initial fall in plasma FFA followed by a secondary rise.[14] It is probable that the early fall in FFA is not a direct effect of glucagon but a consequence of glucagon-stimulated insulin secretion demonstrable when glucagon is administered in pharmacologic but not in physiologic doses.[7]

INSULIN/GLUCAGON RATIO

In a net sense, insulin and glucagon exert diametrically opposing actions upon hepatic glucose balance. Glucagon, through powerful gluconeogenic and glycogenolytic effects, promotes hepatic glucose production, while insulin opposes these actions.

The relative concentrations of these two hormones in the portal vein controls the uptake and release of glucose into the blood by the liver.[26] These hormones also control the disposition of other nutrients in tissues through out the body in accordance with energy needs and the supply of exogenous fuels in the environment. An increase in the concentration of insulin perfusing the liver, relative to that of glucagon, decreases glucose production and favors net storage of glucose, sparing available amino acids from gluconeogenesis for other purposes such as protein synthesis. An increase in glucagon concentration relative to that of insulin increases glycogenolysis and gluconeogenesis, using available amino acids for glucose production.[26,81]

The calculated molar ratio of insulin to glucagon varies, depending on conditions of energy availability. After a carbohydrate meal, when the liver is storing glucose at a maximal rate, the insulin/glucagon ratio is about 70:1. After an overnight fast, when the liver is producing glucose, the ratio is about 3:1 and after a three-day fast, when gluconeogenesis is maximal, the ratio declines to less than 1:1.[81,83,88]

RADIOIMMUNOASSAY

Radioimmunoassays are based on the ability of an unlabeled antigen (Ag) to competitively inhibit the binding of labeled antigen (Ag*) by antibody (Ab). The process may be viewed as a simple competition in which Ag reduces the amount of free Ab, decreasing the availability of Ab to Ag*. When the assay is performed, Ag* and Ab are incubated together in the presence and absence of samples containing unlabeled Ag. After equilibration free Ag* and Ag*·Ab are separated. Commonly used separation procedures include solid phase absorption, precipitation of Ag*·Ab complexes with either a second antibody or a salt, and chromatoelectrophoreis. Ag*·Ab (or free Ag*) is then determined by *radioactive* counting. The antigen concentration of the sample is determined by comparing the diminished Ag* binding of the sample to that of a standard curve obtained by adding graded, known amounts of Ag to Ag* and Ab. A new standard curve is determined in each assay to allow for variation in antigen binding from assay to assay.[66,78]

GLUCOSE TOLERANCE TESTS

The theory of the glucose tolerance test is that a normal animal should be able to remove a glucose load from the plasma within a specific period of time. If unable to do so, the blood glucose concentration remains elevated longer than normal and the patient is said to have a "reduced glucose tolerance". Many factors alter the ability of the body to remove glucose from the blood. These include a number of incidental conditions such as caloric restriction, restricted previous carbohydrate intake, and surgical, infectious, emotional or other stress.[25,94] A glucose tolerance test performed on a patient who has not been well fed, or who has been seriously ill or emotionally disturbed immediately before the test can not be interpretated critically. To avoid these difficulties it usually is important that the patient has had an adequate carbohydrate intake for several days prior to the examination.

The oral glucose tolerance test (OGTT) consists of orally administering a standard dose of glucose to a fasting patient and measuring the blood glucose at standard intervals over a three hour period. The OGTT is used extensively in human medicine to evaluate the endocrine function of the pancreas. The major advantage in using the OGTT is that the test simulates the normal sequence of digestion whereas the intravenous glucose tolerance (IVGTT) bypasses the gastrointestinal hormone release which has been shown to augment insulin secretion.[44,57,75,90] A serious disadvantage of the OGTT is its poor reproducibility.[59] Since the OGTT is based on the gastrointestinal absorption of glucose, small changes in the rate of gastric emptying and intestinal absorption can affect the tolerance curve significantly.

Both the OGTT and the IVGTT have been used in veterinary medicine to diagnosis pre-diabetic states based on glucose intolerance.[35,36,40,42,53,54] The IVGTT has been studied extensively in normal dogs and dogs with various types of pancreatic disease. The K-value has been adapted as the measure of glucose disappearance based on the concept that clearance of excess blood glucose is an exponential function.[2,19,60]

PREVIOUS REPORTS ON H-IVGTT IN DOGS

Greve, et al.[36] provided guidelines for use of the glucose tolerance test and calculation of a K-value in order to establish the degree of glucose intolerance when the plasma glucose is not markedly increased, i.e., prediabetes.

Mahaffey and Anderson[54] extended this work into an extensive study of the H-IVGTT in the dog using normal dogs, 50% partial pancreatized dogs and partially pancreatectomized dogs with staphylococcal alpha-toxin induced pancreatitis. The K-value was found to clearly separate these groups of dogs.

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THE EFFECTS OF PARTIAL PANCREATECTOMY AND ACUTE STAPHYLOCOCCAL ALPHA-TOXIN PANCREATITIS ON THE PLASMA GLUCOSE, INSULIN AND GLUCAGON DURING A H-IVGTT IN THE DOG

INTRODUCTION

The bihormonal control of plasma glucose is mediated by insulin and glucagon released from islet tissue of the pancreas. The importance of insulin in the regulation of plasma glucose was recognized before the role of glucagon was determined. The radioimmunoassay (RIA) technique for plasma insulin was developed over a decade ago by Yalow and Berson and has allowed accurate measurement of plasma insulin. Insulin was well suited for early RIA techniques because the hormone is very similar between species and is readily immunogenic. In addition, there is only one type of functional insulin released from the pancreas. Early RIA measurements of glucagon were extremely variable resulting in conflicting theories of glucagon phsyiology. These early RIA studies of glucagon were hampered by lack of specificity of anti-glucagon antibodies and by cross-reactions with a similar substance which did not have strong gluconeogenic properties and was believed not to be true glucagon.

The development of highly specific anti-glucagon antibody has clearly separated glucagon from a cross-reactivity polypeptide with glucagon-like immunoreactivity (GLI).[5,28,31] Pancreatic or true glucagon secreted by alpha cells has potent gluconeogenic and glycogenolytic properties. Its secretion is inhibited during hyperglycemia in the normal animal. GLI does not have gluconeogenic or glycogenolytic properties and its biologic functions are largely unknown.[23,28,31,33,35,36] However, when glucose is administered orally, GLI and insulin are released into the plasma; it is proposed that GLI augments the oral glucose stimulation of insulin release from the pancreas.

While insulin is produced only in the pancreas, true glucagon is produced and secreted from alpha cells in the pancreas and stomach in the dog. True glucagon plasma concentration correlates directly with the hyperglycemia of diabetes mellitus,[6,7,23,32,34] trauma,[19] acute pancreatitis[3] and bacterial infections[24] and may be involved in the hyperglycemia in these conditions.

Dogs that are subjected to total pancreatectomies are found to have undetectable immunoreactive insulin (IRI) within 48 hours. As IRI approaches undetectable levels, plasma glucagon remains normal and then increases to levels of 8 to 10 fold the normal values. The total plasma glucagon is found to consist of an increase in both true glucagon and GLI.[5,22,35,36] This data originally lead investigators to search for other sources of glucagon production in the body. In the dog, cells indistinct from the alpha cells of the pancreas, both morphologically and histochemically, are found in the fundus of the stomach.[28] These cells secrete glucagon biologically and immunologically identical to pancreatic glucagon.[35] In the upper small intestine of the dog are found alpha-like cells which secrete only GLI.[28] In man, alpha cells are present in the stomach and duodenum and alpha-like cells are found in the upper small intestine.[2]

Totally pancreatectomized dogs are similar to human juvenile diabetics and canine diabetics in that IRI is greatly reduced and plasma true glucagon is elevated. Exogenous insulin administered to these patients decreases plasma glucose and glucagon simultaneously.[6,22,23] It is theorized that insulin is essential for membrane transport and intracellular metabolism of glucose by the alpha cell. When insulin concentration is very low, as in human juvenile diabetes and canine diabetes, the alpha cell can not recognize hyperglycemia and increases secretion of

true glucagon which further contributes to the hyperglycemic state.[6,22,31,36]

Canine diabetes mellitus is similar to human juvenile diabetes in that IRI is very low and incapable of increasing significantly when hyperglycemia is present.[21,26,31] Manns and Martin[21] measured the plasma glucose, IRI and total plasma glucagon during a H-IVGTT in control and naturally occuring diabetes in the dog. Their data for the diabetic dog were similar to data for juvenile diabetic humans in that insulinopenia, hyperglucagonemia, and hyperglycemia were present after a 12-hour fast.

Somatostatin is a hormone that completely inhibits secretion of insulin and glucagon, and when administrated to a human juvenile diabetic with very low IRI, hyperglycemia decreases as the secretion of true glucagon is suppressed. With cessation of somatostatin administration, true glucagon secretion increases and plasma glucose increases to the hyperglycemic range without IRI significantly changing throughout the experiment.[2,8,9,18,26,27]

Totally pancreatectomized dogs have no detectable IRI and elevated true glucagon, GLI and plasma glucose. Sun et al.[29] showed removal of 50% of the pancreas is required before the exponential decline in plasma glucose concentration (k-value) is altered during the high-dose intravenous glucose tolerance test (H-IVGTT). Greve and Anderson,[10] Greve et al.[11] and Mahaffey and Anderson[20] characterized the H-IVGTT in normal dogs, dogs with 50% partial pancreatectomies and dogs with 50% partial pancreatectomies combined with staphylococcal alphatoxin pancreatitis and showed that although the fasting plasma glucose levels remained in the normal range, each group had distinctly different H-IVGTT k-values.

The purpose of this present study was to explore the relationship between pancreatic injury, plasma glucose, plasma insulin, plasma glucagon

and the k-value of normal dogs, dogs with 50% partial pancreatectomies, and dogs with 50% partial pancreatectomies combined with staphylococcal alpha-toxin pancreatitis.

MATERIALS AND METHODS

Eight adult mongrel dogs weighing 17.2 to 28.2 kg were allotted to 2 groups as follows: Group I (5 dogs)--50% partial pancreatectomy and staphylococcal alpha-toxin induced pancreatitis affected dogs (acute pancreatitis); and Group II (3 dogs)--50% partial pancreatectomy dogs. Each dog served as a normal control prior to surgery. Food was withheld for 12 to 16 hours before all experimental procedures.

The distal half of each pancreatic lobe was removed surgically on day 5 from all dogs as described by Mahaffey and Anderson.[20] The ventral pancreatic duct was catheterized through a transverse incision in the duodenum opposite the ductal papilla and 0.1 ml/kg of a 1:2 dilution of staphylococcal alpha-toxin was infused into the pancreatic duct under light manual pressure (Group I); the duct was not ligated. The dogs of Group II received no toxin.

The high dose intravenous glucose tolerance test (H-IVGTT) as modified by Dyck and Moorhouse[4] was performed on days 1, 6 (day after surgical operation), and 9. Glucose (k g/kg as a 50% solution) was injected over a 30-second period into a cephalic vein; that vein was not used again the same day. Blood samples were collected from a peripheral vein prior to (0) and 5, 10, 15, 30, 45, 60, 90 and 120 minutes after injection of glucose. The k-value, calculated from the H-IVGTT, has been described.[4,10,11,20]

Samples were collected for insulin and glucagon assay at the same times the glucose samples were collected during the H-IVGTT. Radioimmunoassay for immunoreactive insulin[12] and true glucagon were performed on blood collected in tubes to which Trasylol[a] had been added. The highly

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[a] Bayer A 128, FBA Pharmaceuticals, Inc., New York.

specific antibody to true glucagon was obtained from the laboratory of R. H. Unger.[b]

Clotted blood samples were taken for blood chemical determinations prior to each glucose tolerance test. The following analytical methods were used: glucose (hexokinase method),[16] amylase[17] (using commercial dye substrate),[c] lipase,[14] alkaline phosphatase,[1] glutamic pyruvic transaminase[15] and creatinine.[13]

All dogs were euthanitized and necropsied on day 9.

Data was analyzed by analysis of variance with unequal subclasses with treatment as the only source of variation.

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[b] Department of Internal Medicine, The University of Texas Health Science Center and Veterans Administration Hospital, Dallas, Texas.

[c] Amylochrome[R], Division Roche Diagnostics, Hoffmann-La Roche, Inc. Nutley, New Jersey.

RESULTS

The packed cell volume (P.C.V.) and hemoglobin (Hb) decreased significantly** on day 6 compared to day 1 in both groups I and II (Table 1). However, there was no significant change ($p > .10$) on day 9 compared to day 6.

The total leukocyte (W.B.C.) count increased above the normal range on day 6 in each group (Table 1) and returned to near normal in group I but remained elevated in group II on day 9. There was a change in the distribution of leukocytes on day 6; neutrophilia, lymphopenia, monocytosis and eosinopenia were present. The leukocyte distribution returned to normal on day 9 (Table 1).

Among the chemistries measured, only alkaline phosphatase (A.P.) increased above the normal range on days 6 and 9. Serum amylase (Table 1) and lipase activity did not change significantly ($p > .10$) or increase above the normal range throughout the experiment in either group. Urea nitrogen (U.N.), creatinine and glutamic pyruvic transaminase (G.P.T.) did not change during the experiment.

The fasting, morning plasma glucose was measured each day for each dog and the means determined for each group (Table 2). In group I, the mean plasma glucose elevated slightly but not significantly ($p > .10$) on days 7 and 8. In group II, the mean plasma glucose remained unchanged throughout the experiment.

The concentration of plasma glucose during the H-IVGTT was plotted from 5 minutes to 60 minutes and the removal of plasma glucose (mg/dl/min) was determined. The rate of glucose removal from the plasma decreased in a similar manner in both groups (Table 3). The decrease in glucose removal

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** $p \leq .05$

rate in group I was significantly* lower on day 6 compared to day 1 and significantly** lower on day 9 compared to day 6. The decline in glucose removal rate in group II was not significant ($p > .10$) on day 6 compared to day 1 but was significantly* lower on day 9 compared to day 6. The decline in both groups on day 9 was not significant ($p > .10$) between groups.

The k-values of group I decreased significantly*** on day 6 compared to day 1 and significantly** on day 9 compared to day 6. In group II, the k-values also decreased significantly** on day 6 compared to day 1 and significantly* on day 9 compared to day 6. There was a significant** difference between groups I and II on day 9 with group I having the lower k-value.

The fasting, morning plasma IRI was measured each day of the experiment for each dog and the means determined for each group (Table 2). There was no change in means of IRI for either group throughout the experiment.

The total amount of IRI measured during the H-IVGTT was calculated by adding the IRI values obtained at 5, 10, 15, 30, 45 and 60 minutes minus the initial fasting plasma IRI from each of the individual time measurements. This calculation for the net increase in measured IRI during the H-IVGTT will be designated ΣI_t. The decrease in the ΣI_t was not significant ($p > .10$) on day 6 compared to day 1 for either group. However, ΣI_t was significantly* decreased for each group on day 9 compared to day 1. Comparisons between the groups can not be drawn because the normal values

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* $p \leq .10$

** $p \leq .05$

*** $p \leq .001$

of ΣI_t for group II on day 1 are markedly greater than the ΣI_t of group I on day 1 (control period). It appears that the randomly chosen dogs of group II had markedly greater ΣI_t values than the dogs of group I. Graphs of the plasma glucose, IRI and glucagon are presented to show the relation of the hormones to glucose concentration during a H-IVGTT (figures 1 and 2).

The fasting, morning plasma glucagon was measured each day of the experiment for each dog and the daily mean determined for each group (Table 2). There was a significant* increase in the fasting plasma glucagon on day 6 and day 7 of group I. The elevation of plasma glucagon of group I on days 6 and 7 was not significantly ($p > .10$) greater than the elevation of group II for the same days.

The total glucagon measured during the H-IVGTT was calculated by adding the plasma glucagon values obtained at 5, 10, 15, 30, 45 and 60 minutes. This calculated figure will be symbolized by ΣG_t. In each group, on day 6, the ΣG_t was significantly* greater than the control value calculated for day 1. On day 9, in each group, the ΣG_t returned to control levels. There was a significant* difference between group I and II on day 6, with the increase of ΣG_t greater for group I.

Histopathological examination of the remnant pancreatic tissue of all dogs showed only slight morphological changes. There was no observable difference between the pancreases of group I and those of group II.

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* $p \leq .10$

DISCUSSION

Manns and Martin[21] documented the hyperglucagonemia of naturally occurring canine diabetes mellitus. Diabetic humans have been shown repeatedly to have elevated plasma glucagon levels and it has been strongly suggested that the hyperglucagonemia is an integral part of the pathogenesis of diabetes mellitus in man.[27,31] Hyperglucagonemia and hyperglycemia can be produced readily in totally pancreatectomized dogs indicating that other sources of glucagon are present. Our results show that a transient, mild hyperglucagonemic and insulinopenic state can be produced experimentally by using the model for canine pre-diabetes described by Mahaffey and Anderson.[20]

Our data for the H-IVGTT and K-values agree with Greve et al.[11] for normal dogs and dogs with 50% partial pancreatectomy. Our data for each group of dogs (normal, 50% partial pancreatectomy and acute pancreatitis) agree with Mahaffey and Anderson; 20 except that the k-values of our pancreatitis dogs were not decreased as much as in that study. Serum amylase and lipase activities and histopathological examination of the pancreatic remnants indicated that we did not induce as severe a pancreatitis as Mahaffey and Anderson.

Since the fasting, morning plasma glucose and IRI did not change throughout the experiment and true glucagon remained in or above the normal control range, we conclude that neither the partial pancreatectomy or the acute pancreatitis decreased the ability of the remaining pancreas to synthesize sufficient IRI to maintain the fasting plasma glucose and fasting true glucagon within the normal range.

The k-value and plasma glucose removal rate decreased significantly on day 6 as compared to day 1 and on day 9 as compared to day 6 in both groups. The decrease was more severe in the acute pancreatitis dogs (group I). This pattern of continual loss of endocrine-pancreatic function from day 6 to day 9 was similarly noted by Mahaffey and Anderson.[20] When the pancreas was challenged to respond maximally (H-IVGTT), dificiencies in the measured amount of IRI released during theH-IVGTT (ΣI_t) could be noted from the control period. The ΣI_t decreased moderately in both groups on day 6 as compared to day 1 (control period) and the ΣI_t decreased further on day 9 as compared to day 6 in both groups. The further reduction in insulin-releasing ability of the pancreas more than 24 hours after surgery could be due to progressive damage to pancreatic tissue; the normal activity of serum amylase and lipase suggest this was not the case. The lower ΣI_t on day 9 may represent progressive inability of the remaining beta cells to maintain the optimum insulin secretion necessary to establish normoglycemia.

In contrast to the decrease in ΣI_t on day 6 and 9, the ΣG_t increased significantly on day 6 and then decreased into the control range by day 9. It can be seen in figures 1 and 2 that the hyperglucagonemia of day 6 is due to an elevated basal level which returned to normal on day 9. The hyperglucagonemia on day 6 was nevertheless responsive to the inhibiting effects of hyperglycemia and hyperinsulinemia during the H-IVGTT. This transient hyperglucagonemia was probably due to the stress of the surgery on day 5 and is evident by the neutrophilia, lymphopenia, monocytosis and eosinopenia in the mean leukocyte differentials on day 6 (Table 1). Trauma is associated with hyperglucagonemia in man.[19] Experimentally, stress in animals causes hyperglucagonemia which can be prevented by β-adrenergic blocking agents.[19,31]

It is apparent the model of canine pre-diabetes by Mahaffey and Anderson[20] produces an insulin deficient state that can only be detected during a H-IVGTT even though the fasting plasma glucose and IRI remains in the normal range. This is compatible with spontaneous canine pre-diabetes. However, the significant hyperglucagonemia of spontaneous canine diabetes is not present in this pre-diabetes model.

TABLE 1: Hemogram and serum chemistry means ± one S.D. for Groups I and II on days 1, 6 and 9.

Variable	Day	Group I	Group II
Hemogram			
P.C.V. (%)	1	49.2 ± 3.1	47.3 ± 4.0
	6	45.2 ± 4.4	44.7 ± 2.5
	9	43.2 ± 2.6	44.0 ± 3.6
Hb. (g/dl)	1	17.1 ± 0.9	16.1 ± 1.7
	6	15.4 ± 1.7	14.9 ± 0.6
	9	14.9 ± 0.8	15.1 ± 1.1
W.B.C. (/mm^3)			
total	1	15120 ± 8691	15833 ± 3233
bands	1	901 ± 1232	257 ± 222
neutrophils	1	9980 ± 7386	12733 ± 1955
lymphocytes	1	2022 ± 645	2521 ± 766
monocytes	1	1129 ± 668	510 ± 394
eosinophils	1	1095 ± 603	1214 ± 432
total	6	26640 ± 6679	23700 ± 18784
bands	6	360 ± 504	0
neutrophils	6	22577 ± 4289	20933 ± 18312
lymphocytes	6	1121 ± 1664	963 ± 1035
monocytes	6	1999 ± 825	1270 ± 990
eosinophils	6	40 ± 90	261 ± 432
total	9	17920 ± 5954	23266 ± 11107
bands	9	714 ± 1192	0
neutrophils	9	10881 ± 3760	20063 ± 8193
lymphocytes	9	2718 ± 976	2573 ± 506
monocytes	9	2299 ± 1266	613 ± 141
eosinophils	9	1285 ± 870	1190 ± 1074
Chemistries			
Amylase (dye units)	1	800 ± 262	1026 ± 214
	6	1376 ± 554	1077 ± 686
	9	1088 ± 237	1094 ± 52
U.N. (mg/dl)	1	13.2 ± 3.8	19.5 ± 1.2
	6	12.8 ± 2.9	12.2 ± 5.0
	9	13.8 ± 4.5	15.8 ± 3.8
Creatinine (mg/dl)	1	1.10 ± 0.2	1.33 ± 0.12
	6	1.03 ± 0.15	1.10 ± 0.24
	9	1.04 ± 0.17	1.20 ± 0.06
G.P.T. (IU/L)	1	52.6 ± 49.5	42.3 ± 14.9
	6	77.8 ± 55.4	59.3 ± 20.0
	9	47.8 ± 17.1	41.0 ± 12.8
A.P. (IU/L)	1	57.8 ± 27.8	33.7 ± 11.9
	6	340.0 ± 117.5	211.7 ± 138.4
	9	141.6 ± 7.4	135.3 ± 78.5

TABLE 2: Fasting and range means of plasma glucose, insulin and glucagon for groups I and II.

Variable	Day	Group I	Range	Group II	Range
Glucose (mg/dl)	1	92.3	80-107	94.3	86-106
	2	88.7	81-106	99.0	88-112
	3	92.7	88-100	96.3	91-101
	4	93.5	86-112	97.0	92-105
	5	94.5	83-123	95.3	85-106
	6	92.0	78-110	88.7	82-98
	7	107.5	97-130	95.0	86-113
	8	107.5	90-127	99.0	90-111
	9	96.5	85-114	96.7	96-98
Insulin (μU/ml)	1	14.3	13-15	12.0	8-17
	2	15.0	13-19	17.0	12-25
	3	15.8	13-21	15.0	10-18
	4	16.5	12-21	19.3	8-30
	5	14.2	12-17	15.7	9-22
	6	15.7	12-19	11.3	7-16
	7	16.3	15-19	17.0	6-33
	8	17.2	16-20	17.0	9-27
	9	16.2	14-18	15.7	9-24
Glucagon (pg/ml)	1	295	250-380	170	72-255
	2	292	180-390	282	236-370
	3	268	163-420	290	260-328
	4	310	230-390	244	181-335
	5	223	100-370	259	192-340
	6	518	425-720	362	236-480
	7	365	240-600	421	302-639
	8	266	200-340	300	153-505
	9	270	160-440	239	132-430

TABLE 3: The mean values for glucose disappearance, K values, ΣI_t and ΣG_t for groups I and II on days 1, 6 and 9.

Glucose Disappearance (mg/dl/min)	1	6.99	7.72
	6	6.05	6.64
	9	4.58	5.32
K values	1	3.04	3.46
	6	2.36	2.82
	9	1.92	2.42
Hormones			
Insulin (ΣI_t) (μU/ml)	1	124	269
	6	96	217
	9	77	194
Glucagon (ΣG_t) (pg/ml)	1	1470	827
	6	2422	1813
	9	1428	1278

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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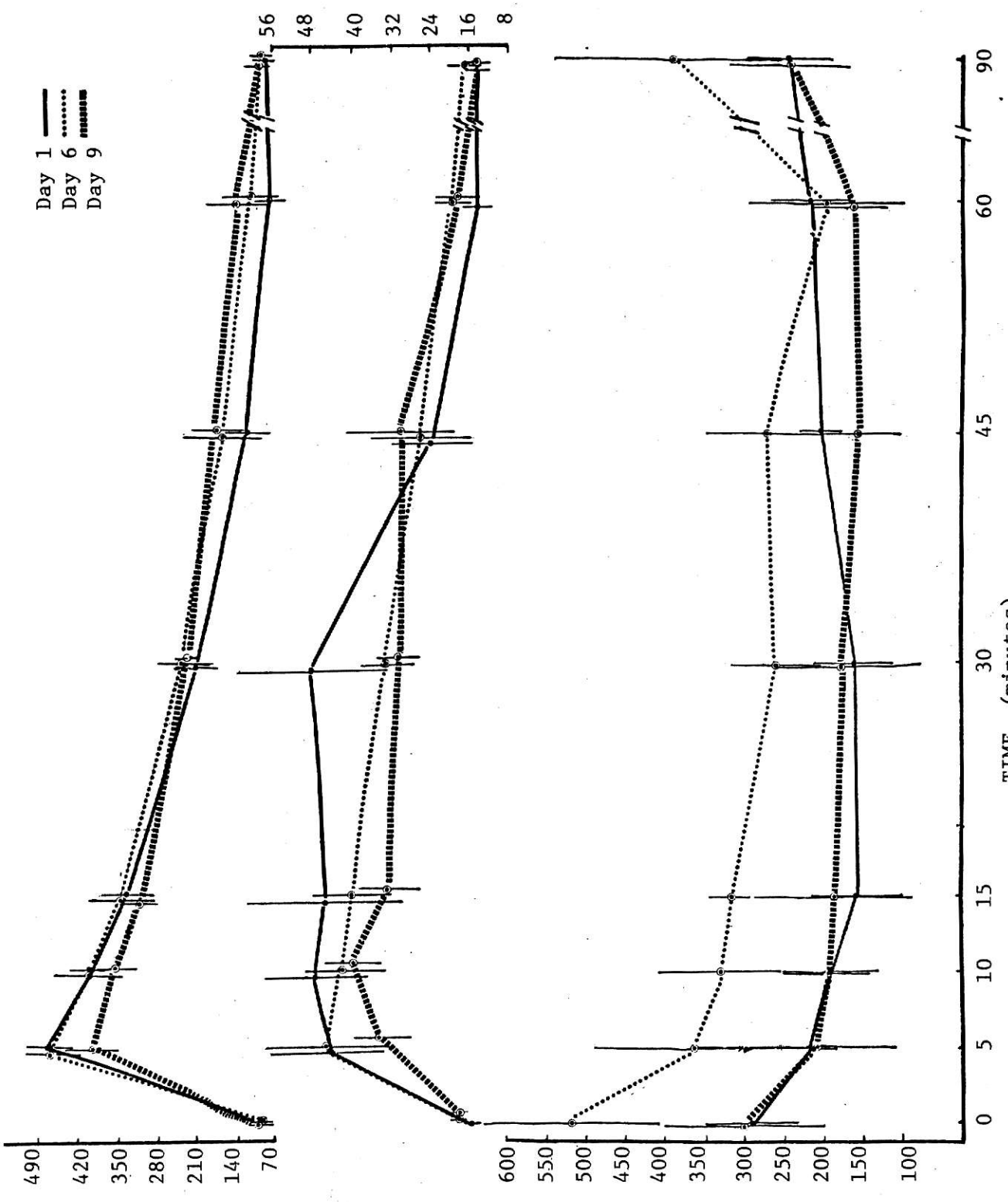

FIGURE 1: The means and standard deviations for plasma glucose, insulin and glucagon during a H-IVGTT for group I.

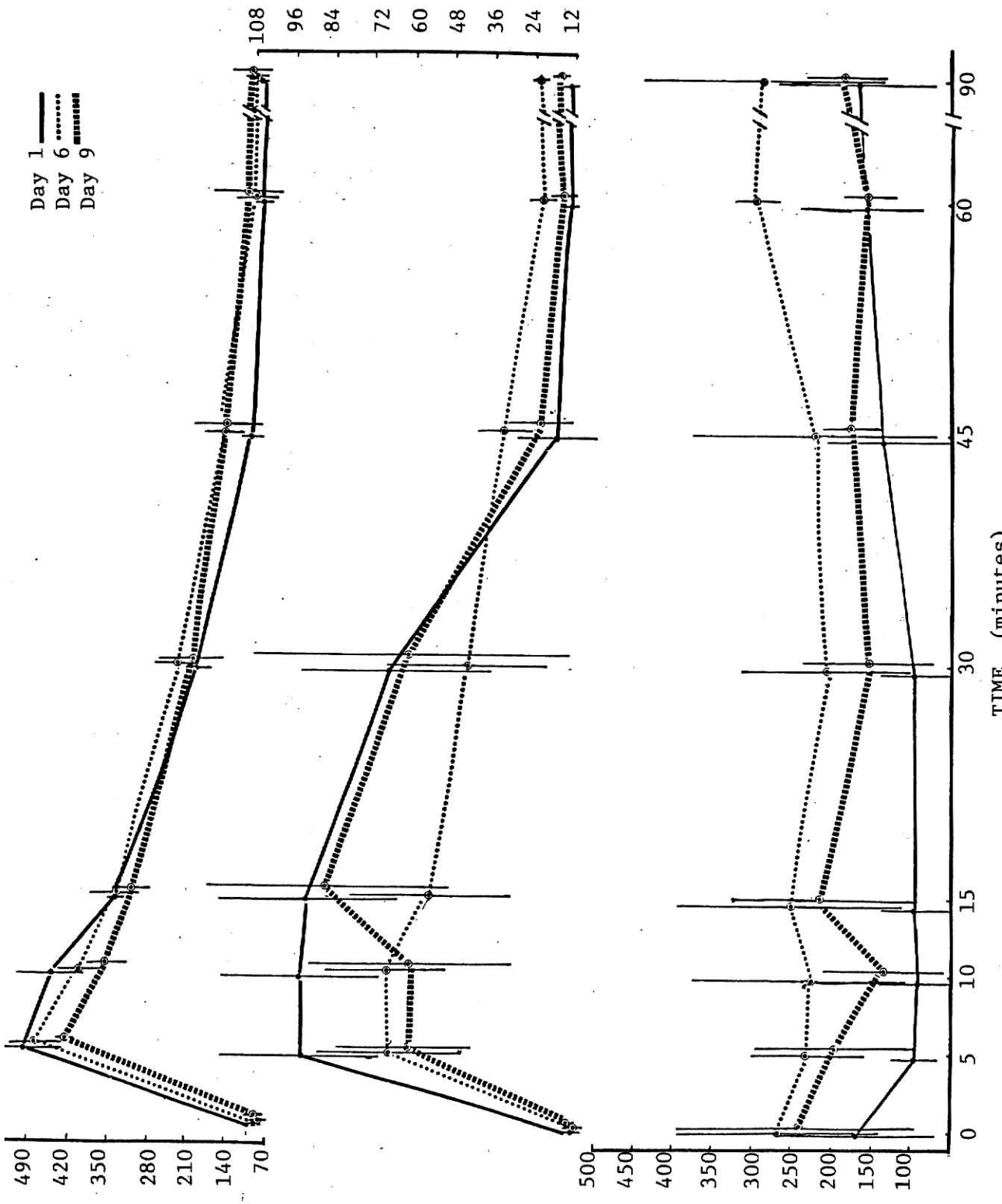

FIGURE 2: The means and standard deviations for plasma glucose, insulin and glucagon during a H-IVGTT for group II.

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APPENDIX

TABLE 4: Means for all dogs of fasting plasma glucose, insulin and glucagon.

Variable	Day	Plasma Level	Standard Error	Range
Glucose (mg/dl)	1	93.3	3.15	80-107
	2	93.8	3.15	81-112
	3	94.5	3.15	88-101
	4	95.2	3.15	86-112
	5	94.9	3.15	83-123
	6	90.3	3.15	78-110
	7	101.2	3.15	86-130
	8	103.2	3.15	90-127
	9	96.6	3.15	88-114
Insulin (μU/ml)	1	13.2	1.14	8-17
	2	16.0	1.14	12-25
	3	15.4	1.14	8-21
	4	17.9	1.14	9-30
	5	14.9	1.14	9-22
	6	13.5	1.14	7-19
	7	16.7	1.14	6-33
	8	17.1	1.14	9-27
	9	15.9	1.14	9-24
Glucagon (pg/ml)	1	232	30.8	72-380
	2	287	34.7	180-390
	3	279	34.7	163-420
	4	277	29.7	181-230
	5	241	34.7	100-370
	6	440	32.8	236-720
	7	393	34.7	240-639
	8	283	34.7	153-505
	9	255	32.8	132-440

TABLE 5: Measured insulin (ΣI_t) and glucagon (ΣG_t) during a H-IVGTT and calculated values of I/G ratio, glucose disappearance and K values.

Group	Dog	Day	Insulin(ΣI_t) (μU/ml)	Glucagon(ΣG_t) (pg/ml)	Insulin/Glucagon	Glucose Disappearance (mg/dl/min)	K values
I	1	1	110	1264	.08703	5.889	3.043
I	1	6	92	2261	.04069	5.248	2.437
I	1	9	68	1541	.04413	4.745	1.554
I	2	1	66	1890	.03492	6.940	3.295
I	2	6	119	2640	.04508	7.560	3.149
I	2	9	88	1995	.04411	5.069	2.111
I	3	1	137	1430	.09580	6.956	2.411
I	3	6	117	1940	.06031	6.307	1.825
I	3	9	128	1150	.1113	3.874	1.461
I	4	1	235	1429	.1645	6.964	2.838
I	4	6	97	3200	.0303	5.385	2.122
I	4	9	70	910	.0769	4.170	1.854
I	5	1	71	1339	.0530	8.179	3.623
I	5	6	56	2067	.0271	5.750	2.283
I	5	9	31	1544	.0201	5.028	2.598
II	7	1	157	1130	.1389	6.137	2.768
II	7	6	171	2599	.0658	6.345	2.275
II	7	9	81	1933	.0419	3.991	1.411
II	8	1	339	867	.3910	9.318	4.057
II	8	6	227	1766	.1285	6.169	2.993
II	8	9	252	862	.2923	5.501	2.635
II	9	1	310	487	.6366	7.702	3.541
II	9	6	254	1075	.2363	7.413	3.264
II	9	9	249	1040	.2394	6.473	3.206

TABLE 6: Individual dog and day measurements of plasma glucose, insulin, glucagon, U.N., creatinine, G.P.T., A.P., amylase, lipase, P.C.V., Hb. and total leukocyte count.

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (U/ml)	Glucagon (pg/ml)	U.N. (mg/ dl)	Cr. (mg/ dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
I	1	1	0	81	15	259	17.0	.98	26	27	808	0.0	44	15.4	11,100
I	1	1	5	400	33	192									
I	1	1	10	350	41	152									
I	1	1	15	286	42	175									
I	1	1	30	196	53	142									
I	1	1	45	109	18	177									
I	1	1	60	78	13	167									
I	1	1	90	78	12	224									
I	1	1	120	78	12	155									
I	1	2	0	87	14	340									
I	1	3	0	88	14	163									
I	1	4	0	86	12	270									
I	1	5	0	90	14	173									
I	1	6	0	99	12	480	11.4	1.01	26	240	934	0.0	45	15	26,100
I	1	6	5	429	27	330									
I	1	6	10	312	34	328									
I	1	6	15	267	37	313									
I	1	6	30	200	32	225									
I	1	6	45	126	18	235									
I	1	6	60	107	16	350									
I	1	6	90	87	16	470									
I	1	6	120	87	14	450									
I	1	7	0	105	15	240									
I	1	8	0	110	16	283									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leukocyte Count
I	1	9	0	99	14	160	18.1	1.1	25	-	802	0.0	40	14.2	15,300
I	1	9	5	462	26	240									
I	1	9	10	413	29	300									
I	1	9	15	365	26	231									
I	1	9	30	277	26	230									
I	1	9	45	221	26	180									
I	1	9	60	201	19	200									
I	1	9	90	117	15	150									
I	1	9	120	98	15	140									
I	2	1	0	97	14	380	12.6	0.94	31	96	1060	0.0	52	17.2	30,500
I	2	1	5	485	37	230									
I	2	1	10	365	37	260									
I	2	1	15	303	32	250									
I	2	1	30	180	18	250									
I	2	1	45	100	13	240									
I	2	1	60	82	13	280									
I	2	1	90	83	14	290									
I	2	1	120	87	15	380									
I	2	2	0	90	14	390									
I	2	3	0	98	13	320									
I	2	4	0	90	17	390									
I	2	5	0	94	17	370									
I	2	6	0	83	19	540	8.1	0.89	30	258	1313	0.0	52	17.6	37,900
I	2	6	5	532	65	310									
I	2	6	10	404	52	230									
I	2	6	15	358	51	320									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
I	2	6	30	190	28	290									
I	2	6	45	134	18	400									
I	2	6	60	93	19	550									
I	2	6	90	85	17	530									
I	2	6	120	86	15	490									
I	2	7	0	97	15	390									
I	2	8	0	102	16	340									
I	2	9	0	88	16	440	17.1	1.1	45	-	1410	1.0	43	15.0	28,100
I	2	9	5	416	30	350									
I	2	9	10	360	42	280									
I	2	9	15	318	37	310									
I	2	9	30	229	26	220									
I	2	9	45	180	32	190									
I	2	9	60	125	17	205									
I	2	9	90	87	17	290									
I	2	9	120	77	14	260									
I	3	1	0	102	13	250	7.9	1.4	22	74	485	0.0	49	17.7	10,300
I	3	1	5	551	29	180									
I	3	1	10	422	41	210									
I	3	1	15	395	44	190									
I	3	1	30	251	52	190									
I	3	1	45	182	28	210									
I	3	1	60	143	21	200									
I	3	1	90	96	14	190									
I	3	1	120	106	16	200									
I	3	2	0	106	19	300									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
I	3	3	0	94	21	420									
I	3	4	0	112	21	390									
I	3	5	0	123	15	290									
I	3	6	0	110	17	430	14.7	1.2	92	514	1162	0.0	46	16.0	20,200
I	3	6	5	560	46	300									
I	3	6	10	493	36	300									
I	3	6	15	438	35	280									
I	3	6	30	327	35	220									
I	3	6	45	269	39	220									
I	3	6	60	198	28	190									
I	3	6	90	132	21	180									
I	3	6	120	112	17	200									
I	3	7	0	130	19	270									
I	3	8	0	122	19	280									
I	3	9	0	103	16	200	12.0	1.3	49	139	1111	0.0	47	16.0	12,600
I	3	9	5	403	37	200									
I	3	9	10	344	45	190									
I	3	9	15	317	37	150									
I	3	9	30	261	35	140									
I	3	9	45	220	46	140									
I	3	9	60	169	24	130									
I	3	9	90	132	14	160									
I	3	9	120	120	23	150									
I	4	1	0	107	15	336	10.8	1.1	140	54	934	0.0	51	17.7	10,300
I	4	1	5	472	65	263									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leukocyte Count
I	4	1	10	434	67	100									
I	4	1	15	382	74	120									
I	4	1	30	261	68	150									
I	4	1	45	155	34	190									
I	4	1	60	103	17	270									
I	4	1	90	89	17	340									
I	4	1	120	89	15	350									
I	4	2	0	81	16	180									
I	4	3	0	88	17	209									
I	4	4	0	90	16	230									
I	4	5	0	83	12	100									
I	4	6	0	85	16	720	12.7	0.99	79	408	1692	0.0	43	14.5	23,099
I	4	6	5	450	35	600									
I	4	6	10	366	29	440									
I	4	6	15	330	35	320									
I	4	6	30	233	36	360									
I	4	6	45	177	36	300									
I	4	6	60	135	22	460									
I	4	6	90	91	21	500									
I	4	6	120	81	18	500									
I	4	7	0	98	16	600									
I	4	8	0	94	20	200									
I	4	9	0	90	16	260	5.6	0.94	45	136	959	0.0	44	15.3	17,300
I	4	9	5	360	28	160									
I	4	9	10	318	33	130									
I	4	9	15	306	31	100									
I	4	9	30	231	31	60									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
I	4	9	45	168	26	80									
I	4	9	60	130	17	120									
I	4	9	90	101	16	240									
I	4	9	120	91	13	180									
I	5	1	0	80	14	290	13.1	0.87	77	43	380	0.0	52	17.7	12,700
I	5	1	5	347	54	210									
I	5	1	10	297	54	-									
I	5	1	15	253	48	-									
I	5	1	30	125	20	-									
I	5	1	45	69	8	-									
I	5	1	60	67	11	-									
I	5	1	90	80	14	-									
I	5	1	120	75	10	-									
I	5	2	0	86	13	-									
I	5	3	0	100	15	-									
I	5	4	0	97	16	-									
I	5	5	0	94	13	-									
I	5	6	0	78	13	-	15.6	0.86	81	961	833	0.6	33	11.8	8,600
I	5	6	5	321	51	-									
I	5	6	10	277	56	-									
I	5	6	25*	259	58	-									
I	5	6	35*	148	24	-									
I	5	6	45	90	19	-									
I	5	6	60	67	15	-									
I	5	6	90	57	14	-									
I	5	6	120	100	17	-									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
I	5	7	0	118	15	-									
I	5	8	0	127	16	-									
I	5	9	0	114	17	-	17.8	0.8	5140	470	833	0.2	37	13.0	23,300
I	5	9	5	512	58	-									
I	5	9	10	453	53	-									
I	5	9	15	388	33	-									
I	5	9	30	300	21	-									
I	5	9	45	232	16	-									
I	5	9	60	199	14	-									
I	5	9	90	143	17	-									
I	5	9	120	128	16	-									
I	6	1	0	87	15	255	18.0	1.24	44	38	1035	0.0	50	17.7	13,400
I	6	1	5	513	46	224									
I	6	1	10	380	47	222									
I	6	1	15	331	32	98									
I	6	1	30	167	-	113									
I	6	1	45	103	23	206									
I	6	1	60	79	13	221									
I	6	1	90	73	18	204									
I	6	1	120	76	17	237									
I	6	2	0	82	14	-									
I	6	3	0	88	15	-									
I	6	4	0	86	17	-									
I	6	5	0	83	14	-									
I	6	6	0	97	17	425	14.0	1.2	162	280	2323	0.03	40	13.1	25,100
I	6	6	5	471	46	283									

TABLE 6:

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leukocyte Count
I	6	6	10	384	44	388									
I	6	6	15	341	30	347									
I	6	6	30	237	-	212									
I	6	6	45	-	20	221									
I	6	6	60	129	18	191									
I	6	6	90	100	13	280									
I	6	6	120	89	13	326									
I	6	7	0	97	18	-									
I	6	8	0	90	16	-									
I	6	9	0	85	18	297	13.1	1.0	73	150	1414	0.0	42	14.2	16,300
I	6	9	5	350	18	130									
I	6	9	10	331	40	170									
I	6	9	15	283	38	190									
I	6	9	30	176	-	320									
I	6	9	45	110	16	220									
I	6	9	60	95	18	217									
I	6	9	90	92	17	300									
I	6	9	120	84	18	290									
II	7	1	0	86	8	255	20.9	1.2	38	20	1035	0.0	45	15.2	19,300
II	7	1	5	427	49	110									
II	7	1	10	380	52	144									
II	7	1	15	328	47	138									
II	7	1	30	209	35	143									
II	7	1	45	128	19	105									
II	7	1	60	100	12	235									
II	7	1	90	80	11	274									
II	7	1	120	83	5	290									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
II	7	2	0	88	12	370									
II	7	3	0	91	10	328									
II	7	4	0	92	8	335									
II	7	5	0	85	9	340									
II	7	6	0	98	7	480	10.6	0.84	39	356	707	0.0	42	14.2	37,900
II	7	6	5	410	42	303									
II	7	6	10	418	52	370									
II	7	6	15	368	29	418									
II	7	6	30	260	37	330									
II	7	6	45	190	24	378									
II	7	6	60	141	18	320									
II	7	6	90	119	14	488									
II	7	6	120	-	13	651									
II	7	7	0	86	6	639									
II	7	8	0	96	9	505									
II	7	9	0	96	9	430	12.6	1.1	30	214	1136	0.03	40	13.8	34,600
II	7	9	5	422	38	308									
II	7	9	10	358	23	226									
II	7	9	15	336	26	343									
II	7	9	30	267	17	246									
II	7	9	45	209	15	210									
II	7	9	60	194	16	170									
II	7	9	90	130	14	228									
II	7	9	120	95	10	216									
II	8	1	0	106	11	182	19.6	1.4	59	40	808	0.0	52	18.1	15,300
II	8	1	5	574	99	52									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
II	8	1	10	502	101	50									
II	8	1	15	350	102	80									
II	8	1	30	177	85	113									
II	8	1	45	79	9	250									
II	8	1	60	76	14	140									
II	8	1	90	71	16	139									
II	8	1	120	79	15	141									
II	8	2	0	97	14	240									
II	8	3	0	97	17	283									
II	8	4	0	94	30	215									
II	8	5	0	95	22	244									
II	8	6	0	86	11	370	8.1	1.2	79	199	656	0.0	45	15.0	30,800
II	8	6	5	430	91	245									
II	8	6	10	363	86	234									
II	8	6	15	292	50	209									
II	8	6	30	178	20	196									
00	9	6	45	119	28	212									
II	8	6	60	84	18	300									
II	8	6	90	91	13	235									
II	8	6	120	87	16	441									
II	8	7	0	113	12	322									
II	8	8	0	111	15	153									
II	8	9	0	98	14	132	14.9	1.2	55	135	111	0.0	45	15.8	22,800
II	8	9	5	424	65	160									
II	8	9	10	312	74	113									
II	8	9	15	291	102	122									
II	8	9	30	174	52	76									
II	8	9	45	129	33	140									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leukocyte Count
II	8	9	60	95	18	119									
II	8	9	120	90	17	189									
II	9	1	0	91	17	72	18.6	1.4	30	41	1237	0.0	45	15.0	12,900
II	9	1	5	493	96	105									
II	9	1	10	425	92	83									
II	9	1	15	342	91	33									
II	9	1	30	192	88	35									
II	9	1	45	104	30	63									
II	9	1	60	77	15	96									
II	9	1	90	87	13	101									
II	9	1	120	93	13	225									
II	9	2	0	112	25	236									
II	9	3	0	101	18	260									
II	9	4	0	105	20	181									
II	9	5	0	106	16	192									
II	9	6	0	82	16	236	17.8	1.3	60	80	1868	0.0	47	15.4	2,400
II	9	6	5	497	55	165									
II	9	6	10	405	65	77									
II	9	6	15	370	85	132									
II	9	6	30	230	75	100									
II	9	6	45	133	43	85									
II	9	6	60	82	27	280									
II	9	6	90	73	17	155									
II	9	6	120	86	17	162									
II	9	7	0	86	33	302									
II	9	8	0	90	27	243									

TABLE 6: continued

Group	Dog	Day	Time	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	U.N. (mg/dl)	Cr. (mg/dl)	G.P.T.	A.P.	Amylase	Lipase	P.V.C. (%)	Hb. (g/dl)	Total Leuko-cyte Count
II	9	9	0	96	24	156	20.0	1.2	38	57	1035	0.0	47	15.6	12,400
II	9	9	5	422	82	125									
II	9	9	10	387	86	67									
II	9	9	15	292	89	190									
II	9	9	30	167	118	145									
II	9	9	45	90	20	182									
II	9	9	60	86	17	175									
II	9	9	90	96	18	134									
II	9	9	20	103	17	153									

TABLE 7: The means and standard deviation of plasma glucose, insulin and glucagon during a H-IVGTT at prior to (0) and 5, 10, 15, 30, 45, 60, 90 and 120 minutes.

Insulin (μU/ml)

Group I	0	5	10	15	30	45	60	90	120
1	14.4±0.89	42.0±14.3	46.6±11.9	44.8±17.2	47.8±21.1	23.2± 8.2	15.4±3.6	15.0±2.5	15.0±1.9
6	16.2±2.59	43.8±14.3	39.0± 9.1	37.6± 7.9	32.7± 3.6	26.2±10.4	20.6±4.7	17.6±3.4	15.4±2.1
9	16.0±1.41	32.2± 6.0	37.4± 6.5	31.6± 5.3	29.5± 4.4	29.2±11.1	19.0±2.9	15.8±1.3	16.6±4.0
Group II									
1	12.0±4.6	81.3±28.0	81.7±26.1	80.0±29.1	69.3±30.0	16.3±11.9	13.7±1.5	13.3±2.5	11.0±5.3
6	11.3±4.5	66.0±21.7	67.7±17.2	54.7±28.3	44.0±25.1	32.0± 9.6	21.0±5.2	14.7±2.1	15.3±2.1
9	15.7±7.6	61.7±22.2	61.0+33.5	72.3±40.6	62.3±51.3	22.7± 9.3	15.3±2.1	16.3±2.1	12.0±4.4

TABLE 7: continued

Glucagon (pg/ml)

Group I	0	5	10	15	30	45	60	90	120
1	296± 59	218± 33	189± 63	166± 60	169± 53	205± 24	228±47	250± 63	264± 97
6	519±122	365±133	337± 81	316± 24	261± 63	275± 77	204±92	392±154	393±128
9	299±102	210± 98	193± 63	188± 90	185±111	158± 61	168±50	255± 73	220± 66
Group II									
1	169± 92	89± 32	92± 48	83± 53	97± 55	139± 98	157±71	171± 91	218± 75
6	262±122	237± 69	227±146	253±148	208±116	225±147	300±20	293±174	418±245
9	239±166	198± 97	135± 82	218±113	155± 86	177± 35	155±30	184± 47	208± 52

TABLE 7: continued

Glucose (mg/dl)

Group I	0	5	10	15	30	45	60	90	120
1	94.8±10	484±56	390±36	339±48	211±42	130.0±37	97.0±28	83.8± 9	91.4±11.6
6	94.8±11	488±56	392±66	347±62	237±54	176.0±66	132.0±40	99.0±19	91.0±12.0
9	93.0± 8	398±45	353±37	318±30	234±39	180.0±46	144.0±41	105.8±18	94.0±16.0
Group II									
1	94.3±10	498±74	436±62	340±11	193±16	103.7±15	84.3±13	79.3± 8	85.0± 7.0
6	88.7± 8	479±43	395±29	343±44	223±42	147.0±38	102.3±33	91.3±18	87.0± 1.0
9	96.7± 1	422±1.2	352±38	306±26	203±56	142.0±61	125.0±60	104.0±23	93.0±11.0

THE EFFECTS OF PARTIAL PANCREATECTOMY AND ACUTE STAPHYLOCOCCAL ALPHA-TOXIN PANCREATITIS ON THE PLASMA GLUCOSE, INSULIN AND GLUCAGON DURING A H-IVGTT IN THE DOG

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D.V.M., University of Georgia, 1972

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Infectious Diseases

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1976

ABSTRACT

The relationship between pancreatic injury and the K-value of the high-dose intravenous glucose tolerance test (H-IVGTT), plasma glucose, plasma insulin and plasma glucagon was explored. Two groups of dogs were used: group I had a 50% partial pancreatectomy combined with staphylococcal alpha-toxin pancreatitis, and group II had a 50% partial pancreatectomy. The surgery was done one day 5. One days 1, 6 and 9, H-IVGTT's were performed and radioimmunoreactive insulin (IRI) and true glucagon were measured prior to (0) and 5, 10, 15, 30, 45, and 60 minutes. Various other perameters were measured prior to the H-IVGTT (U.N., CR., S.A.P., S.G.P.T., CBC). Morning, fasting samples of plasma glucose immunoreactive insulin (IRI) and plasma glucagon were measured for each dog, each day of the experiment.

The K-value, as calculated from the H-IVGTT, was found to significantly decrease in both groups on day 6 compared to day 1 and on day 9 compared to day 6. However, the decrease in K-value was greatest for group I on day 9.

A morning, fasting IRI was measured for each dog and did not change significantly for any of the dogs throughout the experiment. The morning, fasting plasma glucose values, likewise, did not change significantly. The fasting, morning glucagon values were significantly increased on days 6 and 7 in both groups with the greater increase in group I. This mild hyperglucagonemia caused a slight but not significant rise in plasma glucose in group I on days 7 and 8.

Although the fasting IRI was normal, deficiencies of insulin secretion could be noted during the H-IVGTT. The total amount of measured IRI released during the H-IVGTT (ΣI_t) decreased significantly on day 6

compared to day 1 and on day 9 compared to day 6 in both groups. The total glucagon released during the H-IVGTT (ΣG_t) increased on day 6 significantly and then removed to normal on day 9 in each group.

We were able to demonstrate that the pancreas, after a 50% partial pancreatectomy, could maintain fasting levels of plasma glucose, IRI and glucagon. The ability of the pancreas to release insulin was impaired during the H-IVGTT (decreased ΣI_t) but the total glucagon measured during the H-IVGTT (ΣG_t) did not decrease after partial pancreatectomy, suggesting another source of glucagon besides the pancreas.