The Effect of GLP-1 (Glucagon-Like Peptide 1) on Insulin Sensitivity in Diabetic Dogs.

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Physiology
University of Toronto

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Abstract

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It is unclear whether GLP-1 increases insulin sensitivity in addition to stimulating insulin secretion. We studied depancreatized dogs to eliminate GLP-1’s incretin effect. Somatostatin was infused to inhibit extrapancreatic glucagon. Basal glucagon levels were restored by intraportal infusion. Basal intraportal insulin was infused to attain plasma glucose levels of ~10 mM. Glucose was clamped at this level for the remainder of the experiment, which includes peripheral infusion of GLP-1 (1.5 pmol/kg.min) and insulin at either a high or low dose. In 12 paired experiments at the high insulin dose, GLP-1 infusion resulted in higher glucose requirements than controls. This was due to greater glucose utilization whereas glucose production was not further suppressed with GLP-1. FFA and glycerol suppression was also greater with GLP-1 than in controls. In 10 experiments at the low insulin dose, GLP-1 infusion did not affect glucose utilization, or FFA and glycerol suppression. Conclusion: GLP-1 potentiates insulin-stimulated glucose utilization in diabetic dogs, possibly due to GLP-1’s potentiation of insulin’s antilipolytic effect.
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NIDDM

NIDDM (non-insulin dependent diabetes mellitus) is a pathological condition characterized by two main defects: impaired insulin secretion and insulin resistance. While it is uncertain which of these two defects appears first in these patients, both are required for NIDDM to develop (1). NIDDM is consequently associated with many metabolic disorders. Because insulin secretion is impaired, coupled with the decrease in insulin action, hyperglycemia develops. Hepatic glucose production is increased and this is due to an increased rate of gluconeogenesis, while glycogenolysis is similar in NIDDM and non-diabetic individuals (2). This effect may, in part, be due to elevated glucagon secretion, a common phenomenon in diabetic subjects. Under the conditions of a hyperinsulinemic euglycemic clamp, whole-body glucose utilization is reduced in NIDDM patients when compared with non-diabetic subjects, due to decreased muscle glucose utilization (3). This may be explained, in part, by a downregulation of GLUT4 glucose transporters in the muscle due to the prevailing hyperglycemic conditions.

Glucose utilization is not significantly different in the adipose tissue or splanchnic tissues, under these conditions, between these groups. Once glucose has been taken into the cell, it can be stored as glycogen or be used for glycolysis. More than 90% of the glucose used for glycolysis by normal individuals is accounted for by glucose oxidation, whereas the remainder is used for anaerobic glycolysis to produce lactate. In both obese and normal-weight people with NIDDM, both glycogen synthesis and glucose oxidation are impaired, with a greater decline in glycogen
synthesis as compared with glucose oxidation (4). The impairment of glucose oxidation and glycogen synthesis can be explained in part by the impairment of insulin's antilipolytic action in diabetes. Increased lipolysis results in increased free fatty acid levels which in turn compete for oxidation with glucose in the Randle Cycle. Increased free fatty acid oxidation results in increased acetyl-CoA levels which inhibit pyruvate dehydrogenase. The increased rate of free fatty acid oxidation results in an increase in the NADH/NAD ratio, which in turn inhibits the Krebs cycle, and also results in citrate accumulation (citrate is synthesized from Acetyl CoA and oxaloacetate). Citrate inhibits phosphofructokinase, eventually resulting in decreased glucose transport into the cell. Acetyl-CoA also results in inhibition of glycogen synthase, an effect which in turn contributes to decreased glycogenesis. The increase in free fatty acid levels also contributes to increased lipid oxidation in the liver which results in an increase in gluconeogenesis and hepatic glucose production. These problems are amplified by the vicious cycle that exists between insulin resistance and insulin deficiency. If it is assumed that insulin resistance is the primary cause of NIDDM, the scenario can be seen as follows: the insulin resistance in the insulin target tissues can lead to chronic hyperglycemia, which in turn could either impair B-cell function due to glucose toxicity or lead to B-cell exhaustion as a result of hypersecretion of insulin to counteract the prevailing hyperglycemia. If insulin deficiency, however, is the primary cause of NIDDM, then the insufficient insulin secretion will result in hyperglycemic conditions which, through various mechanisms including activation of the glucosamine pathway as well as through activation of protein kinase C, will lead to greater insulin resistance in target tissues. Thus, it is quite evident that effective therapy is essential to
control hyperglycemia, which can only aggravate the course of NIDDM.

Glycemic control in NIDDM patients is improved with diet and exercise therapy, approaches which have been shown to enhance primarily insulin action and also improve B-cell function (5). However, if these therapies fail, then therapeutic use of pharmacological agents is required. The ideal drug to be administered to such patients should improve both insulin secretion and insulin action. Sulfonylureas are common drugs currently in use for treatment of NIDDM patients. These agents are insulin secretagogues (6). This form of therapy has its own drawbacks in that the insulin secretion mechanism of sulfonylureas is not glucose dependent, and as such may lead to hypoglycemia. Additionally, sulfonylureas do not enhance insulin gene transcription and thus may lead to B-cell exhaustion as a result of their insulinotropic action. The biguanide metformin has been shown to reduce gluconeogenesis in the liver and improve glucose utilization in the periphery, an effect attributable, in large part, to increased insulin action (7). Additionally, a recent class of drugs known as thiazolidinediones ("glitazones") have been developed. These drugs have potential as therapeutic agents for type II diabetes as their side effects are apparently less than those of metformin. The glitazones have been shown to improve insulin sensitivity in type II diabetic individuals (8). They have been shown to attenuate both the hyperglycemia and the hyperinsulinemia that are characteristic of these patients (9). Neither metformin nor glitazones, however, enhance insulin secretion like sulfonylureas. The search for a pharmacological drug which can aid in the effective glycemic control of NIDDM patients without the risk of hypoglycemic complications have led researchers to investigations of the properties of a new agent, glucagon-like peptide 1 (GLP-1).
GLP-1 is a peptide derived from the preproglucagon gene which is expressed in the L cells of the distal ileum, the alpha cells of the pancreas and some neurons in the Central Nervous System (10,11). The gene contains six exons and five introns. The post-translational processing of the prohormone is tissue-specific and different (12). In the pancreas, the main products are glucagon, glicentin related pancreatic peptide and the major proglucagon fragment. The major fragment is not further processed to GLP-1 and GLP-2 (13). The major products from the intestine are glicentin, oxyntomodulin, GLP-1, GLP-2 and a small intervening peptide called spacer peptide-2. Biologically inactive GLP-1(1-37) is further processed to the truncated form of GLP-1(7-37) by cleaving six amino acids from the N-terminus. The C-terminal amino acid may also be removed and the new C-terminal amino acid is amidated resulting in the GLP-1(7-36) amide isoform (10,14,15). The truncated forms of GLP-1 are biologically active (16). The biological activity and receptor binding are mainly mediated by the N-terminus, although the removal of the last three C-terminal amino acid residues results in partial loss of activity (17). The deletion of one amino acid, a histidine, at the N-terminus resulting in GLP-1(8-37) greatly decreases activity (18).

The term "incretin" was coined by Zunz and Labarre in 1929 to describe the hormones which are produced in the gastrointestinal tract in response to nutrients and which potentiate pancreatic insulin secretion (19). Both truncated forms of GLP-1 have identical effects on insulin secretion (10,17) and are the most potent gluco-incretins so far described (20). The term "gluco-incretin" refers to insulin secretion in a glucose dependent manner, meaning that the
stimulatory effect of gluco-incretins requires the presence of glucose at 5 mM or above (14). Both truncated forms of GLP-1 are active at concentrations as low as 1-10 pM (14).

Plasma GLP-1 concentrations increase after a meal (21). Oral administration of glucose has also been shown to increase GLP-1 concentrations (13). Intravenous glucose infusion however does not stimulate GLP-1 release (22). GLP-1 plasma levels increase within 10 to 15 minutes after the intake of mixed food. Since the GLP-1 secreting L cells are found in the distal ileum and proximal colon, it is unlikely that they only respond to luminal nutrients. Early GLP-1 secretion has been shown to be induced by neural or hormonal signals from the proximal gut (23). Gastric inhibitory peptide has been shown to be one of the signals (24).

**GLP-1 receptor**

GLP-1 is a peptide which must bind to a cell membrane bound receptor so that its biological activity can be transmitted into the interior of the cell. Insulinoma cell lines and isolated rat islets (15,25) that have a GLP-1 receptor have provided a model to study GLP-1 binding. In several studies on pancreatic cells, GLP-1 binds to the receptor with a Kd, a measure of binding affinity, of between 0.2 nmol/l and 3.3 nmol/l depending on the particular cell line (15). Scatchard analysis of binding in RINm5f cells revealed a single class of binding sites (26). In addition, the covalent linkage of a radioactive form of the peptide to the receptor revealed on the Western Blot, a single band of 63 kDa (27). Taken together, the studies above point to the existence of a single GLP-1 receptor. Glucagon has been able to displace GLP-1 from the receptor with a much lower affinity than GLP-1 (28,29). This correlates well with the fact that glucagon is much less potent than GLP-1 at secreting insulin in the pancreatic B-cell (17,30). The GLP-1 receptor is specific for GLP-1, yet one study has suggested that exendin
(9-39)amide acts through a common receptor on pancreatic B-cells (31).

The GLP-1 receptor has been associated with class II of the superfamily G-protein-coupled receptor (32,33). Bell localized the human GLP-1 receptor gene to chromosome band 6p21 using fluorescence in situ hybridization (34). The rat pancreatic receptor gene has recently been cloned by Thorens (14) and encodes a 463 amino acid peptide. Based on the groups of hydrophobic amino acids, the chain contains 7 transmembrane domains. It is structurally related to secretin, parathyroid hormone, calcitonin, vasoactive intestinal peptide, growth hormone releasing hormone and glucagon receptors, as reviewed by Bell (34).

EFFECTS OF GLP-1

Mechanism of GLP-1 induced insulin secretion

Glucose-induced insulin secretion by B-cells requires the uptake of glucose by glucose (GLUT 2) transporters. The glucose metabolism within the cell results in a rise in the cytoplasmic ATP/ADP ratio. This in turn induces the closure of an ATP-dependent K+ channel. This block in K+ efflux depolarizes the plasma membrane, leading to the opening of a voltage-gated calcium channel. The resulting increase in intracellular calcium concentration triggers the exocytosis of insulin-containing secretory granules (35-37) and thus increases insulin secretion.

Among the first effects of GLP-1 on the mechanisms of insulin secretion found, was its ability to increase cAMP levels (17,38). However, studies have also shown that at higher supraphysiological concentrations GLP-1(7-37) decreases cAMP production (16,17,38). This
response to GLP-1(7-37) or GLP-1(7-36) amide was shown to be due to a rapid and reversible homologous desensitization of the GLP-1 receptor. Agonist-induced receptor desensitization has been shown for other G-protein receptors (39).

It was postulated that GLP-1 could increase cAMP levels by either stimulating its production by adenylate cyclase or inhibiting phosphodiesterase from degrading cAMP. Goke et al. found that guanine nucleotides (GTP and two others) significantly reduced the binding of GLP-1(7-36) amide to membranes of RINm5F (40). They also found, using the Scatchard method, that guanine nucleotides decreased the affinity of GLP-1 for receptors. From this information, it was likely that GLP-1 is coupled to the adenylate cyclase system by a nucleotide regulatory protein subunit and that the cAMP increase is not due to the inhibition of the phosphodiesterase. Several other groups have confirmed that the GLP-1 receptor is coupled to the adenylate cyclase system most likely by a G-protein (14,33,38,39).

The induction of glucose competence by GLP-1 stemmed from its ability to modulate a step in the glucose signalling pathway. Holz and Habener (41,42) hypothesized that a likely target for GLP-1’s modulation was the ATP-sensitive K+ channel. The authors conducted studies on single B-cells using patch clamp techniques and observed a synergistic interaction between glucose and GLP-1. The glucose and GLP-1 inhibit the activity of the ATP-sensitive K+ channel. Holz and Habener proposed a model in which glucose competence (sensitivity of the B cell to the insulin secretory effect of glucose) is a result of crosstalk between signalling systems. In this model, the GLP-1 G-protein coupled receptor activates adenylate cyclase and increases intracellular cAMP levels. Activated PKA then increases the affinity of the channel for ATP, resulting in channel closure.
It is still unclear whether the increased affinity of the K+ channel for ATP results from phosphorylation of this channel by PKA. Holz and Habener showed that GLP-1 decreased the activity of K+ ATP-sensitive channels, however they did not show that cAMP dependent PKA phosphorylates the ATP-sensitive K+ channel. It is not clear whether the channel itself can be phosphorylated. In addition, the decreased activity of the ATP-sensitive K+ channel may be an indirect effect of PKA and cAMP because one electrophysiological study on RINm5F insulinoma cells failed to detect any direct effect of either the catalytic subunit of protein kinase A or of increased cAMP levels on ATP-sensitive K+ channel activity (43).

Lu et al. have demonstrated that GLP-1 increased cAMP levels, raised the free cytosolic calcium level and stimulated insulin secretion in a glucose dependent manner in transformed hamster insulin tumour (HIT) cells (44). To determine the Ca2+ source, they used nimodipine, an inhibitor of the L-type voltage-dependent Ca2+ channel, and found that the increase in Ca2+ induced by GLP-1 was abolished and glucose-stimulated insulin secretion was greatly reduced. They also used chelating factor EGTA in the extracellular fluid and found that the increase in Ca2+ levels was abolished. Nimodipine and EGTA did not inhibit the GLP-1 stimulatory effect on cAMP accumulation. They concluded that GLP-1 potentiates glucose-stimulated insulin secretion by increasing extracellular Ca2+ influx through voltage-dependent Ca2+ channels.

However, Wheeler et al. have shown that GLP-1 can control other sources of Ca2+ (45). They used a monkey kidney cell (COS-7) transfected with a high affinity rat GLP-1 receptor by Thorens (14). They found that EGTA did not abolish the initial transient elevation of intracellular Ca2+ due to exposure of glucose and GLP-1. This points to the endoplasmic
reticulum as a source of Ca2+. They applied Ca2+ L type blocker verapamil and other blockers instead of EGTA and again found that they could not stop the rise in intracellular Ca2+ levels. GLP-1 was found to activate PLC and increase inositol levels in the cells. In this study, the authors linked GLP-1 to two G-protein-coupled signalling pathways, the adenylate cyclase system and PLC. The related receptor for glucagon has been shown to stimulate adenylate cyclase and phosphoinositol turnover in liver cells and mobilize intracellular Ca2+. The related receptors for PTH and GH-releasing hormone have been associated with the stimulation of cAMP, phosphoinositol turnover, and Ca2+ mobilization. Wollheim et al. have suggested that PKC might inhibit the ATP dependent K+ channel like other secretagogues (46). This inhibition might be through a phosphorylation. PKC might also sensitize the exocytosis machinery to Ca2+ levels. In Wollheim’s study, they demonstrated that D-glyceraldehyde: 1) increases the intracellular concentration of DAG (activator of PKC) before any change in the intracellular ATP concentration, and 2) closes the ATP dependent K+ channel and stimulates insulin secretion. They also showed that activators of PKC mimicked the effect of glyceraldehyde on ATP sensitive K channels. In another study, the authors found that the PKC inhibitor, staurosporine, reduced the effect of GLP-1 on insulin secretion (47). This would support the hypothesis that GLP-1 is linked to PKA and possibly PLC. However, other studies have shown opposite results (48). Thorens, the investigator who originally cloned and transfected the GLP-1 receptor to the COS-7 cell, also studied the effects of GLP-1 on the production of inositol phosphate and mobilization of intracellular calcium (33). He could not find any effects. Thorens also repeated the study in stable chinese hamster lung fibroblasts and several insulinoma cell lines expressing GLP-1 receptors and found no production of inositol
phosphate and mobilization induced by GLP-1. The contrasting results do illustrate the point that cell lines can be variable and perhaps even small differences in methods can lead to differences in results. Cell lines are a more convenient and cheaper model than islets to study the mechanisms of glucose-induced insulin release. Islets are difficult and expensive to isolate. Unfortunately, most cell lines exhibit differences in their glucose stimulated insulin secretory responses compared to normal islets.

**Insulin-releasing properties of GLP-1**

The insulin releasing properties of GLP-1 were first reported in 1987 using the perfused rat pancreas (49,50). Kreymann et al. reported that in man, GLP-1 plasma concentrations increased after a meal and that a GLP-1 infusion increased insulin infusion (21). GLP-1 seems to be only effective in increasing insulin secretion in the presence of elevated glucose levels. In several studies in the perfused rat pancreas model, insulin was secreted only in the presence of glucose levels above 5 mmol/l (51). In another study, the metabolic inhibitor mannoheptulose abolished the response of the perfused rat islets to GLP-1 (52). This indicates that glucose must be metabolized for GLP-1 to enhance insulin secretion. GLP-1 was found to be glucose dependent in subjects with NIDDM (53). GLP-1 has been found to increase both phases of insulin secretion (16,54).

Holz and Habener have reported that GLP-1 confers glucose sensitivity to glucose resistant B-cells, a phenomenon they refer to as glucose competence (41,42). Holz and Habener used the patch-clamp recording technique to do electrophysiological studies of single B-cells isolated from dispersed islets of Langerhans. The majority of the cells showed little or no depolarizing response when initially exposed to a stimulatory concentration (10 mM) of glucose.
The authors indicate that this is expected since under the in vitro conditions, the B-cells are no longer regulated by circulating hormones that maintain the glucose-competent state. The simultaneous exposure of glucose and GLP-1 rendered the initially glucose-insensitive B-cells fully responsive. Moreover, the exposure of the two substances did not need to be simultaneous. The prior application of either glucose or GLP-1 followed by a ten minute wash out period, 'primed' the cells to be responsive when the second substance was added. They postulated that this 'priming' effect was due to prolonged action of cystolic second messengers and/or slow dissociation of GLP-1 from its receptor.

Effects of GLP-1 on insulin gene transcription

GLP-1 has been found to stimulate insulin gene transcription and insulin accumulation in secretory granules in insulinoma cells and rat islet cell lines (38,51,55,56). GLP-1 may regulate insulin gene transcription through a cAMP-dependent PKA mechanism. Fehmann and Habener have proposed a model of how GLP-1 stimulates insulin biosynthesis. GLP-1 stimulates adenylate cyclase activity which leads to increased cAMP levels. PKA is activated by the increased cAMP levels and phosphorylates the CREB protein and activates it. The CREB protein binds to the insulin gene and enhances transcription. Thus, GLP-1 by stimulating transcription can replenish the intracellular supply of insulin depleted by secretion by potent secretagogues like itself and thus avoid beta cell exhaustion. Sulphonylureas, in contrast do not enhance insulin gene transcription.

Other Effects of GLP-1 Independent of its Incretin Effect
The possibility that GLP-1 might also regulate glucagon and somatostatin has led to studies of GLP-1 effects on A and D cells. GLP-1 has been shown to increase somatostatin secretion in rat islets (57) as well as in isolated perfused canine and rat pancreases (30). However, GLP-1 has been shown to either inhibit (58) or have no effect (16) on glucagon secretion. Since hyperglycemia contributes to the impaired glucose tolerance in NIDDM patients (59), the effect of GLP-1 on glucagon secretion are very important. GLP-1 receptors have been found on B-cell lines (26,39), and have been found on the somatostatin secreting cell line RIN1027-B2 (60). Specific binding sites for $^{125}$I-GLP-1 have been found on A, B and D cells in isolated rat islets (57) but mRNA for the GLP-1 receptor could not be found in the A cells using Northern RNA blotting techniques (61). However, a recent study has found GLP-1 receptor gene expression on A cells using reverse transcription PCR followed by Southern blot analysis (62). Whether the suppression of glucagon is a direct effect mediated through the receptors in the A cells or indirect via somatostatin stimulation by GLP-1, studies exist which illustrate that GLP-1 has a suppressive effect on glucagon levels, independent of its incretin effect (58,63).

GLP-1 has also been shown to delay gastric emptying (64). This effect leads to a lowering of blood glucose levels since the delay in gastric emptying slows the absorption of nutrients which in turn attenuates post-prandial glucose peaks. This action of GLP-1 mimics the effects of dietary fibres and acarbose and could be important in post-prandial glucose control. However, since the action of GLP-1 on insulin secretion is glucose dependent, if the postprandial glucose levels are less, this could potentially diminish the incretin effect of GLP-1.

These blood glucose lowering actions of GLP-1 (the incretin effect, suppression of
glucagon secretion and delayed gastric emptying) have created much interest in the pursuit of GLP-1 as a pharmacological treatment for NIDDM. The interest in GLP-1 stems from the fact that the incretin effect elicited by GLP-1 is known to be glucose dependent and thus protect patients from the risk of hypoglycemia associated with insulin replacement therapies and the use of drugs like sulfonylureas. However, there is also some evidence that GLP-1 may improve insulin action, independent of the properties listed above. There is, however, much controversy surrounding this last point. A number of studies have been performed to investigate this potential property of GLP-1.

**Effects of GLP-1 on Insulin Action**

A number of *in vitro* studies have been performed in order to determine whether GLP-1 receptors are present in the insulin sensitive tissues. Much of this work has only been published in abstract form. The studies can be subdivided into two groups, those using binding methodology and those investigating the presence of mRNA for the GLP-1 receptor. Delgado et al. have found the presence of GLP-1 binding in rat skeletal muscle (65), while Villanueva-Penacarrillo et al. have found the presence of GLP-1 binding in the rat liver (66). Blackmore et al. however found no evidence for presence of GLP-1 receptors in isolated liver hepatocytes using binding studies (67). Valverde et al. have reported the presence of GLP-1 binding in rat adipose tissue (68) and Merida et al. have also found GLP-1 binding sites in human adipose tissue (69). Kieffer et al. found that GLP-1 binds to 3T3-L1 adipocytes (70). These results from binding studies should be taken with caution, since GLP-1 is known to be structurally related to a number of different hormones that bind to G-coupled receptors (34). Thus, positive
binding studies do not necessarily reflect the presence of GLP-1 receptors due to the possibility of cross binding to one of these receptors. More definitive evidence can be obtained from studies that have attempted to locate GLP-1 receptor gene expression in these tissues. Egan et al. have found the presence of mRNA for the GLP-1 receptor in fat, skeletal muscle and liver tissues of rats using reverse transcription PCR followed by Southern blotting (71). Wheeler et al. found GLP-1 receptor gene expression in rat muscle and liver using Northern blot hybridization (45). Thorens et al. (14) however found no mRNA for the GLP-1 receptor in rat liver or muscle. Bullock et al. (72) found no mRNA for the GLP-1 receptor in skeletal muscle, liver or adipocytes in rats using in situ hybridization. Campos et al. found the presence of receptor gene expression for GLP-1 in the mouse liver (73). Wei et al. (74) could find no expression of the pancreatic GLP-1 receptor in human skeletal muscle, liver or adipose tissue using RNase protection. They did not however exclude the possibility that a pharmacological levels of GLP-1 bind to different receptors in these tissues. This possibility is supported by recent data by Kieffer et al. (70) and Montrose-Rafizadeh et al. (75).

The presence of a receptor does not necessarily indicate that there is an action. *In vitro* studies investigating the presence of an insulin-like or insulin-potentiating action in these insulin sensitive tissues have yielded conflicting results. Furnsinn et al. looked at skeletal rat muscle and found that GLP-1 failed to affect glycogen synthesis (76). Nakagawa et al. found that GLP-1 had no insulin-like effects on glucose metabolism in isolated rat hepatocytes (77). However, a number of studies also indicate that there are insulin-like or insulin-potentiating actions of GLP-1 *in vitro*. One study (78) performed on rat skeletal muscle shows that glycogen synthesis is stimulated in the presence of GLP-1, as is true in the presence of insulin. Glucose oxidation
and glucose utilization were also measured and found to increase significantly with GLP-1. Yang et al. (79) found that GLP-1 enhanced insulin-stimulated glycogen synthesis in rat L6 muscle cells. Valverde et al. also have found a potent glycogenic effect of GLP-1 in rat liver (80). Yamatani et al. have found that GLP-1 inhibits glucagon induced glycogenolysis in the perfused liver (81). Wang et al. have reported that GLP-1 increases glucose transport and transporters (GLUT1 and GLUT4) in 3T3-L1 adipocytes (82). Oben et al. have found that GLP-1 enhances [14C]acetate incorporation into fatty acids in explant of rat adipose tissue (83). Egan et al. have also found that GLP-1 enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes (71), increasing glucose uptake and glucose incorporation into fatty acids. Miki et al. have reported that GLP-1 enhances insulin-stimulated glucose uptake and decreases intracellular cAMP content in isolated rat adipocytes (84). However, on fat, there appear to be two opposite effects of GLP-1. Ruiz-Grande et al have found that isolated rat adipocytes exhibit increased lipolytic action in the presence of GLP-1 (85). Furthermore, this effect was found to be mediated by an increase in intracellular cAMP levels which was mediated by GLP-1. This was determined to be the same effect that glucagon elicited in this tissue through the same mechanism (increased cAMP levels). Furthermore, GLP-1 did not compete with [125I]glucagon in binding to the glucagon receptor. Thus, the effect was attributed to GLP-1’s action through its own receptor.

In vivo studies have also yielded controversial results. There are many studies illustrating negative results in vivo. Radziuk et al. (86) studied the effects of GLP-1 infusion on systemic and tissue glucose fluxes in normal pigs and found no effect of GLP-1 independent of its incretin effect. Myers et al. (87) found no effect of GLP-1 administered subcutaneously
in normal dogs during a hyperinsulinemic hyperglycemic (\(\sim\) 150 mg/dl) clamp using somatostatin. Freyse (63) found no effect of GLP-1 in insulin-deprived diabetic dogs, independent of its suppression of glucagon. Toft-Neilson (88) found that there were no insulin-independent effects of GLP-1 on glucose disposal in healthy humans during an intravenous glucose tolerance test using somatostatin. Orskov (89) applied a hyperinsulinemic, euglycemic clamp, using somatostatin, in healthy males and found no effect of GLP-1 in these subjects. More recently, Ahrens et al. (90) concluded that GLP-1 has no effect on the low insulin sensitivity in NIDDM patients during a hyperinsulinemic, euglycemic clamp. However, these authors’ conclusion was based on experiments where insulin levels were different between the GLP-1 treated and control groups, because the incretin effect was not abolished.

Positive results have also been found in in vivo studies using GLP-1. However, some of these studies were inappropriately designed to study GLP-1 effect on insulin sensitivity since care was not taken to control other effects of GLP-1 which may influence the measure of insulin sensitivity. Tominaga et al. (91) found that GLP-1 enhances insulin-stimulated glucose uptake in peripheral tissues but does not increase insulin-stimulated suppression of hepatic glucose production in normal rats during a euglycemic clamp. Similarly, Van Dijk et al. (92) found that in addition to stimulating insulin secretion, GLP-1 increased the metabolic clearance rate in fed and fasted rats. In both of these studies, somatostatin was not used, and thus, it is not possible to determine if the results observed were consequences of the incretin effect. Mizuno et al. (93) found that 4 weeks of GLP-1 treatment can augment insulin action in peripheral tissues during a euglycemic clamp in obese diabetic rats (OLETF). The use of chronic GLP-1 treatment in this study, however, may result in improved insulin sensitivity due to GLP-1’s incretin effect and
the consequently improved glycemic control.

However, other studies which have controlled for the incretin effect have found an insulin-like or insulin-potentiating action of GLP-1 in vivo. D’Alessio (94) used either GLP-1 or saline infusion in healthy humans, followed with a bolus of glucose. The insulin and glucose data were analyzed using Bergman’s minimal model. Insulin secretion increased with GLP-1 vs. control as expected. However, the calculated glucose effectiveness at basal insulin levels (which is an index of non-insulin mediated glucose disposal) also increased with GLP-1 vs. control. These studies were conducted with pharmacological doses of GLP-1. Subsequent studies at physiological doses of GLP-1 resulted in similar findings (95). Recently, D’Alessio et al. (96) have reported that GLP-1 has a direct effect on suppressing hepatic glucose production, independent of its effects on pancreatic hormones.

Gutniak studied the effects of GLP-1 on glucose metabolism under the conditions of a euglycemic clamp in IDDM patients (97). Glucose levels were clamped at the same levels in both experiment and control groups, and the rise in insulin levels was matched between groups. Glucose requirement to maintain euglycemia was found to be significantly greater in the group receiving GLP-1 infusion. This finding indicates that GLP-1 had an insulin-like or insulin-potentiating effect in these subjects. However, glucagon levels were not clamped. This could be an important consideration since GLP-1 is known to suppress glucagon secretion (58,63). Additionally, tracer was not used so it is not possible to determine whether this increase in glucose requirement was due to changes in glucose production or utilization. We devised a protocol similar to the one used by Gutniak but with the additional features of clamping glucagon and using tracer.
**AIM:** To determine whether GLP-1 has an insulin-like or insulin-potentiating action on glucose production and/or glucose utilization *in vivo*, independent of the incretin effect, at constant glucagon levels in diabetic dogs.
Methods:

Experimental animals and preparation.

The study was performed on eight post-absorptive, depancreatized male dogs. Our experiments were done in depancreatized dogs to abolish GLP-1 stimulation of insulin secretion since depancreatized dogs have no endogenous insulin secretion. Depancreatized dogs are a model of selective insulin deficiency, because glucagon (IRG 3500) is secreted by the oxyntic cells in the gastric mucosa of dogs (98). Pancreatic and extrapancreatic glucagon has been found to have identical biological effects on glycogenolysis and gluconeogenesis (99). Mongrel dogs weighing 22-34 kg and of at least one year of age were selected after a 3 week conditioning period during which time they were immunized for distemper, hepatitis, parvo virus, and rabies. To prevent infections, the dogs were given antibiotics orally twice a day for 8 days, starting 3 days before surgery (500 mg, Cephalexin, Novopharm, Toronto, Ontario, Canada). On the day of the surgery, the cephalosporin antibiotics were given i.v. (1g Kefzol, Cefazolin sodium: Eli Lilly, Toronto, Ontario, Canada, b.i.d.). The dogs were fasted for 24 hours before surgery. The animals were given an I.M. injection of atropine (0.05 mg/ml Atropine Sulfate, M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada) to stop throat secretions and a neuroleptic (.5 mg/kg Atravet, Ayerst Laboratories, Montreal, Quebec, Canada) to sedate the animal. They were then placed under general anaesthesia induced with an intravenous dose (.02mg/kg) of an ultra short acting barbiturate, sodium thiopental (Abbott Laboratories, Montreal, Quebec, Canada) and maintained with 0.5% halothane (Halocarbon Laboratories, New Jersey, USA) in carrier gas consisting of 60% nitrous oxide (Canox, Toronto, Ontario, Canada) and 40% oxygen.
The surgeries were done in the Department of Comparative Medicine of the University of Toronto under sterile conditions. The abdominal cavity was opened with a midline laparotomy and visceral fat was removed as necessary to allow easy access to the spleen and pancreas. The pancreas was completely removed while care was taken to preserve duodenal vascularization through the pancreatoduodenal vessels.

In all the dogs, a silastic cannula (0.04" internal diameter, Baxter Healthcare Corporation, McGaw Park, IL) was inserted into the portal vein through a branch of the splenic vein and advanced until the tip was approximately 1.0 cm beyond the point of confluence of the splenic vein with the portal vein, ie. approximately 5 cm from the branching point of the portal vein into its left and right bifurcations to the liver. Three silastic cannulae (one 0.04" internal diameter and two 0.03" internal diameter) were inserted into the jugular vein and advanced into the superior vena cava depending on the dog size. In addition a silastic cannula (0.04" internal diameter) was inserted into a carotid artery and advanced into the aortic arch. The carotid cannula served for arterial sampling and the jugular and portal cannulae served for infusions. The thicker jugular line was used for the viscous glucose infusate. The cannulae were tunneled subcutaneously and exteriorized at the back of the neck. They were filled with heparin (1000 U/ml, Hepalean, Organon Teknika, Ontario, Canada) and bandaged around the dog’s neck. After the end of surgery, antibiotics analgesics (0.3mg Buprenorphine HCL; Rickett & Colman Pharmaceuticals, Hull, Great Britain) were given intramuscularly to prevent post-surgery pain. In addition, a small dose of NPH insulin (Iletin II NPH insulin, Eli Lilly, Co. Indianapolis,
USA.) was injected subcutaneously to regulate glycemia. The amount of food given to these diabetic dogs was slowly increased after surgery until they received a diet of 400 g of dry chow (minimum 25% protein, minimum 9% fat, maximum 4% fibre, maximum 12% moisture, maximum 10% ash, maximum 2% added minerals, Purina Mills, St. Louis, MO) mixed with 670 g of canned meat (10% protein, 5% fat, 78% moisture, Derby Pet Food, Brampton, Ontario, Canada) once a day. The dog food was supplemented with folic acid (5mg, Novopharm Ltd, Toronto, Ontario, Canada) and iron (70mg ferrous gluconate, Novopharm Ltd, Toronto, Ontario, Canada) to assist erythrocyte production and thus prevent anemia due to the blood sampling during the experiments. The dogs received in addition, pancreatic enzymes (Cotazym, Organon Canada, Toronto, Ontario, Canada) orally to replace the lost exocrine function of the pancreas. Once the diabetic dog had eaten half of his food, regular porcine (Iletin II regular insulin, Eli Lilly Co., Indianapolis, USA) and NPH porcine insulin in a 1:2 ratio, was injected subcutaneously to control glycemia. The amount of glucose in the urine was determined daily (Chemstrip, Mannheim Boehringer, Laval, Quebec, Canada) and was used as an indicator of glycemia. The insulin dose was adjusted accordingly to keep glycosuria at below 1%, and blood glucose on the two days before experiments maintained at 8-10 mM. Porcine insulin does not induce the formation of anti-insulin antibodies for at least 2 months (100), thus allowing accurate measurements of plasma insulin. The cannulae were regularly flushed (every 4-5 days) with saline and filled with heparin to maintain patency and avoid clotting and the neck incision was cleaned with peroxide and sprayed with a topical antibacterial agent (Topazone, Austin, Quebec, Canada). Body weight, body temperature, hematocrit, stools and food intake were monitored regularly. A minimum of two
weeks elapsed between the surgery and the first experiment, and the dogs were given at least one week rest between experiments for effective erythrocyte production and thus preventing anemia. All procedures were in accordance with the Canadian Council on Animal Care standards and were approved by the Animal Care Committee of the University of Toronto.

Only dogs who were healthy, with a hematocrit above 35% and 2 days of relatively well controlled diabetes (blood glucose 8-10 mM) were allowed to undergo experiments. The dogs received the normal amount of food the day before the experiment and received a lower amount of insulin. The regular insulin dose was unaffected while the NPH insulin was reduced to 1/2 or 1/3 of the previous day’s dose. This was done to obtain early morning hyperglycemia and thus facilitate the control of blood glucose levels by i.v. insulin. After the experiment the food was given and a reduced insulin dose was injected as appropriate, to avoid overlap with the regular daily dose. The experiments were performed after at least a 18 hour overnight fast.
**Experimental design**

Figure #1 shows the experimental protocol. The depancreatized dogs were hyperglycemic at the start of the experiment (445.3 ± 41.8 mg/dl) because they had received a reduced NPH insulin dose, the day before. In these hyperglycemic dogs, regular porcine insulin was infused intraportally, initially starting at a high dose. The dose was then gradually reduced to basal levels in the goal of obtaining constant moderate hyperglycemia (160-200 mg/dl). These levels of glycemia are below the renal glucose threshold of dogs which is approximately 240 mg/dl (101). We wished to study this diabetic model at hyperglycemia which usually characterizes the diabetic state but took care to avoid glycosuria, because when glycosuria is present, tracer-determined rate of disappearance does not reflect metabolic glucose utilization. When glucose levels declined below 300 mg/dl, a bolus of tracer (35 uCi of HPLC-purified 3-[3-H] tritiated glucose, New England Nuclear, Boston, MA) was given and a continuous tracer infusion (0.25 uCi/min) was started at time = -150 to enable the measurement of glucose production and glucose utilization. The approximate ratio of the bolus vs the continuous infusion is equal to the ratio of the pool of glucose in the dogs (in normal dogs, this is equal to 25% of the dog’s mass) vs the basal endogenous glucose production rate (approximately 2.5 mg/kg.min.) (102). In diabetic dogs, the bolus has to be increased because the glucose pool is greater. The bolus is designed to decrease the tracer equilibration period (103). The 3-[3-H] glucose was HPLC-purified to remove contaminants which have been shown to induce errors in the determinations of glucose production (104,105). At the same time as tracer infusion was initiated, somatostatin (Bachem, California, USA) and glucagon (ELi Lilly, Indianapolis, USA) were infused at 0.8 ug/kg.min and 0.65 ng/kg.min respectively.
Somatostatin infusion is required to inhibit glucagon secretion from the gastric mucosa, an extrapancreatic supply of glucagon. Glucagon was infused intraportally to maintain basal glucagon levels throughout the experiment. The infusions of somatostatin and glucagon are required to clamp the glucagon levels so that GLP-1’s effect of suppression of glucagon secretion will not influence results.

The tracer equilibration period was at least 120 minutes long. The tracer infusion, and the low dose portal insulin infusion were continued throughout the experiment. At time = -30 minutes, basal sampling started every 10 minutes for 30 minutes. At time = 0, insulin was infused peripherally at either a high dose (0.9 mU/kg.min) or a low dose (0.125 mU/kg.min), with or without simultaneous GLP-1 (7-36) amide (Saxon Biochemicals GMBH, Bachem California) infusion (1.5 pmol/kg.min). The high dose insulin treatment results in postprandial insulin levels while the low dose insulin treatment results in fasting insulin levels. All insulin infusions were prepared in saline containing approximately 3% (v/v) of the dog’s own plasma. The plasma was added to allow insulin to bind to albumin and improve mixing and avoid insulin sticking to the glass beaker used for preparation of the infusate.

Plasma glucose was clamped at the initial pre-clamp glycemic levels for 3-hours with a variable exogenous dextrose infusion (25% dextrose, Abbott Laboratories, Montreal, Quebec, Canada) which was adjusted accordingly to plasma glucose concentrations determined every 5 minutes. Glucose was clamped to isolate the effects of insulin per se from the effect of lowering glycemia and to avoid any release of counterregulatory hormones. The glucose infusion was spiked with 3-[3-H] glucose tracer according to Finegood et al. (106,107) to prevent the decline in the glucose specific activity during the glucose clamp, and thus, minimize errors which are
associated with the use of one-compartment, fixed-pool volume model method for calculations of glucose production (108,109). The amount of tracer in the dextrose infusate was based on estimates of suppression of glucose production and dextrose requirements. The following equation by Finegood et al. (106), modified as in Giacca et al. (110) to account for partial suppression of glucose production, was used to calculate the specific activity of the dextrose infusate.

\[
S_{A\text{inf}} = I \times \left[ \frac{G_{\text{INF}(ss)}/R_{a(b)}}{-F \times 1000} \right] \times \frac{\text{GINF}(ss)}{} \times \frac{\text{BW}}{}
\]

where:

- \(S_{A\text{inf}}\) = specific activity of the dextrose infusate (uCi/g)
- \(I\) = constant tracer infusion rate (uCi/min)
- \(G_{\text{INF}(ss)}\) = steady state glucose infusion rate (mg/kg.min)
- \(R_{a(b)}\) = basal glucose production (mg/kg.min)
- \(F\) = steady state suppression of glucose production
- \(F = \frac{R_{a(b)} - R_{a(ss)}}{R_{a(b)}}\)
- \(R_{a(ss)}\) = steady state glucose production (mg/kg.min)
- \(\text{BW}\) = weight of the dog (kg)

The following equation was used to calculate the amount of tracer to be added to cold glucose to obtain the \(S_{A\text{inf}}\) required.

\[
T_{\text{INFvol}} = \frac{S_{A\text{inf}} \times G \times V}{T_{\text{INF}} + [S_{A\text{inf}} \times (G-C)]}
\]

where:

- \(T_{\text{INFvol}}\) = ml of tracer infusate to be added to dextrose infusate
- \(S_{A\text{inf}}\) = specific activity of the dextrose infusate (uCi/g)
- \(V\) = final volume of the dextrose infusate (ml)
- \(T_{\text{INF}}\) = radioactive concentration of tracer infusate (uCi/ml)
- \(G\) = cold glucose concentration in the dextrose infusate (g/ml)
- \(C\) = concentration of carrier glucose in the tracer infusate (g/ml)
The SAginf for the high dose was based on initial GINF, Ra(b) and F estimates of 4.0 mg/kg.min, 3.5 mg/kg.min and 0.85, respectively. The SAginf for the low dose was based on initial GINF, Ra(b) and F estimates of 1.2 mg/kg.min, 2.7 mg/kg.min and 0.4, respectively. These initial values were continuously updated according to experimental results.

Arterial samples were taken every 10 minutes for 30 minutes in the basal period and then every 10 minutes in the first and third hour and every 15 minutes in the second hour of the hyperinsulinemic clamp. The blood samples were taken for insulin, glucagon, GLP-1, free fatty acids, lactate, glycerol, alanine, and for 3-[3-H]-glucose. The blood samples for 3-[3-H]-glucose and insulin analysis were collected in tubes containing 2.5 mg of sodium fluoride (Fisher), to prevent glucose degradation, and dried heparin (50 U.S.P. units per 1.0 ml sample) as anticoagulant. The samples for glucagon, GLP-1 and free fatty acid analysis were collected in tubes containing 0.125 ml of 24 mg/dl ethylenediamine tetraacetic acid (EDTA;Sigma) to prevent clotting and 0.125 ml of Trasylol (2000 kallikrein IU; FBA, NY) per 2.5 ml of blood. Trasylol is a broad-spectrum proteolytic inhibitor that protects against loss of glucagon immunoreactivity. Blood samples for alanine, glycerol and lactate were collected in tubes containing an equal volume of 10% perchloric acid (BDH Inc. Toronto, Ont.) to deproteinize the sample by precipitation thereby clearing the sample. The sample must be clear to reduce errors in the fluorometric assay methods. Within an hour after collection, the samples were centrifuged at 800g at 4°C. The supernatant was stored at -20C for later analysis.
PROTOCOL
Paired experiments in overnight fasted depancreatized dogs with carotid, jugular and portal cannulations.

Low portal insulin dose

Somatostatin

Portal Glucagon

3-\textsuperscript{3}H-glucose tracer

Insulin (0.9 or 0.125 mU/kg.min) ± GLP-1 (1.5 pmol/kg.min)

Variable glucose infusion

Sampling period


**Plasma Insulin Assay**

The plasma insulin assay was done by using a kit from Pharmacia AB, Uppsala, Sweden 1994. Pharmacia Insulin RIA is a double antibody radioimmunoassay. Insulin in the sample competes with a fixed amount of $^{125}\text{I}$-labelled insulin for the binding sites on the specific antibodies. Bound and free insulin are separated by the addition of a second antibody immunoadsorbent followed by centrifugation and decanting. The radioactivity in the pellet is then measured. The radioactivity is inversely proportional to the quantity of insulin in the sample. Each package of Pharmacia Insulin RIA contains reagents for 100 tubes, sufficient for 44 samples and one standard curve in duplicate.

A standard curve is first constructed using insulin standards with known concentrations (0, 3, 10, 30, 100 and 240 uU/ml) in duplicate. We used dog insulin standard. Dog insulin has the same primary structure as pork insulin. Samples are also assayed in duplicate. 100 ul are pipetted into the appropriate vials. Then $^{125}\text{I}$-insulin is added (50 ul to all the tubes) and then the antibody is added (50 ul to all the tubes). At this point, the contents of the tubes should all be green. The rack is then shaken to ensure mixing and is incubated for 2 hours at room temperature. Following this, the suspension is decanted and is incubated for 1/2 hour at room temperature. Then the supernatant is centrifuged at 10 minutes at 1500g. The tubes are decanted and the tubes are then left to stand 1/2 minute upside down on absorbent paper. For the most optimal results, the tube rack should be moved (upside down) onto a dry absorbent paper tapped a couple of times against the absorbent paper to get rid of residual drops. The
precipitate is firmly packed and none of it is lost with vigorous tapping. The tubes are then counted to determine the radioactivity.

The calculation of results is as follows:

1. The counts (B) for each of the standards and unknowns are expressed as a percentage of the mean counts of the "0-standard" (Bo).

   \[
   \text{% activity bound} = \frac{B(\text{standard or unknown})}{Bo} \times 100\%
   \]

2. The percentage values obtained for the insulin standards are plotted against the concentration on a lin-log paper and a standard curve is constructed.

3. The concentration of the unknown samples is read from the standard curve.

The coefficient of interassay variation as determined on reference plasma was less than 7%.

Plasma Glucagon Assay

The plasma glucagon assay was performed using the kit from DPC. Antibody glucagon is an $^{125}\text{I}$ radioimmunoassay designed for the quantitative measurement of glucagon in EDTA plasma. The DPC's Double Antibody Glucagon Procedure is a sequential radioimmunoassay. After preincubation of the blood sample with anti-glucagon antibody, $^{125}\text{I}$ labelled glucagon competes with the unlabelled glucagon in the sample for antibody sites. After incubation for a fixed time, separation of bound from free glucagon is achieved by the PEG-accelerated double-antibody method, followed by centrifugation. The precipitate containing the antibody-bound fraction is then counted, and the glucagon concentrations are read from a calibration curve. The whole assay involves two overnight incubations.
Glass tubes rather than plastic tubes should be employed for the assay, due to the tendency of glucagon to adsorb to plastic surfaces. Just prior to the assay, the 2000 pg/ml Glucagon ('Master') Calibrator is diluted in the Glucagon Zero Calibrator. Each dilution is vortexed thoroughly before proceeding to the next dilution. 200 ul of the zero calibrator A is pipetted into NSB tubes (non-specific binding), and into the maximum binding tubes, and 200 ul of each of the remaining calibrators B through F (287, 144, 29, 14, 7 pmol/l respectively) into appropriate tubes (this was done in duplicate). Then 200 ul of each sample and each control is pipetted into appropriate tubes (this was also done in duplicate). 100 ul of Glucagon Antiserum (BLUE) is then added to all tubes except the NSB and T tubes, and they are vortexed. The rack is then covered and incubated for 24 hours at 2-8 C. The racks are covered to avoid evaporation. Then 100 ul of $^{125}$I-glucagon (clear) is added to all tubes, which are all subsequently vortexed. The rack is then covered again and incubated for another 24 hours at 2-8 C. The T-tubes (total counts) are counted as they do not need further processing. Then 1.0 ml of cold Precipitating Solution (red) is added to all the tubes, and vortexed. The tubes are then centrifuged for 15 minutes at 1500xg. The supernatant is aspirated and the precipitate is counted for 1 minute.

To obtain results in terms of concentration from the logit-log representation of the calibration curve, first for each pair of tubes the average NSB-corrected counts per minute are calculated:

Net Counts = Average CPM minus Average NSB CPM

Then the binding of each pair of tubes as a percent of maximum binding (MB) is determined, with the NSB-corrected counts of the A tubes taken as 100%:
Percent Bound = (Net Counts/Net MB Counts) x 100%

Using the logit-log graph paper provided with the kit, the Percent Bound is plotted along the vertical axis against the Concentration along the horizontal axis for each of the nonzero calibrators, and the straight line is drawn to approximate the path of these points. Results for the unknowns may then be read from the line by interpolation.

The coefficient of interassay variation as determined on reference plasma was less than 16%.

**Plasma Glucose Assay**

Plasma glucose concentrations were measured by the glucose oxidase method (111) on a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). A 10 ul sample of plasma containing D-glucose is injected into a solution containing oxygen and glucose oxidase. The glucose reacts with oxygen in the following reaction which is catalysed by glucose oxidase,

\[
\text{D-glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Glucose Oxidase}} \text{gluconic acid} + \text{H}_2\text{O}_2
\]

In this reaction oxygen is used at the same rate of glucose to form gluconic acid. A polarographic oxygen sensor is used to detect oxygen consumption which is directly proportional to the glucose concentration in the sample. Results are obtainable within thirty seconds after sample addition. The data are accurate to ±3 mg/dl. The analyzer was calibrated before use and frequently during the experiment with the 150 mg/dl Beckman Certified Glucose Standard.

**Plasma Specific Activity Assay**
For the determination of 3-[3-H] glucose specific activity, 200 ul of plasma (done in duplicate) was deproteinized in equal volumes (400 ul each) of 5% (w/v) zinc sulphate and 0.3 N barium hydroxide (BDH) which had been titrated and adjusted for strength. 1 ml of distilled water was then added to each sample. The samples were deproteinized to reduce quenching of the emitted energy from the tritium label by the protein when being counted. A 1ml aliquot of the supernatant was then evaporated to dryness to eliminate tritiated water. After addition of 1 ml of water and 10 ml of liquid scintillation solution (Ready Safe, Beckman), the radioactivity from 3-[3-H]-glucose was measured in a beta-scintillation counter (Camberra Packard). Aliquots of the infused glucose tracer and of the labelled glucose infusate were diluted with pre-tracer equilibration plasma (2 dilutions of 1:1000 and 1:50 respectively) and assayed together with the plasma samples. The coefficient of interassay variation as determined on reference plasma was 15%.

**Plasma Free Fatty Acid Assay**

The FFA concentrations were determined with the flurometric method of Miles et al. (112). This procedure uses the equations given below (113) and a fluorometer (Sequoia-Turner, Model 450 Fluorometer) to produce NAD+ and allow the determination of plasma FFA concentrations.

\[
\text{FFA} + \text{CoA} + \text{ATP} \xrightarrow{\text{acyl CoA synthetase}} \text{acyl CoA} + \text{AMP} + \text{PPi}
\]

\[
\text{AMP} + \text{ATP} \xrightarrow{\text{myokinase}} 2\text{ADP}
\]

\[
2\text{ADP} + 2\text{phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} 2\text{ATP} + 2\text{pyruvate}
\]

\[
2\text{pyruvate} + 2\text{NADH} \xrightarrow{\text{lactate dehydrogenase}} 2\text{lactate} + 2\text{NAD}^+
\]

A standard curve (1-7 nM) is made by diluting oleate (Sigma) in potassium phosphate buffer. The standards, samples and control plasma were pipetted into glass tubes. Tris assay
buffer (NADH, ATP, PEP, myokinase, pyruvate kinase, lactic dehydrogenase, acyl-CoA synthetase (all Sigma) was added to the tubes which were then mixed and allowed to sit for 10 min. at room temperature. The tubes were then preread in the fluorometer. CoA was then added to allow the reactions to take place. The tubes were mixed and incubated for 75-90 minutes before being postread in the fluorometer. The postreading was subtracted from the prereading to determine the decrease in fluorescence due to NADH oxidation. A standard curve was constructed and the sample readings were interpolated off the curve. The interassay variation, as determined by reference plasma measurements, was 9%.

**Plasma Metabolite Assay**

Plasma metabolites (lactate, alanine and glycerol) were determined using an enzymatic fluorometric continuous-flow assay (114) with a fluoromicrophotometer (Aminco, Silver Spring, MD).

The lactate measurements were based on the following reaction:

\[
\text{lactate} + \text{NAD}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{pyruvate} + \text{NADH} + \text{H}^+ 
\]

Measurement of alanine was based on the following reaction:

\[
\text{L-alanine} + \text{NAD}^+ \xrightarrow{\text{alanine dehydrogenase}} \text{pyruvate} + \text{NADH} + \text{NH}_4^+ 
\]
Measurement of glycerol was based on the following reactions:

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{L-glycerol-1-phosphate} + \text{ATP} \\
\text{L-glycerol-1-phosphate} + \text{NAD}^+ \xrightarrow{\text{glycerol-3-phosphate dehydrogenase}} \text{dihydroxyacetone phosphate} + \text{NADH} + \text{H}^+
\]

For each metabolite assay, NADH was measured using a fluoronephelometer with a 350-nm excitation filter and a 468-nm emission filter.

The coefficients of interassay variation for lactate, alanine and glycerol, as determined from reference plasma, were less than 5%, 4% and 9%, respectively.

**GLP-1**

This assay was performed by Dr. Brubaker as follows:

2 ml of TFA (trifluoroacetic acid, pH 2.5 with diethylamine) was added to 1 ml of plasma. The sample was then collected by reversed-phase adsorption to C18 silica (SEPPAC, Waters Association, Milford MA). The sample was then dried in vacuo for GLP-1(7-36NH2) RIA. The antibody (GLP-1(7-36NH2)) was obtained from Affinity Research Products, Nottingham, U.K. and was C-terminal specific. This entire procedure was done in singlicate for each sample provided.
The coefficient of interassay variation, as determined on reference plasma was 16%.
Calculations

GP was calculated as the endogenous rate of appearance measured with 3-[3-H]-glucose. A modified one-compartmental model of Steele was used to account for the exogenously infused mixture of labelled and unlabelled glucose. Data were smoothed with the optimal segments routine using the optimal error algorithm (115,116). The glucose turnover data was calculated using a custom made EXCEL spreadsheet on a 486 DX2, Packard Bell computer.

Statistics

The data were expressed as mean ± standard error of the mean (SEM). The calculations were done on the last 90 minutes when a new steady state was obtained. Since the dog experiments were paired (each dog had a GLP-1 and control experiment), we used two way analysis of variance to detect differences between experimental groups. Whenever necessary, the data were log transformed to equilibize variances. The two variables used in the analysis were dog and treatment. Data were also analyzed with non-parametric statistics. In particular, variables were ranked (Probit transformed) and normal scores were computed from the ranks. The ANOVA was then performed on these normal scores. Data were also analyzed within each group for detection of differences between the experimental periods. Calculations were performed with SAS software (SAS Statistical Analysis System, Cary, NC).
Results

High Insulin Dose

The following results are based on a peripheral insulin dose of 0.9 mU/kg.min with n=6 for both GLP-1-infused and saline-infused (control) dogs. The low portal insulin dose required to achieve and maintain moderate hyperglycemia in these dogs was not significantly different between groups (GLP-1: 0.36 ± 0.09 vs. control: 0.32 ± 0.09 mU/kg.min). The basal insulin levels were 5.5 ± 0.9 and 6.5 ± 2.2 uU/ml and rose to 45.6 ± 7.1 and 43.4 ± 4.7 uU/ml after peripheral insulin infusion in the control and GLP-1 groups respectively (figure #2).

There were no significant differences in insulin levels at any time between these groups. The basal plasma glucagon levels were 81.8 ± 19.6 and 83.6 ± 20.6 ng/l in the GLP-1 and control groups respectively. These levels dropped slightly but insignificantly during the experiment to 70.6 ± 11.4 and 72.3 ± 14.4 ng/l respectively. There were again no significant differences in glucagon levels between groups (figure #3). The basal values of GLP-1 were not significantly different between groups (GLP-1: 12.0 ± 1.6, n=3, vs. control: 17.5 ± 8.4 pg/ml, n=3). With GLP-1 infusion, the GLP-1 levels rose to 380.4 ± 38.9 pg/ml (n=3) whereas GLP-1 levels in controls remained at basal levels (15.3 ± 2.9 pg/ml, n=3) (figure #4). The assays for three dogs still need to be performed.

The basal plasma glucose levels were 177.0 ± 6.3 and 182.8 ± 5.0 mg/dl in the control and GLP-1 groups respectively and were maintained at this level throughout the course of the experiment (control: 178.3 ± 5.6 vs. GLP-1: 186.0 ± 6.2 mg/dl) (figure #5) by the use of variable peripheral glucose infusion using "hot ginf". The plasma glucose specific activity was
maintained constant throughout the course of the experiment (control BASAL: 3.19 ± 0.31 vs.
CLAMP 3.22 ± 0.17 uCi/gm, GLP-1 BASAL: 3.18 ± 0.36 vs. CLAMP: 3.09 ± 0.20 uCi/gm) (figure #6). The glucose infusion rate (GINF) was significantly higher in the GLP-1-infused dogs than in the control dogs at this insulin dose (GLP-1: 10.95 ± 1.98 vs. control: 7.84 ± 1.50 mg/kg.min, p < 0.001) (figure #7). The calculated values for glucose production were found to be not significantly different between the GLP-1 and control groups in both basal (GLP-1: 2.92 ± 0.18 vs. control: 3.38 ± 0.25 mg/kg.min) or clamp periods (GLP-1: 2.22 ± 0.30 vs. control: 2.40 ± 0.68 mg/kg.min) (figure #8). Therefore, the suppression of glucose production was unaffected by GLP-1. The calculated values for glucose utilization during the basal period were not significantly different between the GLP-1 and control groups (GLP-1: 3.24 ± 0.15 vs. control: 3.42 ± 0.12 mg/kg.min). However, the glucose utilization values for the clamp period were significantly higher in the GLP-1-infused dogs than in saline controls (GLP-1: 13.05 ± 1.97 vs. control: 10.21 ± 1.74 mg/kg.min, p < 0.001) (figure #9), which indicates that GLP-1 enhanced insulin’s stimulation of glucose utilization. The basal free fatty acid levels in the GLP-1 and control groups were not significantly different between groups (GLP-1: 1080.9 ± 155.8 vs. control: 1169.4 ± 167.3 uEq/l). During the clamp period, however, the insulin-induced suppression of free fatty acids was significantly greater in the GLP-1 infused dogs than in the control group. Free fatty acids were 375.3 ± 103.0 uEq/l with GLP-1 and 524.4 ± 101.1 uEq/l in controls (figure #10). The basal levels of glycerol were not significantly different between groups (GLP-1: 154.3 ± 55.6 vs. control: 136.3 ± 52.7 umol/l). During the clamp, glycerol levels decreased to lower levels in the GLP-1 group than
in the control group (GLP-1: 77.9 ± 17.5 vs. **control**: 125.6 ± 51.8 umol/l, p<0.05) (figure #11).

The basal lactate levels were not significantly different between groups (**GLP-1**: 1097.8 ± 510.6 vs. **control**: 781.8 ± 208.1 umol/l). Insulin infusion significantly increased lactate levels in the control group while the lactate levels were not increased significantly in the GLP-1 infusion group. The lactate levels during the clamp, however, were also not significantly different between groups (**GLP-1**: 952.4 ± 227.2 vs. **control**: 989.7 ± 134.5 umol/l) (figure #12). The basal levels of alanine were not significantly different between groups (**GLP-1**: 564.0 ± 146.7 vs. **control**: 576.6 ± 125.7 umol/l). During the clamp, alanine decreased to levels that were slightly lower with GLP-1, but not significantly different than control (**GLP-1**: 371.7 ± 97.8 vs. **control**: 453.3 ± 108.2 umol/l) (figure #13).

**Low Insulin Dose**

The following results are based on a peripheral insulin dose of 0.125 mU/kg.min with n=5 for both GLP-1-infused and saline-infused (control) dogs. The low portal insulin dose required to achieve and maintain moderate hyperglycemia was not significantly different between groups (**GLP-1**: 0.19 ± 0.09 vs. **control**: 0.25 ± 0.15 mU/kg.min). The basal insulin levels in these dogs were 6.2 ± 1.5 and 6.0 ± 2.2 uU/ml and rose to 13.3 ± 1.4 and 12.3 ± 2.9 uU/ml after peripheral insulin infusion in the GLP-1 and control groups respectively (figure #14). There were no significant differences in insulin levels at any time between groups. The basal plasma glucagon levels were 93.6 ± 18.0 and 71.5 ± 15.3 ng/l in the GLP-1 and control groups respectively. These basal levels were significantly different (p<0.01).
dropped slightly but insignificantly during the clamp to $81.9 \pm 19.9$ and $60.4 \pm 7.9$ ng/l respectively. The clamp levels were again significantly different between groups ($p<0.01$) however, the change from basal was not significantly different between the groups (figure #15). The basal values of GLP-1 were not significantly different between groups (GLP-1: $16.8 \pm 1.1$, n=3, vs. control $15.8 \pm 2.4$, n=3). With GLP-1 infusion, GLP-1 levels rose to $446.3 \pm 76.7$ (n=3) in the GLP-1 infused dogs whereas GLP-1 levels remained at basal levels in controls ($11.7 \pm 0.7$, n=3). (figure #16). The assays for two dogs still need to be performed.

The basal plasma glucose levels were $175.3 \pm 8.6$ and $180.8 \pm 6.0$ mg/dl in the GLP-1 and control groups respectively and were maintained at this level throughout the course of the experiment (control: $182.2 \pm 6.9$ vs. GLP-1: $178.6 \pm 7.1$ mg/dl) (figure #17) by the use of variable peripheral glucose infusion using "hot ginf". The plasma glucose specific activity was maintained constant throughout the course of the experiment (control BASAL: $3.09 \pm 0.19$ vs. CLAMP $3.27 \pm 0.19$ uCi/gm, GLP-1 BASAL: $2.82 \pm 0.17$ vs. CLAMP: $3.01 \pm 0.26$ uCi/gm) (figure #18). The glucose infusion rate (GINF) was not significantly different in the GLP-1-infused dogs than in the control dogs at this insulin dose (GLP-1: $1.66 \pm 0.14$ vs. control: $1.59 \pm 0.41$ mg/kg.min) (figure #19). The calculated values for glucose production were found to be significantly different between the GLP-1 and control groups in both basal (GLP-1: $3.65 \pm 0.28$ vs. control: $3.20 \pm 0.22$ mg/kg.min, $p<0.01$) and clamp (GLP-1: $2.66 \pm 0.18$ vs. control: $2.25 \pm 0.25$ mg/kg.min, $p<0.01$) periods. However, the decrease from basal was not significantly different compared to control (figure #20). The calculated values for glucose utilization were significantly different between the GLP-1 and control groups in both basal (GLP-1: $3.77 \pm 0.25$ vs. control: $3.29 \pm 0.19$ mg/kg.min, $p<0.01$) and clamp (GLP-1:
4.25 ± 0.28 vs. **control**: 3.87 ± 0.38 mg/kg.min) periods. The increase from basal was significant for both groups (p < 0.01), however, this increase was not significantly different between the groups (figure #21). The basal free fatty acid levels in the GLP-1 and control groups were not significantly different between groups (**GLP-1**: 1144.3 ± 121.6 vs. **control**: 1207.6 ± 185.5 uEq/l). During the clamp period, the suppression of free fatty acids was not significantly affected by GLP-1 infusion. Free fatty acid levels were 930.3 ± 156.7 uEq/l in the GLP-1 group and 928.4 ± 155.8 uEq/l in controls (figure #22). The basal levels of glycerol were not significantly different between groups (**GLP-1**: 88.1 ± 7.1 vs. **control**: 81.3 ± 11.9 umol/l). Glycerol levels were not significantly affected by insulin infusion in the control group or the insulin plus GLP-1 infusion in the GLP-1 group. The clamp glycerol levels were also not significantly different between groups (**GLP-1**: 72.9 ± 4.6 vs. **control**: 80.5 ± 16.1 umol/l) (figure #23).

The basal lactate levels were not significantly different between groups (**GLP-1**: 865.5 ± 67.1 vs. **control**: 901.3 ± 340.4 umol/l, n=3). Neither insulin infusion in the control group or insulin with GLP-1 infusion significantly affected lactate levels. The clamp lactate levels were also not significantly different between groups (**GLP-1**: 774.7 ± 205.7 vs. **control**: 697.7 ± 170.0 umol/l, n=3) (figure #24). The assays for two dogs still need to be performed. The basal levels of alanine were not significantly different between groups (**GLP-1**: 721.1 ± 180.8 vs. **control**: 714.9 ± 240.5 umol/l). Alanine levels were not significantly lowered from basal by insulin infusion in the control group or by insulin plus GLP-1 infusion in the GLP-1 group. The clamp alanine levels were also not significantly different between groups (**GLP-1**: 776.7 ± 233.6 vs. **control**: 649.1 ± 202.9 umol/l) (figure #25).
Figure #2: Plasma insulin levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

PLASMA INSULIN LEVEL
(HIGH INSULIN DOSE)

--- INS
n=6

--- INS + GLP-1
n=6

uU/ml

TIME (min)
Figure #3: Plasma glucagon levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

**PLASMA GLUCAGON LEVEL**

(HIGH INSULIN DOSE)

- INS
  - \( n = 6 \)
- INS + GLP-1
  - \( n = 6 \)

pg/ml

TIME (min)
Figure #4: Plasma GLP-1 levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLP 1
( HIGH INSULIN DOSE )

- - - INS
n=3

- - - INS+GLP-1
n=3

*p < 0.001

TIME (min)
Figure #5: Plasma glucose levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

PLASMA GLUCOSE
(HIGH INSULIN DOSE)

-- INS
n=6

- INS+GLP-1
n=6

TIME (MIN)

mg/dl

-50 0 50 100 150 200
Figure #6: Plasma glucose specific activity during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

PLASMA GLUCOSE SPECIFIC ACTIVITY
(HIGH INSULIN DOSE)

--- INS
n=6

--- INS + GLP-1
n=6

uCi/gm

TIME (min)
Figure #7: Glucose infusion during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCOSE INFUSION
(HIGH INSULIN DOSE)

--- INS
n=6

--- INS+GLP-1
n=6

* p < 0.001

TIME (MIN)
Figure #8: Glucose production during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCOSE PRODUCTION
(HIGH INSULIN DOSE)

- - - - INS
n=6

INS+GLP-1
n=6

mg/kg.min

-50 0 50 100 150 200

TIME (MIN)
Figure #9: Glucose utilization during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCOSE UTILIZATION (HIGH INSULIN DOSE)

- INS
  n=6

- INS+GLP-1
  n=6

* p < 0.001

TIME (min)
Figure #10: Free fatty acid levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

FREE FATTY ACIDS
(HIGH INSULIN DOSE)

* p<0.01
Figure #11: Plasma glycerol levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLYCEROL
(HIGH INSULIN DOSE)

- - - INS
n=6

--- INS+GLP-1
n=6

* p < 0.05

TIME (min)
Figure #12: Plasma lactate levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

LACTATE
(HIGH INSULIN DOSE)

ins
n=6

INS+GLP-1
n=6

umol/l

TIME (min)
Figure #13: Plasma alanine levels during high dose peripheral insulin infusion (0.9 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)
Figure #14: Plasma insulin levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

PLASMA INSULIN LEVEL (LOW INSULIN DOSE)

\[ \text{INS} \quad n=5 \]
\[ \text{INS+GLP-1} \quad n=5 \]

\[ uU/ml \]

TIME (min)
Figure #15: Plasma glucagon levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCAGON
(LOW INSULIN DOSE)

--- INS
n=5

--- INS + GLP-1
n=5

TIME (min)

pg/ml
Figure #16: Plasma GLP-1 levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLP 1  
( LOW INSULIN DOSE )

--- INS  
n=3

--- INS+GLP-1  
n=3

* p<0.001
Figure #17: Plasma glucose levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

PLASMA GLUCOSE
(LOW INSULIN DOSE)

n=5

INS

INS+GLP-1

TIME (min)

mg/dl
Figure #18: Plasma glucose specific activity during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)
Figure #19: Glucose infusion during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCOSE INFUSION
(LOW INSULIN DOSE)

--- INS
n=5

--- INS+GLP-1
n=5

mg/kg.min

TIME (MIN)
Figure #20: Glucose production during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)
Figure #21: Glucose utilization during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

GLUCOSE UTILIZATION
(LOW INSULIN DOSE)

- INS
  n=5

- INS+GLP-1
  n=5

mg/kg.min

TIME (min)
Figure 22: Free fatty acid levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

FREE FATTY ACIDS
(LOW INSULIN DOSE)

- INS
- INS + GLP-1

n=5

TIME (min)
Figure #23: Plasma glycerol levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)
Figure #24: Plasma lactate levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

LACTATE
(LOW INSULIN DOSE)

- INS
  n=3

- INS+GLP-1
  n=3

TIME (min)
Figure #25: Plasma alanine levels during low dose peripheral insulin infusion (0.125 mU/kg.min) with or without GLP-1 (1.5 pmol/kg.min)

ALANINE (LOW INSULIN DOSE)

\(-\)\text{INS} \quad \bullet \text{INS+GLP-1}

n=5

TIME (min)

umol/l

-50 0 50 100 150 200
Our results obtained with the high insulin dose indicate that, in depancreatized diabetic dogs, GLP-1 potentiated insulin action, during a hyperglycemic (160-200 mg/dl) hyperinsulinemic clamp at constant glucagon levels. This was due to a significant effect of GLP-1 on enhancing the insulin-stimulated glucose utilization. Free fatty acids were further suppressed by GLP-1 than in the control experiments as were the glycerol levels which indicates that GLP-1 potentiated insulin’s antilipolytic effect. Our results obtained at the low insulin dose, on the other hand, indicate that GLP-1 did not have any insulin-like or insulin-potentiating effect at levels of insulin close to the fasting range.

The prominence of insulin resistance in our depancreatized diabetic dogs is evident through the analysis of a number of experimental variables. The portal insulin dose necessary to attain and achieve moderate hyperglycemia was 0.34 ± 0.06 mU/kg.min in the dogs used for the high dose experiments and 0.22 ± 0.12 mU/kg.min in the dogs used for the low dose experiments. These values indicate that the dogs were insulin resistant since our studies were performed at plasma glucose levels of ~180 mg/dl and the basal insulin secretion rate which maintains euglycemia (~100 mg/dl) in normal dogs is 0.2-0.25 mU/kg.min. A number of other measured experimental variables are consistent with the state of insulin resistance. 1) The glucose requirements in our diabetic dogs were comparable to those in normal dogs exposed to lower dose insulin infusions (117). 2) Despite being infused with a dose of insulin that was not lower than the basal insulin secretion rate in normal dogs, and despite being hyperglycemic, our diabetic dogs had a glucose production which was substantially greater (in both the high and low
dose experiments) than the 2.5 mg/kg.min known to be characteristic of normal dogs. The suppression of glucose production by insulin was impaired as compared to normal dogs. This indicates the presence of hepatic insulin resistance. 3) The insulin-stimulated glucose utilization was comparable to normal dogs at a lower insulin dose (117), indicating peripheral insulin resistance. 4) The basal levels of free fatty acids were also higher in diabetic dogs, in accordance with an impairment in insulin’s antilipolytic action in the diabetic state. The suppression of free fatty acids and glycerol at both the high and low insulin doses was also impaired in our depancreatized dogs.

Our results indicate that GLP-1 has an effect on enhancing the impaired peripheral insulin sensitivity in depancreatized dogs. Since the effect was found only to occur at the high and not at the low insulin doses, and therefore requires a given amount of insulin to be present, we conclude that GLP-1 does not act via an independent mechanism from that of insulin. In practical terms, this implies that GLP-1 would improve insulin sensitivity at postprandial insulin levels (comparable to the levels attained at the high insulin dose infusion) while leaving insulin sensitivity unaffected at fasting insulin levels (comparable to the levels attained at the low insulin dose infusion).

The improvement in insulin sensitivity was found to be due to enhancement of peripheral glucose utilization, while the hepatic glucose production was unaffected. In an attempt to localize the effect on glucose utilization, in collaboration with Dr. Wheeler, we investigated the gene expression of the GLP-1 receptor in the insulin sensitive tissues of dogs (liver, muscle and adipose tissue). Our preliminary results, obtained using reverse transcription PCR followed by Southern blotting, indicate that there is GLP-1 receptor gene expression in both muscle and
adipose tissue. No gene expression for the GLP-1 receptor could be detected in the liver. This is consistent with a peripheral effect of GLP-1 that we found in our in vivo study. However, RT-PCR only allows for detection of a portion of the GLP-1 mRNA transcript. More definitive evidence for the presence of gene expression for the GLP-1 receptor would be attained if the entire transcript were found. It is important to note however that the presence of receptor mRNA and protein in the various tissues is not an indication that GLP-1 will act through that receptor. Our studies were conducted in depancreatized diabetic dogs in order to eliminate the incretin effect. However, there have also been reports that GLP-1 might decrease the clearance of insulin in normal humans (118,119). In our studies, we found no such effect of GLP-1 on insulin clearance at either the high or low insulin dose. This is reflected in the fact that insulin levels were matched at all time points for both experiment and control groups at either insulin dose. Since these dogs were depancreatized, there was no endogenous insulin secretion. So we controlled the rate of appearance of insulin and since the levels were the same in both experiment and control groups, GLP-1 had no effect on insulin clearance. Gutniak et al. (97) and Orskov et al. (89) also did not see any effect of GLP-1 on insulin clearance.

Glucagon levels were maintained at basal levels in both GLP-1 infused and control groups, at both doses, throughout the course of the experiment. This was desired so as prevent GLP-1’s glucagonostatic effect from interfering with the results of the study. In the low insulin dose study, the glucagon levels were significantly different in both basal and clamp periods (being higher in GLP-1-infused dogs vs. control) although the glucagon levels were clamped at basal levels in either case. This may explain why there were significantly higher basal and clamp glucose production values since glucagon stimulates glucose production. Basal and clamp
glucose utilization and clearance were also significantly higher in the GLP-1 group than the control group. This indicates that the GLP-1 group had greater peripheral insulin sensitivity and lower hepatic insulin sensitivity than the control group. However, the response of glucose production and utilization to GLP-1 did not differ from that to saline.

The levels of plasma lactate in the high dose studies would be expected to rise in accordance with increased glucose utilization in the muscle (the major tissue involved in insulin-stimulated glucose uptake) since an increased rate of glycolysis would be anticipated. However, we found plasma lactate to be lowered, although not significantly, by GLP-1 treatment. This raises the possibility that GLP-1 also improves glucose oxidation. These results would be in accordance with those of Villanueva-Penacarrillo et al. (78) who found that acute incubation of rat skeletal muscle in solution media with GLP-1 significantly increased both glucose utilization and glucose oxidation by the muscle. The plasma lactate levels were also not significantly affected by GLP-1 at the low insulin dose, although this was expected as GLP-1 had no effect on insulin sensitivity at this dose.

The GLP-1 assay results indicate that GLP-1 levels rose from basal to levels that were approximately 2-3 times the levels attained by Orskov et al. (89) in GLP-1 infusion studies in humans with GLP-1 infusion at rates of approximately 1/2 of our infusion rate. The GLP-1 levels in our dogs should theoretically be two times higher than in these reported levels by Orskov. This difference may be due to differences in the RIA for GLP-1 used between studies, but may also indicate that GLP-1 clearance in dogs is lower than in humans.

The plasma alanine levels were significantly lowered by high dose insulin infusion, consistent with results by Giacca et al. (110) in depancreatized diabetic dogs at this same
Although there is a trend for alanine levels to be further decreased with GLP-1, the plasma alanine levels were not significantly different at the high insulin dose, suggesting that GLP-1 does not substantially enhance insulin’s antiproteolytic effects. However, future studies with better methodology should be performed to investigate the effect of GLP-1 on protein metabolism. At the low peripheral insulin dose, insulin had no effect on the lowering of alanine levels, probably due to the insulin-resistant state of these dogs. Again, the low dose plasma alanine levels were also unaffected by GLP-1, consistent with the finding that GLP-1 did not exhibit any extrapancreatic effects at the low dose.

The plasma lactate and alanine results also indicate that insulin’s effect of limiting the flow of gluconeogenic substrates is also not significantly influenced by GLP-1. While GLP-1 did significantly lower glycerol levels at the high dose, glycerol is only very marginally used as a substrate for gluconeogenesis and as such the possibility that GLP-1 exerts an effect on limiting the rate of gluconeogenesis is not very likely.

The plasma free fatty acid levels are significantly lowered in the presence of GLP-1 at the high insulin dose compared to control. Since glycerol also declined to a greater extent with GLP-1, this indicates that GLP-1 potentiated insulin’s antilipolytic effect. Part of the effect of GLP-1 on enhancing insulin sensitivity might be secondary due to the FFA lowering action of insulin. This concept can be explained in view of Randle’s cycle in which free fatty acids and glucose compete for oxidation in the Kreb’s cycle. The lowering effect of free fatty acid levels would reduce competition between glucose and fatty acids and allow more glucose to be oxidized (and in effect improve insulin sensitivity). The effect of FFA on glucose utilization is known to be delayed in humans, but Sindelar et al. (120) and Rebrin et al. (121) as well as Wiesenthal
et al. (122) have shown that this effect is quicker in dogs.

The finding that GLP-1 potentiates insulin’s antilipolytic effect is supported by the presence of GLP-1 receptor gene expression in dog adipose tissue, as found by Dr. Wheeler. However, a direct effect on muscle glucose metabolism cannot be discounted since gene expression for the GLP-1 receptor has also been detected in the dog muscle samples by Dr. Wheeler. In addition, the effect of GLP-1 may not necessarily be mediated by GLP-1 receptors in adipocytes or muscle cells. Since increased blood flow can also increase muscle glucose utilization, the possibility that GLP-1 may have an indirect effect on glucose utilization by increasing blood flow should be investigated. The possibility of GLP-1-induced hemodynamic effects is supported by one recent study which shows that GLP-1 increases blood pressure in rats (123).

Our results reporting the absence of an insulin-potentiating effect of GLP-1 on glucose production are also supported by the absence of GLP-1 receptor mRNA in the dog liver, as found by Dr. Wheeler. Our results with relation to hepatic insulin sensitivity are puzzling, however, in that GLP-1 was found to have no effect on hepatic glucose production at the high insulin dose, while enhancing the antilipolytic action at this dose. Decreased plasma free fatty acids would in fact be expected to reduce the insulin resistance in the liver and improve insulin’s suppressive effect on hepatic glucose production. Elevated plasma FFA levels enhance cellular FFA uptake in the liver by the mass action effect (124). This in turn stimulates lipid oxidation and accumulation of Acetyl-CoA, which acts to inhibit pyruvate dehydrogenase and stimulate pyruvate carboxylase (125). This leads to an increased flux of three carbon precursors into gluconeogenesis. The stimulation of lipid oxidation also provides increased levels of ATP, a
continued source of energy for driving gluconeogenesis (126). Thus, these effects should act to increase hepatic glucose production. A decrease in plasma FFA levels would consequently result in a reduction of the elevated rate of gluconeogenesis, thus improving insulin sensitivity in this tissue. However, what is seen from our results is that hepatic glucose production is not affected by GLP-1. One likely explanation is that suppression of FFA is not very important for the suppression of hepatic glucose production in the depancreatized diabetic dog. Giacca et al. (110), who used the same depancreatized dog model during a hyperinsulinemic hyperglycemic clamp (~ 10 mM) with the same peripheral insulin dose (0.9 mU/kg.min) without a glucagon clamp, found that hepatic glucose production was suppressed to a much greater degree than in our study. The glucagon levels were suppressed in this study consistent with the suppressive effect of insulin on glucagon secretion. Thus, it is possible that in depancreatized diabetic dogs, glucagon is more important than FFA in the regulation of hepatic glucose production. Another possible explanation for this effect is that a reduction in hepatic glucose production related to decreased levels of FFA may be balanced by a glucagon-like effect of GLP-1 which might increase hepatic glucose production. Since GLP-1 arises from the same precursor molecule as glucagon (proglucagon), GLP-1 might also exhibit some glucagon-like catabolic effects. The receptor for GLP-1 is structurally related to a number of other G-protein coupled receptors, including glucagon (34). Indeed, GLP-1 has been shown to bind to the glucagon receptor, with less affinity than glucagon itself (28,29). This presents the possibility that at pharmacological doses, GLP-1 may bind to the glucagon receptor in the dog liver. The study by Ruiz-Grande et al. (85) support the concept that GLP-1 can exhibit glucagon-like catabolic effects in the adipose tissue. However, this study determined that GLP-1 exerted this effect through its own
receptor via a cAMP-mediated pathway. In this study by Ruiz-Grande et al., GLP-1 did not compete with $[^{125}\text{I}]$glucagon in binding to the glucagon receptor. However, the study of cross-binding in adipocytes could not be a good model of what occurs in the liver. In the adipocytes, glucagon receptors are scarce, and GLP-1 receptors appear to be present. In the liver, a larger number of glucagon receptors are present and the GLP-1 receptor appears to be absent. Thus, the use of a pharmacological dose of GLP-1 might allow for the interaction of GLP-1 with the glucagon receptor since there would be no competing GLP-1 receptors in the dog liver. However, it should be noted that if such a catabolic effect of GLP-1 were to exist, it should have resulted in a decrease in suppression of FFA with GLP-1 than saline in the low insulin study since the antilipolytic action of GLP-1 was not found at this dose. Since the suppression of FFA was not affected by GLP-1 at the low insulin dose, it remains to be determined if in fact such a catabolic effect of GLP-1 exists.

**Comparison of results with other in vivo studies**

D’Alessio et al (96) have recently studied GLP-1 infusion in humans while clamping insulin and glucagon at basal levels and have found a direct effect of GLP-1 on hepatic glucose production. Our results, however, indicate that there is no direct effect of GLP-1 on hepatic glucose production. There are some important differences however in the methodology used between studies. One important difference is the use of a control in our study. The D’Alessio study involved the use of experimental values taken prior to GLP-1 infusion as a control to compare with values measured after GLP-1 infusion. This creates the possibility that the decline in hepatic glucose production was, in part, a time-dependent effect, since under fasting
conditions, hepatic glucose production decreases over time. In our study, for each subject, a control study was performed in the precise manner that the GLP-1 infusion study was performed with the exception that peripheral GLP-1 infusion was replaced with saline. Thus, any difference between groups could be accounted for by an effect of GLP-1. Hvidberg et al. (127) have found that hepatic glucose production in healthy man is suppressed by GLP-1, but this effect was attributed to GLP-1’s effect on pancreatic hormones. The above study by D’Alessio is the only one which reports that GLP-1 has a direct effect on hepatic glucose production in vivo.

There are a number of investigators who claim that GLP-1 has no peripheral effects. Orskov et al. (89) have found no effects on peripheral or hepatic insulin sensitivity during a hyperinsulinemic, euglycemic clamp using somatostatin in healthy man. The methodology used in this study however differs from ours in a number of ways. Orskov used a lower dose of GLP-1 than used in our experimental protocol (approximately 1/2). This however may not explain why these investigators could not detect an improvement in peripheral insulin sensitivity since Myers et al. (87) also found no such effect in normal dogs under the conditions of a hyperglycemic (~150 mg/dl) clamp, but with the use of a total quantity of GLP-1 which was substantially higher than that used in both Orskov’s study and our study. In both our study and Orskov’s study, GLP-1 was administered intravenously, whereas Myers et al. administered it subcutaneously. There are two major differences between Orskov’s and Myers’ studies and ours: 1) The glucose clamps performed by Orskov et al. and Myers et al. were initiated with insulin and saline. Thereafter, saline was replaced with GLP-1 in half of the studies. In our study, GLP-1 was initiated together with insulin, and it might be speculated that this may have
facilitated detection of a GLP-1 effect, and 2) Both studies were performed using normal subjects (humans for Orskov and dogs for Myers), whereas our study was performed in diabetic dogs. Orskov's and Myers' results therefore illustrate that in subjects with high insulin sensitivity (as is present in nondiabetics), GLP-1 does not significantly enhance insulin sensitivity. However, the diabetic state is one in which the phenomenon of insulin resistance is quite well established. The greatly reduced insulin sensitivity is a feature which allows for a modest increase in insulin sensitivity to be detected as was determined in both our study and in the study by Gutniak et al. (97) in IDDM patients. Additionally, Villanueva-Penacarrillo et al. (128) have found that adipose tissue from both IDDM and NIDDM patients has increased GLP-1 binding. This indicates that adipose tissue in diabetics may be a site where increased insulin action due to GLP-1 could be detected more readily. In addition, it cannot be discounted that the potentiation of insulin's effect by GLP-1 is glucose dependent. To determine whether or not this is the case, our studies should be repeated using euglycemic clamps.

D'Alessio et al. found in healthy humans that GLP-1 increased glucose effectiveness (the effect of glucose on its own disposal) in an insulin-independent manner at both pharmacological (94) and physiological (95) GLP-1 levels. However, Finegood and Tzur (129) have recently questioned the validity of using Bergman's minimal model for this quantification of glucose effectiveness when comparing groups with different B-cell secretory responses as is the case when GLP-1 and control groups are compared. Toft-Nielson et al. (88) found no insulin-independent effect of GLP-1 on glucose effectiveness in healthy humans. This may again be due to the nondiabetic conditions of their subjects. However, our results support the notion that GLP-1 would have no such insulin-independent effect, even in diabetic subjects, since the
enhancement of glucose utilization seen was shown to be dependent on the insulin dose, as the
effect was not present at the low dose insulin infusion.

Freyse et al. (63) found that in insulin-deprived depancreatized diabetic dogs, there were
no effects of GLP-1 independent of the suppression of glucagon. However, in these dogs, any
extrapancreatic blood glucose lowering effect would be masked by the effect of decreased
glucagon concentrations. Furthermore, the use of completely insulin-deprived subjects does not
allow for measurement of an enhancement of insulin action, but rather an insulin-like action.
Our results indicate as well that GLP-1 would not have an enhancing effect on insulin action in
the absence of insulin (based on an extrapolation of results from low insulin doses to a state of
no peripheral insulin infusion).

Ahrens et al. (90) found that GLP-1 had no effect on insulin sensitivity during a
euglycemic clamp in NIDDM patients. However, in this study somatostatin was not used to
inhibit the incretin effect. Instead the classical M/I method of calculation was used to measure
insulin sensitivity, where M is the glucose infusion rate, and I is the plasma insulin
concentration. However, a crucial assumption of the use of this approach to measure insulin
sensitivity is that M and I are linearly related (130). Since the reported insulin levels between
the GLP-1 and control groups are substantially different and NIDDM subjects are known to be
very insulin resistant, such a linear relationship may not hold. Under such conditions, it is
possible that a modest effect of GLP-1 on insulin sensitivity may be undetectable with this
calculation. Clamping insulin at the same level, as was done in our study, would minimize this
potential error.

Tominaga et al. (91), Van Dijk et al. (92) and Minuzo et al. (93) all found positive
effects for GLP-1 on insulin sensitivity. However, the methodology used in each case may be questioned. Tominaga found that GLP-1 has an enhancing effect on peripheral glucose utilization, while having no effect on hepatic glucose production in normal rats through use of euglycemic clamps. However, somatostatin was not used to inhibit the incretin effect, indicating that increased insulin levels may be responsible for this observed effect. Van Dijk found that GLP-1 increased the metabolic clearance rate in both fed and fasted rats. Again, however, the incretin effect was not controlled for. Thus, higher insulin levels may be responsible for the observed effect. Mizuno et al. found that 4 weeks of GLP-1 treatment can enhance insulin action in peripheral tissues in obese diabetic rats during a euglycemic clamp. However, the incretin effect was not controlled for. Thus, higher insulin levels may be responsible for the observed effect. Mizuno et al. found that 4 weeks of GLP-1 treatment can enhance insulin action in peripheral tissues in obese diabetic rats during a euglycemic clamp. However, the incretin effect was not controlled for. Thus, higher insulin levels may be responsible for the observed effect.

Recently, Scrocchi et al. (131) found that GLP-1 knockout mice are hyperglycemic and hypoinsulinemic in the fed state, consistent with absence of GLP-1’s incretin effect, however, they have normal circulating insulin levels and are hyperglycemic in the fasting state, indicating insulin resistance. However, again the insulin resistance may be secondary to the hyperglycemic state due to lower insulin levels. Gutniak et al. (97) found that GLP-1 increased the glucose disposal rate during a euglycemic clamp in IDDM patients. The glucagon levels, however, were not clamped or measured. Thus, this effect may be due to increased suppression of glucagon secretion rather than an independent effect on glucose disposal by GLP-1. This study was
performed without the use of tracer. Since tracer was not used, glucose production and glucose utilization were not determined. However, it was assumed that since a high peripheral insulin dose (0.8 mU/kg.min) was infused, 1) glucose production would be completely suppressed and 2) the improvement in insulin sensitivity was due to an increase in glucose utilization. Based on previous studies in humans (132), the assumption of complete suppression of glucose production with the peripheral insulin dose used by Gutniak et al. would be reasonable in normal subjects. However, this assumption might be questioned in diabetic subjects (133). Thus, a further suppressive effect of GLP-1 on glucose production could not legitimately be discounted as a cause of the improved insulin sensitivity observed.

In vitro studies and potential mechanisms for GLP-1’s extrapancreatic effects

There is very much controversy related to the mechanism of GLP-1’s peripheral effect. Drawing on studies of the mechanism of its effect on B-cell lines and studies of its incretin effect, most of these studies point towards an effect mediated by increased cAMP levels. However, this is the same mechanism of action used by catabolic hormones such as glucagon and catecholamines. Since these actions are antagonistic to those of insulin, cAMP appears to be an unlikely candidate.

Recently, Miki et al. (84) have found that GLP-1 improves insulin-stimulated glucose uptake in rat adipocytes, while the intracellular cAMP content was found to decrease. In the same study, insulin when present at 1 nM was found to have the same effect on cAMP levels. Furthermore, Ruiz-Grande et al. (85) have found that GLP-1 exhibited a lipolytic effect in rat adipocytes, which was found to be mediated by increasing cAMP levels. These studies show
that GLP-1 might have a dual effect on adipocyte lipolysis and support the concept that GLP-1’s insulin-potentiating action is unlikely to be mediated through a cAMP mechanism.

Villanueva-Penacarrillo et al. (78) found that GLP-1 had a potent glycogenic effect in rat skeletal muscle. Yang et al. (79) also found that GLP-1 enhanced insulin-stimulated glycogen synthesis in rat L6 muscle cells, and this may be accompanied by a decrease in cAMP levels. It has recently been found that the GLP-1 receptor can couple to both stimulatory and inhibitory G proteins (75). Galera et al. (134) have recently found that a glycogenic effect is also observed in BC3H-1 myocytes and suggest that this effect may be mediated by inositolphosphoglycans and diacylglycerol. Diacylglycerol is also known to activate protein kinase C. Wheeler et al. (45) have found that in COS cells transfected with the GLP-1 receptor, the GLP-1 receptor is coupled to phospholipase C. Thus, GLP-1’s effect may be mediated via a Ca2+/protein kinase C mediated mechanism. However, this remains controversial since Thorens et al. (33) found that GLP-1 is not coupled to phospholipase C in the same cells. GLP-1’s action in the periphery might also be due to the presence of other receptors which are coupled to Ca2+ and protein kinase C, to which GLP-1 could bind at pharmacological doses (as suggested by the studies of Kieffer et al. (70) and Montrose-Rafizadeh et al. (75)). The effect of protein kinase C on insulin action is an area of intense debate since many investigators argue that protein kinase C antagonizes the action of insulin. This may be due to the actions of different isoforms of PKC. One study (135) has illustrated that in the L6 rat skeletal muscle cell line, PKC activation via the phorbol ester TPA acutely increases PP-1 activity, an effect which was attributed to increased MAPK activity by increased PKC activation. PP-1 is an intermediate in the glycogen synthesis pathway. Thus, this effect of stimulating PP-1 can potentially result in increased
glycogen synthesis. This finding adds support to the reported results of Villanueva-Penacarrillo et al. (78) and Galera et al. (134) who found potent glycogenic effects of GLP-1 in rat skeletal muscle and BC3H myocytes respectively. The glycogenic effect of GLP-1 could be potentially very interesting in the treatment of NIDDM patients since these patients are known to have a reduced ability to synthesize glycogen. Also, studies exist which indicate that in muscle tissue, protein kinase C can act to enhance PI-3 kinase activity (136). Activation of PI-3 kinase is known to increase glucose uptake, an effect which is proposed to be due to an increase in glucose transporter translocation, although the mechanism behind this effect is still unclear. The findings of an increase in glycogen synthesis reported in muscle could be important in explaining in part why we see an increased peripheral glucose utilization in our diabetic dogs. Thus, although a reduction in the level of free fatty acids in our study suggested that the improvement in insulin sensitivity might be a secondary effect, it appears possible that GLP-1 could have a direct effect on muscle, the main target tissue for insulin-stimulated glucose utilization. The improvement in glycogen synthesis and glucose oxidation as illustrated by Villanueva-Penacarrillo et al. in rat skeletal muscle provide a sink for glucose through glycolysis and the Krebs Cycle, which would help to facilitate glucose uptake in the cells by depleting the intracellular glucose concentration. It is also possible that GLP-1 directly enhances glucose transport and glucose transporter translocation in muscle, although there are no reports on GLP-1 effects on these parameters in muscle tissue. It should be mentioned that the glycogenic effect of GLP-1 in muscle is still controversial, as Furnsinn et al. (76) indicate that there is no effect of GLP-1 on glycogen synthesis in rat skeletal muscle. These results are somewhat puzzling since Furnsinn et al. reported that their study was performed in the same manner as the one by
Villanueva-Penaccarillo et al. Future studies into the potential effect of GLP-1 in improving glycogen synthesis are needed. We are unable to substantiate this effect with the results from our study since glycogen levels were not measured in our dogs.

Although there is evidence that an action of GLP-1 directly on muscle to improve glucose utilization could exist, the contribution to this peripheral effect of a lowering of plasma free fatty acid levels cannot be discounted. Paradoxically, in adipose tissue, Ruiz-Grande et al. (85) have found that GLP-1 exhibited a lipolytic action which was mediated by cAMP, an effect which was also elicited by glucagon. This illustrates that lowering of free fatty acids by GLP-1 does not seem plausible through a mechanism mediated by cAMP. In keeping with a proposed action of GLP-1 via protein kinase C however, there are studies which illustrate that increased PKC activity in rat adipocytes (137,138) enhances glucose uptake in these cells, which could explain FFA lowering by enhancing the rapid reesterification of FFA into triglycerides. PKC activation by GLP-1 might help to explain how GLP-1 enhanced insulin-stimulated glucose uptake in the studies by Egan et al. (71) in 3T3-L1 adipocytes and by Miki et al. (84) in isolated rat adipocytes. Furthermore, protein kinase C has been shown to be involved in enhancing triglyceride synthesis from glucose (137,139-143). This insulin-like action of GLP-1 may be explained by an increase in glucose transporter translocation as described by Wang et al. (82) who found that GLP-1 increases glucose transport and also glucose transporter (GLUT1 and GLUT4) translocation in 3T3-L1 adipocytes. Oben et al. (83) also found that GLP-1 enhanced the incorporation of [14C]acetate into fatty acids in explants of rat adipose tissue. Egan et al. (71) have also reported both effects of increased glucose uptake and incorporation into fatty acids in 3T3-L1 adipocytes. The elevated intracellular fatty acid levels resulting from fatty acid
synthesis can result in promotion of reesterification into triglycerides by a mass action effect (144). The high level of fatty acids may also act in an inhibitory fashion on hormone sensitive lipase (145). Another point of interest relates to the regulation of the two major enzymes that regulate FFA levels in the adipocyte. When fat in the form of circulating lipoprotein triglyceride is to enter the adipose tissue cells, lipoprotein lipase is activated to break down this lipoprotein triglyceride in the capillary lumen. The liberated FFA can then be uptaken by the cell. Inside the adipose tissue cells, the fatty acids are incorporated into triglycerides and the level of fatty acids is controlled by hormone-sensitive lipase which acts to stimulate breakdown of triglycerides into free fatty acids and glycerol. Insulin has a stimulatory effect on lipoprotein lipase and an inhibitory effect on hormone-sensitive lipase. Adipose tissue lipoprotein lipase is most active postprandially (146). Since this is true, the involvement of GLP-1, a peptide whose secretion is stimulated in the presence of nutrients in the gut, might be of some interest. However, GLP-1 was found to have no effect on lipoprotein lipase activity (147). However, this study illustrated that GLP-1 has no insulin-independent effects on lipoprotein lipase activity but did not examine whether GLP-1 enhances the stimulation of lipoprotein lipase induced by insulin. Our study cannot clarify whether GLP-1 has an effect on lipoprotein lipase since our dogs were studied after an overnight fast. Our results indicate that at the high insulin dose, both plasma free fatty acid and glycerol levels were significantly lower in the GLP-1 infused dogs than in control experiments. This suggests that GLP-1 may enhance insulin’s inhibition of hormone-sensitive lipase. The inhibition of hormone-sensitive lipase by insulin has been reported to be due to a dephosphorylation of this enzyme (148,149). Insulin may do this in one of two ways. Insulin has been shown to both decrease cAMP levels and cAMP dependent protein kinase activity, thus
reducing the phosphorylated state hormone-sensitive lipase (150). Therefore, GLP-1’s effect may be that of further lowering cAMP levels, through coupling of the GLP-1 or related receptor to inhibitory G proteins. In addition, insulin has been reported to dephosphorylate hormone-sensitive lipase through the activation of protein phosphatases (137). These protein phosphatases include PP-1 which has been shown to be activated by protein kinase C in the L6 rat muscle cell line (135). This effect may also occur in adipocytes, however this remains to be determined. Protein kinase C has recently been shown to inhibit hormone-sensitive lipase gene expression in 3T3-F442A and BFC-1 adipocytes (151). This indicates that protein kinase C would have a chronic effect in inhibiting hormone-sensitive lipase activity. From this information, it is conceivable that protein kinase C could have also have an acute effect on inhibiting hormone-sensitive lipase activity. This property should be investigated in future studies as a potential mechanism for an enhanced antilipolytic action of GLP-1.

Thus, it is evident that GLP-1 might improve peripheral insulin sensitivity through a number of actions that may enhance many actions of insulin in insulin target tissues. The possibility of a hepatic effect of GLP-1 has also been reported by some investigators. Valverde et al. (80) found that GLP-1 has a potent glycogenic effect in rat liver. Yamatani et al. (81) also found that GLP-1 inhibits glucagon-induced glycogenolysis in rat liver. These findings imply that GLP-1 does have some insulin-like hepatic effects in the rat. We found no evidence for the insulin-like or insulin potentiating effect of GLP-1 on hepatic glucose production in dogs. Thus, the possibility arises that there are species-specific effects of GLP-1. However, a recent study (77) with the same methodology used by Valverde et al. (80) failed to reproduce this effect also in rats. Murayama et al. (152) also found that GLP-1 did not affect glucose, ketone body and
CONCLUSIONS:

Our study indicates that GLP-1 has an insulin-potentiating action on peripheral glucose utilization \textit{in vivo}, independent of the incretin effect, at constant glucagon levels, in diabetic dogs. This may in part be due to potentiation of insulin’s antilipolytic action. However, it still needs to be determined whether the insulin sensitizing effect of GLP-1 can also be shown in NIDDM. A dual effect of GLP-1 on insulin secretion and action would make it an excellent candidate for the treatment of NIDDM, a pathological condition characterized by both reduced insulin secretion and insulin resistance.

Our results which indicate that GLP-1 enhances insulin action suggest that lower insulin levels would be required to maintain good diabetic control with GLP-1 than without. This would be beneficial in the treatment of NIDDM in view of the fact that chronic hyperinsulinemia is associated with an increased risk of cardiovascular disease. Whether the insulin-potentiating effect of GLP-1 on its own might contribute to this risk of cardiovascular disease remains to be determined.


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