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Effects of Glucagon-like Peptide 1 (GLP-1) on Glycemia Control and Its Metabolic Consequence after Severe Thermal Injury – Studies in an Animal Model

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Abstract

Background—Hyperglycemia with insulin resistance is commonly seen in severely burned patients and tight glycemia control with insulin (TGCI) may be beneficial in this condition. The most potent insulinotropic hormone GLP-1 stimulates insulin secretion in a glucose dependent manner. Since infusion of GLP-1 never reduces glucose levels to below ~70mg/dl, the risk of hypoglycemia associated with TGCI is reduced. In this study we investigated the metabolic effects of GLP-1 infusion after burn injury (BI) in an animal model.

Methods—Male CD rats were divided in 3 groups: BI with saline (B), BI with GLP-1 treatment (B+GLP) and sham burn (SB). BI was full thickness 40% TBSA. The B+GLP group received GLP-1 infusion via osmotic pump. Fasting blood glucose (FG), plasma insulin and plasma GLP-1 levels were measured during intraperitoneal glucose tolerance tests (IGTT). Expressions of caspase 3 and bcl-2 were evaluated in pancreatic islets. In a sub-set of animals, protein metabolism and total energy expenditure (TEE) were measured.

Results—Fasting GLP was reduced in B compared to SB or B+GLP. B+GLP showed reduced FG, improved IGTT, with increased plasma insulin and GLP-1 responses to glucose. GLP-1 reduced protein breakdown and TEE in B+GLP versus B, with improved protein balance. Increased expression of caspase 3 and decreased expression of bcl-2 in islet cells by BI were ameliorated by GLP-1.

Conclusions—BI reduced plasma GLP-1 in association with insulin resistance. GLP-1 infusion improved glucose tolerance and showed anabolic effects on protein metabolism and reduced TEE after BI, possibly via insulinotropic and non-insulinotropic mechanisms.

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INTRODUCTION

Hyperglycemia associated with insulin resistance is a common metabolic response in severely burned patients; even those without a previous history of diabetes. Recent clinical evidence suggest that mean glucose level is an independent predictor of mortality in trauma patients [1,2,3,4]. Tight control of plasma glucose level using exogenous insulin therapy has proved to be beneficial to critically ill patients [5,6,7,8]; especially trauma patients [8]. However, intensive insulin therapy is associated with a potential risk of life-threatening hypoglycemia; which counter-balances the beneficial effect of controlling hyperglycemia [9]. Furthermore, it has been shown that fluctuations in plasma glucose levels during insulin therapy are associated with increased mortality in surgical ICU patients [10]. Therefore a safer agent for glycemic control which can prevent dramatic changes of blood sugar level and hypoglycemia would significantly improve the care of these patients.

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide secreted by the L-cells of the intestinal epithelium in response to intestinal nutrients [11,12]. It is formed by proteolytic cleavage of proglucagon and is the most potent insulinotropic hormone on pancreatic beta cell secretion. GLP-1 has been demonstrated to enhance glucose-stimulated insulin secretion and improves glucose uptake by skeletal muscle, hepatic tissue and fat cells [13]. It also exerts proliferative, neogenic and antiapoptotic effects on pancreatic beta cells [14]. Numerous studies have shown that GLP-1 and its agonists are effective agents for controlling hyperglycemia in the treatment of type 2 diabetic patients [15,16,17]. GLP-1 virtually stops stimulating insulin secretion when plasma glucose levels are reduced to approximately 70 mg/dl [18]. Thus, compared with insulin, GLP-1 has the potential to be a safer agent for controlling hyperglycemia without the risk of iatrogenic hypoglycemia. Before its clinical application for hyperglycemia control, it would be important to have a comprehensive knowledge of the metabolic and insulinotrophic effects of GLP-1 in animal models of critical injury. Since the major metabolic features of burn injury are hypermetabolism, severe muscle wasting and hyperglycemia, the present study was designed to examine the effects of GLP-1 on glucose homeostasis, protein kinetics, and energy expenditure in an animal model of burn injury. Plasma levels of insulin and GLP-1 after thermal injury were also monitored and the effects of GLP-1 on pancreatic beta cells after thermal injury were also evaluated.

MATERIALS AND METHODS

The study was conducted in two stages. We first determined the effect of chronic GLP-1 infusion on glucose tolerance in burned and sham treated animals. Secondly, we explored the effect of GLP-1 on burn injury induced alterations in energy and protein metabolism and expression of caspase 3 and bcl-2 in burned animals with and without GLP-1 treatment.

Animal model

A total 42 male CD rats (Charles River Breeding Lab, Wilmington, MA) weighing ~400 gram (416.6 ± 14.3 g, Mean $\pm$ SD) were used for the study. Animals were housed in the Center for Comparative Medicine (CCM) of the Massachusetts General Hospital. The animals were acclimatized to the environment for at least 5 days after delivery and were maintained on a regular 12-h light/dark cycle (6:00 PM to 6:00 AM) with free access to food and water. To maintain a relative stable metabolic condition, all animals were trained to be positioned in cone-shaped plastic bags (Harvard Apparatus Holliston, MA) for two hours every day, starting 5 days prior to the day of surgical preparation as described by Reigle et al. [19].

Surgical preparation of the animals was as follows: One polyethylene catheter (Braintree Scientific, Inc, MA) was implanted into left carotid artery. Another polyethylene catheter connected to a micro-osmotic Pump (Alzet model 2001, Cupertino, CA) containing GLP-1 (GLP-1 7-36 peptide, Sigma-Aldrich Corp, Louis, MO), 1 mg/ml, pumping rate 1 μ l/h; B +GLP-1 group) or saline (SB and B groups) was inserted into the left jugular vein. Thus, GLP-1 was delivered intravenously at a rate of approximately 40 ng/kg/min. For animals used in the stable isotope infusion studies, an additional polyethylene catheter was inserted into the left jugular vein for infusion of the tracers. All procedures were conducted under anesthesia produced by intra-peritoneal injection of pentobarbital (Hospira Inc, Lake Forest, Illinois; 50mg/kg body weight). All catheters were filled with heparin-saline (100 unit/ml) and capped with stainless steel plugs (LS22, Instech Solomon, Plymouth Meeting, PA). The micro osmotic pumps were embedded under the skin of the neck. After removal of fur from the dorsal surface of each animal, a full-thickness thermal injury of 40% total body surface area (TBSA) was produced by placing the animal in a template exposing 40% TBSA to boiling water for 10 sec. The animals were immediately resuscitated by intra-peritoneal injection of saline (40 ml/kg) and placed in individual metal cages with free access to food and water; daily food intake was monitored. Sham burned rats were treated in the same manner as the burned animals with the exception that they were exposed to room temperature rather than boiling water.

Intraperitoneal Glucose Tolerance Tests (IGTT)

Intraperitoneal glucose tolerance tests (IGTT) were performed on three groups (n = 8 each) of animals, sham burn, burn, and burn plus GLP-1 treatment. The tests were performed on day 6 post-burn injury. After an overnight fast (from 5 pm to 8 am), the rats were placed in individual cone-shaped plastic restraining bags and each animal received an intraperitoneal injection of 10% glucose solution (1g/kg body weight). Ten minutes before the injection, an arterial blood sample (0.4 ml) was collected into a tube containing dipeptidyl peptidase IV inhibitor (DPP4-010, Linco Research, Inc., St. Charles, MA) for the determination of baseline blood glucose level, plasma insulin level and plasma GLP-1 level; this was followed by additional blood sampling at 5, 15, 30, 60, 90, and 120 min after injection. The concentrations of blood glucose were determined with a blood glucose meter (Ascensia, Bayer Corporation, Mishawaka, IN), and the plasma was immediately separated by centrifugation (1000 g $\times$ 10 min at 4°C) and stored at -80°C for determinations of GLP-1 and insulin levels. Areas under the blood insulin and glucose time-concentration curves were calculated using the trapezoidal rule.

Pancreatic tissue collection and Islet Isolation

On completion of the IGTT, the animals were euthanized by overdose of pentobarbital. Pancreatic tissue was harvested and islets were isolated by digestion with collagenase P (Roche Molecular Biochemicals) in PBS for 10min at 37 °C using a wrist-action shaker [20,21]. Islets were isolated by hand picking under microscopic guidance and processed for Western blot analysis of caspase 3 and bcl 2 as described below.

Stable Isotope Studies

Stable isotope studies were conducted on the three groups of animals that were treated in the manner described above. These studies were designed to measure whole body energy expenditure and protein metabolism in sham burn (n=6), burn (n=7) and GLP-1 treated burned (n=5) animals. The stable isotope studies were conducted on day 6 post burn when burned animals were in a hypermetabolic state. The total energy expenditure measurements were conducted on all animals after an overnight fast (food was removed 5 pm the day before) using an indirect calorimeter system specifically designed for rodents (CaloSys 994620 series, TSE Systems, Inc. Midland, MI); for 2.5 hours, the rates of total oxygen

consumption and carbon dioxide production were automatically calculated and reported by the system. At the end of each measurement, the animal was removed from the metabolic chamber, placed in a cone-shaped restraining bag and a primed constant infusion of L-1- ^{13}C -leucine (99% ^{13}C , Cambridge Isotope Laboratories, Inc. Andover, MA) was performed for 2 hours (priming dose 8.93 μmol , infusion rate 0.149 $\mu\text{mol} \cdot \text{min}^{-1}$) for measurement of whole body protein metabolism. Blood samples (0.7 ml) and expired air samples were collected prior to and at 90, 100, 110, and 120 min after beginning the tracer infusion. Isotope enrichments of 1- ^{13}C - α -ketoisocaproate (KIC), a surrogate for intracellular leucine enrichment in plasma [22] and $^{13}\text{CO}_2$ in expired air were measured. The rates of whole body protein synthesis and breakdown were calculated based on plasma leucine kinetics using previously described methods [23].

Measurement of Plasma Levels of Insulin, GLP-1 and 1- ^{13}C -ketoisocaproate, and $^{13}\text{CO}_2$ in Expired Air

The plasma levels of insulin and the active form of GLP-1 were measured using commercially available Elisa kits (Linco Research, Inc., St. Charles, MA). Stable isotope enrichment of plasma 1- ^{13}C -ketoisocaproate was measured on silylquinoxalinol (SQ) derivatives using gas chromatography-mass spectrometry methods as described before [23]. Enrichment of $^{13}\text{CO}_2$ in samples of expired air were analyzed using isotope ratio mass-spectrometry [23].

Western Blot Analysis

Bcl-2 and caspase-3 (indicators of pancreatic islet anti-apoptosis and apoptosis) were assessed by Western Blot Analysis. The total protein in isolated islets was extracted with T-PER® Tissue Protein Extraction Reagent (Pierce Rockford, IL) after adding Halt™ Protease Inhibitor Cocktail Kit (Pierce Rockford, IL) and Western blot analysis was performed as follows: 50 μg of total protein from each sample was subjected to SDS-PAGE using 4–12% gradient Bis-Tris gels (Invitrogen, Carlsbad, CA) under reducing conditions. Proteins were then transferred to nitrocellulose membranes (Invitrogen, Carlsbad, CA) using an electrotransfer system (XCell II Blot Module, Invitrogen, Carlsbad, CA). Nonspecific proteins were blocked with 0.2% nonfat dry milk in borate buffer (Kirkegaard & Perry Laboratories, Inc. Gaithersburg, MD) for 1.5h. Blots were incubated with a primary antibody, followed by a secondary antibody (horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit antibody at 1:4000 dilution; all the antibodies used in this study were purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Blots were washed with 1x Tris-buffered saline/Tween (Sigma-Aldrich Corp, Louis, MO) for 30 mins. between steps. Antibody C-2 (1:200 dilution) was used for detection of bcl-2 (28 kDa), antibody H-277 (1:200 dilution) was used to detect caspase-3 (35kDa), and antibody (1:2000 dilution) C4 was used to visualize beta-actin (43kDa). Signal intensities were analyzed using the Quantity One Image Program (Version 4.6.3, Bio-Rad Laboratories, Inc. Hercules, CA). Levels of actin were used to normalize the levels of bcl-2 and caspase 3 as a control for loading differences in total amounts of protein. The protein levels of bcl-2 and caspase 3 were presented as their relative percentages compared to the SB group (100%; Figure 4).

Statistical Analysis

All results were expressed as mean $\pm$ SEM. For the IGTT studies, areas under the curves (AUC) for plasma glucose and insulin levels following injection were calculated using the trapezoidal rule. Statistical analysis was performed by one-way analysis of variance (ANOVA) and individual means were compared by the Student-Neuman-Keul's (S-N-K) test. P values less than 0.05 were considered to be statistically significant. Prostate (Polysoftware International, Pearl River, NY) software was used for analysis.

RESULTS

Intraperitoneal Glucose Tolerance Test (IGTT): Blood Glucose Levels

The results of the IGTTs are shown in Fig 1A. On post burn day 6, there was significant difference in fasting glucose levels among these groups ($F=11.88$, $P<0.004$), B vs. SB groups (mean $\pm$ SEM; 111.3 ± 7.6 vs 97.1 ± 6.2 mg/dl, $P<0.001$). Fasting glucose levels in B+GLP-1 animals (99.4 ± 4.5 mg/dl) were similar to levels in SB animals ($p=0.478$) but significantly lower than in B animals. ($p=0.001$). There was no significant difference in plasma glucose levels at 5 and 15 min post glucose injection among the three groups. However, at all later time points, plasma glucose levels remained significantly higher in the B group, as compared with the SB group and the B+GLP-1 group ($p<0.01$). AUC's of the blood glucose response curves (0 min to 120 min.) are shown in Fig 1B. One-way ANOVA demonstrated a significant difference among the three groups ($F= 4.55$, $p=0.0227$). There was a higher AUG in the B group as compared to the SB ($p=0.001$) and B+GLP-1 Group ($p=0.001$), indicating that GLP-1 treatment improves glucose tolerance in burned animals. The AUG of B+GLP-1 Group was not statistically different from the SB group ($p= 0.106$) although the value was higher than SB group.

Intraperitoneal Glucose Tolerance Test (IGTT): Plasma GLP-1 levels

Plasma GLP-1 levels measured in the 3 groups of animals on post burn day 6 during IGTT (at baseline and at various times after glucose administration) are shown in Figure 2. At baseline, there was a difference among three groups ($F= 78.15$; $P<0.0001$). the B group animals showed significantly lower levels of GLP-1 as compared to the SB group (7.74 ± 4.49 vs. 21.73 ± 7.22 pmol/L, $p=0.001$) and this difference persisted for 120 min ($p<0.001$ at each time point with ANOVA and S-N-K tests). Glucose administration resulted in a two-fold increment in plasma GLP-1 level in the SB group; but not in the B group. As expected, GLP-1 infusion markedly increased plasma GLP-1 level and AUG in B+GLP-1 animals as compared to B and SB animals.

Intraperitoneal Glucose Tolerance Test (IGTT): Plasma Insulin Levels

The insulin levels in different groups of animals before and during the IGTTs are shown in Figure 3A. On post burn day 6, fasting insulin levels were lower in B and B+SB groups as compared to SB group (ANOVA: $F= 6.01$ $p=0.008$; SB vs. B; $p=0.008$; SB vs. G+GLP-1, $p=0.026$). There was no statistical difference between the B and B+GLP-1 groups although there was a higher value of plasma insulin in B+GLP-1 group as compared to B group. Following bolus intraperitoneal injection of glucose during the IGTT test, there was an immediate increase in plasma insulin level in all 3 groups of animals. AUC's of the blood insulin response curves are shown in Fig 3B. AUC during the first 30 minutes after glucose injection were significantly lower in B group animals as compared to SB animals (ANOVA Test $F= 14.32$; $p=0.0001$; SB vs. B, $p= 0.001$) indicating a reduced immediate insulin response to intravenous glucose. B+GLP-1 animals showed an increase in insulin response comparing to B group ($p=0.008$), but the AUC of B+GLP-1 was still significantly lower than that in SB animals ($p=0.027$).

Expressions of caspase-3 and bcl-2 in pancreatic tissues

Western blot analysis of bcl-2 and caspase 3 expression in isolated pancreatic islets was conducted to assess the extent of apoptosis of pancreatic islet cells after burn injury and burn injury plus GLP-1 treatment. These parameters have been reported in the literature to assess beta cell apoptosis (23, 24, 25) The protein levels in each analysis were normalized to actin level. The data are presented as a relative percentage of expressions in the B and B+GLP-1 groups as compared to the SB group (Fig. 4). One-way ANOVA of the bcl-2 and caspase 3

expression data revealed significant main effects of treatment (bcl-2, $F=35.12$, $P<0.001$; caspase 3, $F=31.93$, $P<0.001$). Expression of bcl-2 was significantly decreased in the burn group ($64.9\pm7.0\%$ of SB, $P<0.001$), however, this reduction was significantly reversed in the B+GLP-1 group ($94.6\pm2.2\%$ of SB; $P<0.001$ compared to B). Expression of caspase 3 was significantly enhanced in the B group ($157.0\pm11.0\%$ of SB, $P<0.001$), but was reduced in B+GLP-1 group ($105.6\pm9.0\%$ of SB, $P<0.001$ compared to B). Thus, burn injury reduced islet cell bcl-2 expression and increased caspase 3 expression, but these effects were reversed in animals receiving 6 days of constant GLP-1 infusion.

Protein kinetics

Table 1 summarizes the rates of protein synthesis/breakdown and balance for the 3 groups of animals as measured with L-1- ^{13}C -leucine. One-way ANOVA for the kinetics results did not show significant difference in the rate of whole body protein synthesis among the three groups ($F=1.08$, $p=0.3644$). However, the rate of whole body protein breakdown ($F=4.67$, $p=0.027$) and protein balance ($F=7.87$, $p=0.0046$) were significantly different among the three groups. As expected, burn injury caused a significant increase in the rate of whole body protein breakdown (B vs. SB; $p=0.04$), and a negative protein balance (B vs. SB; $p=0.005$) as compared to the sham burned group. However, burned animals receiving GLP-1 treatment showed a significant reduction in whole body protein breakdown rate (B vs. B+GLP, $p=0.013$) with a predominant reduction in whole body protein breakdown rate (B+GLP, $p=0.027$). The rates of protein breakdown and protein balance were GLP-1 treated animals were not significantly different from the sham burn group. Thus, chronic GLP-1 treatment caused a significantly improved net protein balance in the burned animals.

Energy Expenditure

The measured energy expenditure rate on day 6 after thermal injury showed significant treatment effects among the three groups of animals ($F=6.956$, $p=0.0062$). As expected, at six days after thermal injury, burned animals demonstrated a significantly higher level of energy expenditure as compared to the sham burn group (B vs. SB; 4.75 ± 0.26 vs. 4.18 ± 0.13 Kcal.kg $^{-1}$.h $^{-1}$, $p=0.008$). However, burned animals receiving GLP-1 treatment showed a reduced energy expenditure rate (4.10 ± 0.17 Kcal.kg $^{-1}$.h $^{-1}$, vs. B group; $p=0.01$), which was not significantly different from the sham burn group (B+GLP vs. SB; $p=0.826$).

DISCUSSION

This study was designed to evaluate the metabolic consequence of chronic GLP-1 administration after burn injury. The results establish that as compared to sham burned animals, the burned rats showed higher fasting blood glucose level, reduced blood glucose tolerance, increased energy expenditure and a higher level of protein catabolism, as manifested by a higher rate of protein breakdown with a negative total protein balance. Similar findings have been reported in our previous studies of human burn patients using similar methods [26].

We also demonstrated that higher fasting glucose levels in burned animals are associated with lower levels of GLP-1, suggesting that burn injury results in a relative deficiency of plasma GLP-1. In addition there was a reduced response of plasma insulin to exogenous glucose in burned animals, which was correlated with a compromised plasma GLP-1 response in burned vs. sham burn animals. It has been reported that GLP-1 is the most potent insulinotropic agent which accounts for at least 50% of total insulin secretion after an oral glucose challenge [27] and in diabetics it stimulates insulin secretion in a dose- and glucose-dependent manner [28,29]. Thus, reduced fasting GLP-1 level and/or lack of GLP-1 response to glucose challenge in burned animals could be a contributory factor to burn

injury induced insulin resistance, as demonstrated by the higher blood glucose level and glucose intolerance seen in these animals. Although it has been reported that multiple inflammatory mediators compromise insulin secretion from pancreatic beta cells [12], the present study demonstrated that GLP-1 infusion in burned animals enhanced the response of plasma insulin levels to glucose challenge. Thus, our results support the clinical use of GLP-1 for glycemic control in burn patients.

The mechanism(s) which lead to reduced levels of GLP-1 after thermal injury remains unclear but could be the consequence of decreased synthesis and/or increased degradation. GLP-1 is mainly generated by L cells in the gut, which are distributed throughout both the small and large intestines. In both healthy humans and rodents, plasma GLP-1 increases rapidly after food ingestion [30]. However, in burn patients, ischemic and anoxic injury to the intestinal mucosa [31] may affect synthesis and secretion of GLP-1 by L-cells. Although details of the pathways controlling proglucagon synthesis and the formation of GLP-1 have not been fully elucidated, there is evidence that insulin itself may up-regulate GLP-1 production via receptors on gut L cells [32]. Thus, reduced insulin receptor function after thermal injury may compromise insulin-stimulated GLP-1 production. On the other hand, since GLP-1 is degraded by multiple enzymes including dipeptidyl peptidase IV (DPPIV) [33] and neuropeptidase 24.11 [34], expression of these enzymes and/or alterations in their activities could also be factors regulating plasma GLP-1. Further investigations of the detailed molecular mechanisms for GLP-1 secretion and degradation, and its effect on pancreatic beta cell function after burn injury are clearly warranted.

Since the dose of GLP-1 required to affect metabolic responses in burn patients has not been reported, in the present study, a dose of 40 ng/kg/h was used, which is similar to the effective dose in animal models of diabetes treatment [35]. Due to the short biological half-life of GLP-1 [36], the peptide was continuously infused with an implanted pump. Constant supply of GLP-1 at 1 µg/h significantly increased the plasma level of GLP-1 to an approximate level of 130 pmol/L (Figure 2). We observed that this rate of GLP-1 infusion attenuated burn injury induced hyperglycemia and reduction in insulin secretion in response to bolus administration of glucose, improved glucose intolerance, reduced the hypermetabolic state and improved protein balance (mainly by reducing whole body protein degradation). Thus GLP-1 produced multiple positive metabolic effects in maintaining glucose homeostasis and ameliorating burn induced insulin resistance. It is worth mentioning, that the plasma levels of GLP-1 achieved in burned animals after GLP-1 infusion were in a similar range to those required for effective hyperglycemia control in patients with type 2 diabetes [37].

Numerous studies have investigated apoptosis in different tissues following thermal injury in animal models [38,39] and have shown apoptotic changes in almost every organ or system, indicating a “systemic apoptotic response” [40]. However, this response has not been demonstrated in islet cells. Our study revealed that treatment of burned animals with GLP-1 for 6 days stimulated expression of bcl-2 (proliferation marker) and reduced expression of capsase 3 (apoptosis marker) in pancreatic islet cells. Since GLP-1 receptors are mainly distributed on islet cells, the observed anti-apoptotic effects are primarily targeted at reducing apoptosis of insulin-secreting beta cells. The effects of GLP-1 in protecting islet cells have been reported in patients receiving islet cell transplantation [41,42]. The protection of pancreatic islet cells from apoptosis lends support to our finding that burned animals treated with GLP-1 showed more active insulin response to glucose injection than those treated with saline (Figure 3).

The observed blood glucose control and anabolic effects of GLP-1 in severely burned animals is partially related to its effect in stimulating insulin secretion. Insulin has been

widely used for controlling hyperglycemia and as an anabolic hormone in the metabolic care of burn patients [43,44]. However, insulin infusion significantly increases the risk of hypoglycemia, which increases risk for ventricular arrhythmias; a potentially fatal complication of insulin therapy. Recent reports have indicated that the incidence of hypoglycemia during insulin treatment range from 2.8% [45] to 30% [46]. Furthermore, insulin infusion by itself, cannot overcome the insulin resistance state. In clinical settings, insulin infusion requires frequent monitoring of blood glucose (at least every two hours) which significantly increases the labor intensity to health care providers. In fact, two large-scale studies of tight glycemia control using insulin had to be stopped due to a high incidence of hypoglycemia. Our study indicated that although GLP-1 infusion to burned animals resulted in a peptide concentration that was about 4 fold of the basal levels, it ameliorated burn induced hyperglycemia without causing hypoglycemia, and insulin levels were maintained within the physiological range. Thus, GLP-1 is potentially a safer agent for glycemia control, and as an anabolic agent in burn patients. The safety and efficiency of its clinical applications have been confirmed by recent reports on glucose control in ICU surgical patients [35] and in protection of beta cell function after transplantation [47].

Some of the effects of GLP-1 are independent of its insulinotropic action. For example, GLP-1 treated animals showed a decrease in the hypermetabolic state, which is not seen with insulin treatment. Although the mechanism(s) for these effects have not been fully elucidated, it has been reported that specific GLP-1 receptors existed in liver, skeletal muscle and the center nervous system. Thus GLP-1 may directly affect gluconeogenesis/ glycogenolysis in the liver, glucose transport in skeletal muscle [48,49,50] and energy metabolism in the CNS. The interactions of the GLP-1 signal transduction pathway with the insulin signal transduction pathway, and its effect on energy regulation definitely require further exploration.

In summary, the present study explored certain beneficial metabolic effects of GLP-1 treatment after severe burn injury. In general, the peptide reduced the insulin resistance state and demonstrated anabolic effects in ameliorating hypermetabolism and the protein catabolic state. Since GLP-1 stimulates insulin secretion at physiological levels without the risk of hypoglycemia, it has the potential to become a useful agent for controlling hyperglycemia in severely burned patients.

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Figure 1. Plasma Glucose Levels with IGTT

1A: The fasting glucose level was significantly higher in the burned group, as compared to the sham burn group (mean $\pm$ sem; 111.3 ± 7.6 vs 97.1 ± 6.2 mg/dl, $p < 0.001$). Fasting glucose level in GLP-1 treated burned animals (99.4 ± 4.5 mg/dl), was close to that of sham burned animals ($p = 0.478$), but significantly lower than in burned animals ($p = 0.001$). There was no significant difference in plasma glucose levels at 5 and 15 min post glucose injection among the three groups. However at all the later time points, plasma glucose levels remained significantly higher in the burned group, as compared with the sham burned group and the burned group with GLP-1 treatment (* $P < 0.01$).

1B. AUC's for the blood glucose response curves. AUC of B group is higher as compared to the SB group (* $p < 0.001$) and B+GLP-1 Group (* $p < 0.001$); indicating that GLP-1 treatment reduces glucose intolerance in burned animals. The AUG of B+GLP-1 Group was not statistically different from the SB Group ($p = 0.106$).

Figure 2. Plasma levels of GLP-1

Plasma GLP-1 levels were measured in the three groups of animals on post burn day 6 during IGTT. Burn injury caused a significant decline in the fasting level of GLP-1 as compared to the sham burn group (B vs SB * $p < 0.001$). Glucose administration caused a ~two-fold increment in plasma GLP-1 in the sham burn group, but this was not seen in the burn group. As expected, GLP-1 infusion significantly increased plasma GLP-1 levels compared with sham burned animals.

Figure 3. Plasma levels of Insulin during IGTT

3A: Insulin levels were evaluated during the IGTT tests on Day 6 in SB, B and B+GLP-1 animals (n=8). On post burn day 6, fasting insulin levels were lower in B and B+SB groups as compared to SB group (SB vs. B; $p<0.018$; SB vs. G+GLP-1, $p<0.03$). There was no statistical difference between the B and B+GLP-1 groups although there was a higher value of plasma insulin in B+GLP-1 group versus B group. Following bolus intraperitoneal injection of glucose during the IGTT test, there was an immediate increase in plasma insulin level in all 3 groups of animals.

Figure 3B. AUC's during the first 30 minutes after injection. The AUC in B group was significantly lower than that of the SB animals (* $p<0.0001$); indicating a decreased immediate insulin response to intravenous glucose injection. B+GLP-1 group showed a higher AUC of insulin comparing to B group (# $P<0.008$), but this was significantly lower than that of the SB animals (+ $p<0.027$).

Figure 4.

Relative expression of cell proliferation marker bcl-2 and apoptosis marker caspase 3 in pancreatic islets. Western blot analyses of protein expression in pancreatic islets were conducted and the results were normalized to beta actin level in each group. The relative expression of bcl-2 and caspase 3 in B and B+GLP1 groups were compared with those in the SB group. One way ANOVA of the bcl-2 and caspase 3 expression data revealed significant main effects of treatment. Expression of bcl-2 was significantly decreased in the B group ($64.9 \pm 7.0\%$ of the SB, $*P < 0.001$), however, this reduction was reversed in the B+GLP-1 group ($94.6 \pm 1.0\%$ of SB, $\#P < 0.001$ when compared to B). Expression of caspase 3 was significantly enhanced in the B group ($157.0 \pm 11.0\%$ of SB, $\#p < 0.001$), but it was significantly reduced in the B+GLP-1 ($105.6 \pm 9\%$ of SB; B+GLP-1 vs B; $\#p < 0.001$).

TABLE 1

Protein Kinetics

	Sham Burn (n=6)	Burn (n=7)	Burn + GLP-1 (n=5)
Synthesis	15.6 ± 1.1	16.4 ± 0.9	14.5 ± 0.8
Breakdown	18.4 ± 1.4	22.1 ± 1.4 ^{**}	17.9 ± 0.7 ^{##}
Balance	-2.8 ± 0.4	-5.6 ± 0.7 ^{**}	-3.4 ± 0.2 ^{##}

* Data presented as mean ± SEM in g · kg⁻¹ D⁻¹.

** P<0.01 compared with sham burn group;

P<0.01 compared with burn group.