Effects of a Fat-Rich Diet in the Pancreas of Rats During the Acute Phase of Burns

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ABSTRACT

OBJECTIVES: **Oversupply of nutrients overstimulates beta cells in standard conditions, and severe burn injuries increase the metabolic needs. In this study, we investigated the effects of fat-rich nutrients on the endocrine pancreas during the acute phase of severe burns.**

MATERIALS AND METHODS: **Twenty-one Wistar albino rats were randomly divided into 3 groups. Two groups were fed with standard commercial rat chow (1 with sham procedure and 1 with burn procedure), and the third group (also with burn procedure) was fed a fat-rich diet (60% kcal fat). The burn procedure involved a 25% total body surface area full-thickness burn. Blood samples were taken at 36 hours and 7 days after burn injury or sham procedure. On postburn day 7, skin biopsies were taken and a pancreatectomy was performed. Pancreatic tissues were examined under light microscopy; islets size and cellularity were calculated and investigated immunohistochemically.**

RESULTS: **Plasma glucose, C-Peptide, and insulin levels were similar in all the study groups 36 hours and 7 days after burn induction or sham procedure. There was a significant increase in the number of cells per one islet in the burn group given a fat-rich diet compared with the other groups (P = .05). Caspase-3 was strongly expressed in both groups with burn injuries.**

CONCLUSIONS: **Overconsumption of certain fats can lead to a compensatory response by beta cells that eventually can progress to beta-cell dysfunction during the acute phase of burns. Severe burns induce pancreatic islet cell hyperplasia. Providing excessive fat nutrients during the acute postburn period attenuates this response. Despite the morphophysiological changes observed in the pancreas, all animals in our study were able to achieve a similar glycemic homeostasis.**

KEY WORDS: *Apoptosis, Burn injury, Hyperglycemia, Insulin resistance*

INTRODUCTION

Burn injury results in a unique pathophysiology that generates profound endocrine, inflammatory, metabolic, and immune changes that lead to elevated resting energy expenditures, catabolism, and multiorgan dysfunction. All of these changes cause profound alterations in glucose homeostasis, inducing a hyperglycemic state that subsequently generates a cascade of transient or permanent local and systemic modifications that increase patient morbidity and mortality.[1,2] The excessive inflammatory and metabolic responses seen in the acute phase of burns induce pancreatic beta-cell apoptosis, and the presence of hyperinsulinemic hyperglycemia is indicative of insufficient insulin secretion from beta cells.[3,4] Underlying molecular mechanisms of burn-induced beta-cell failure with acquired insulin resistance is not well known, especially because beta-cell mass cannot be quantified in humans in vivo. However, several experimental studies in animal models and human autopsy have demonstrated apoptosis of beta-cells following inflammatory states and major traumas such as severe burns,[4,5] and in our previous experimental study in a rat model we found a decrease in the number of pancreatic islets with hypercellularity during the acute phase of burn injury.[3] Lipotoxicity before burn contributes to worse clinical outcomes in obese patients after a major trauma.

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Although the effects of high circulating lipids on the liver have been studied extensively, possible effects of a high-fat diet after burn injury have not been evaluated in detail with regard to the pancreatic functions and morphology.[3,5]

Recent studies have described the role of interleukin 1 beta (IL-1β) inducing pancreatic beta-cell apoptosis during the early phase of postburn hyperglycemia through a mechanism similar to that seen in type 1 diabetes.[4] The quick activation of monocytes and macrophages following burn injury induces the release of mature IL-1β and inflammasome components including Caspase-1.[6] Neutralization of IL-1β produces beneficial effects on postburn glycemic control and improves survival.[4] All of these findings suggest that apoptosis in the pancreas of patients with severe burns may be linked to a series of events that can induce Caspase-1 activation and IL-1β release,[6] and these pathways may be influenced by the nutritional status, especially in the early postburn phase. Nutrition has a complex relationship with the hypermetabolic state experienced by patients with burn injuries, and early enteral feeding significantly attenuates the hypermetabolic response. Therefore, early enteral feeding not only minimizes the complications but improves the outcomes, and it is highly recommended to initiate it within the first 24 hours postburn if possible.[7]

Oversupply of nutrients overstimulates beta-cells in standard conditions.[8] Because severe burn injuries will increase metabolic needs, aggressive high-protein enteral feeding is used in the postburn period to improve recovery and healing. In patients with burn injuries, the ability of the body to handle additional amounts of fat is significantly altered; hence, the proportion of fat to be supplemented should be carefully estimated.[9] Together with the high-protein content, there is still inconclusive evidence for improved outcomes in burn patients either with high-fat or high-carbohydrate enteral feeds. In the present study, our goal was to investigate the effects of fat-rich nutrients on the endocrine pancreas, including beta-cell response, in the acute phase of severe burn injuries in an animal model. Our hypothesis was that augmented and uncontrolled lipotoxicity after burn trauma causes more dramatic metabolic stress on beta-cells that are already exhausted with the hypermetabolic and insulin resistant state of burn trauma.

MATERIALS AND METHODS

This study was conducted at the Baskent University Faculty of Medicine (Ankara, Turkey). We obtained 21 male Wistar albino rats weighing 350 to 400 g from the Baskent University Laboratory Animal Breeding Center in Ankara, Turkey. The animals were kept at the Baskent University Laboratory Animal Center under standardized conditions for light and temperature for 15 days before the start of the study to allow proper acclimatization; food and water were provided ad libitum. All experimental procedures adhered to the Guiding Principles in the Use and Care of Animals published by the National Institutes of Health, and the protocol was approved by the Baskent University Animal Care and Ethics Committee (DA/2022/10).

Burn procedure

A well-established method for the induction of a 25% total body surface area (TBSA) full-thickness burn was used.[3,10,11] Rats were anesthetized by a combination of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride injected intraperitoneally. The dorsum of each rat was shaved with clippers and marked for an area equivalent to 25% of the calculated TBSA. A brass plate of 4 cm × 4 cm, with temperature monitored using a thermocouple device of a multimeter (Fluke 116 HVAC), was heated to 250 °C under the flame of a Bunsen burner and placed onto the marked area for 10 seconds to create a full-thickness burn of 25% TBSA.[3,10,11] Immediately after the burn induction, all animals received 2 mL/100 g of lactate Ringer solution via intraperitoneal injection for fluid resuscitation and fentanyl hydrochloride at a dose of 0.02 mg/kg for analgesia. The wound was covered using silver sulfadiazine, and the animals were returned to their cages. The thickness of each burn injury was confirmed by histopathologic examination. Animals from the sham group were handled identically with the exception of burn injury induction.

Pancreatectomy

Animals were anesthetized with a mixed intraperitoneal injection of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride at day 7 postburn. Immediately after anesthesia induction, animals were immobilized in dorsal decubitus, and the abdominal area was shaved using an electrical trimmer. Under aseptic conditions, an upper abdominal midline incision was performed to open the peritoneum. The cecum and the stomach were gently exteriorized to expose the spleen and the pancreas; the spleen with the attached pancreas was gently pulled with blunt broad forceps and exteriorized on a gauze. Afterward, a dissection was performed upward to the spleen to gently separate the pancreatic tissue.

Groups

We divided the 21 Wistar albino rats randomly into 3 groups, each consisting of 7 rats, as follows: sham (S) group, burn (B) group, and burn plus fat-rich diet (B+Nut) group.

The S group animals were fed with standard commercial rat chow and water ad libitum before we started the sham procedure. Anesthesia was given, and the dorsa of each animal was shaved. Ringer lactate solution (2 mL/100 g) was injected intraperitoneally. Wound dressings were

applied on the shaved area were changed every other day. Blood samples were obtained 36 hours after we performed the sham procedures. The same standard diet continued under laboratory conditions for 7 days. Seven days after the sham procedure was performed, blood samples and skin biopsies were obtained, and all the animals underwent a pancreatectomy before being humanely killed.

In the B group, animals were fed with standard commercial rat chow and water ad libitum. The dorsa of each animal was shaved, and a 25% TBSA burn was induced following the method previously described. Resuscitation was accomplished by intraperitoneal injection of Ringer lactate solution. Blood samples were obtained 36 hours postburn. Wound dressings were changed every other day. Animals were fed with the same nutritional protocol as the S group of standard rat chow over the 7 days postburn. On day 7, blood samples and skin biopsies were obtained and a pancreatectomy was performed. After the surgery, animals were humanely killed.

All 7 animals in the B+Nut group were fed with standard commercial rat chow and water ad libitum for 14 days before starting the experimental period. After inflicting a 25% TBSA burn as previously described, all animals were then fed with a fat-rich diet containing 60% kcal from butter origin (Arden Arastirma Deney). Wound dressings were changed every other day. Blood samples were obtained 36 hours postburn. The fat-rich diet protocol continued; after 7 days postburn, blood samples were collected, skin biopsy was taken, and a pancreatectomy was performed before animals were humanely killed.

Measurement of glucose, interleukin 1β, C peptide, and insulin

Serum glucose level was assayed by an enzymatic (Hexokinase/G-6-PDH) method, using an Abbott Alinity c Analyzer according to the manufacturer's specifications. Serum concentrations of IL-1β, C peptide, and insulin (USCN Wuhan USCN Business Co., Ltd.), were evaluated by an enzyme-linked immunosorbent assay using spectrophotometer optical density of 450 nm (Epoch, BioTeck Instruments Inc). The detection limits were as follows: IL-1β <5.4 pg/mL, C peptide <49.9 pg/mL, and insulin <51.6 pg/mL. The intra- and interassay variabilities were 10% and 12%, respectively, for all of these tests.

Hematoxylin and eosin staining process

Tissue samples were fixed in 10% buffered formalin, subjected to routine histological processing, and embedded in paraffin. The paraffin blocks were oriented in such a way that their long axes could be seen after 24 hours of fixation in paraffin; 4-μm thick sections were obtained from each block, stained with hematoxylin-eosin (H&E), and examined under a light microscope. A 3Dhistech Panoramic P250 Flash III scanner was used to digitize the slides.

Image acquisition and analysis

Imaging processing was performed with the ViraPath application (Virasoft Software Inc). The pancreatic islets were marked after images were captured, and calculations of the islet areas and number of cells within the islets were made by the application.

Caspase 3 and interleukin 1β levels

Immunohistochemical staining was conducted in Omnis (Dako) with the EnVision Flex staining kit. Sections were kept at 60 °C for 60 minutes and dewaxed with Clearify solution (Dako) at 25 °C for 1 minute in an autostainer. For heat-induced antigen retrieval, we used citrate buffer (EnV Flex HRS, low pH) for caspase 3 antibody and EDTA buffer (EnV Flex HRS, high pH) at 97 °C for 30 minutes for IL-1β antibody. After sections were rinsed with a wash buffer, sections were incubated with rabbit polyclonal caspase 3 antibody (GTX110543, GeneTex, dilution 1:100) and rabbit recombinant multiclonal anti-IL-1β antibody (clone RM1009, Abcam, dilution 1:100) for 30 minutes. Sections were then incubated with peroxidase solution (EnV Flex peroxidase-blocking reagent, Dako) for 3 minutes. After sections were rinsed, they were reactivated in EnV Flex/HRP solution for 20 minutes and then incubated for 5 minutes with EnV Flex substrate working solution (Dako) for visualization. Sections were then counterstained with hematoxylin. All histopathological analyses were performed under a light microscope (Olympus BX43F) by a pathologist blinded to the type of treatment, and parameters were evaluated semiquantitatively.

Cytoplasmic staining intensity of islet cells was evaluated with caspase. No staining was scored as 0, pale cytoplasmic positivity was scored as 1, and strong cytoplasmic positivity was scored as 2. With IL-1β, scoring was done according to the number of positive cytoplasmic stained cells in the islets. No staining was scored as 0, positive staining in <5 cells in islets was scored as 1, and positive staining in >5 cells in islets scored as 2.

Statistical analyses

We analyzed data using IBM SPSS version 25.0 for Windows. For descriptive statistics, results are shown as number and percent for evaluation of categorical variables. Kolmogorov-Smirnov normality test was used for the conformity of numerical variables to normal distribution, and median (minimum-maximum) values were given since the assumption of normal distribution was not provided. Kruskal-Wallis analysis of variance test was used to analyze differences between groups in terms of numerical variables.

Post hoc comparison tests were used to examine in which groups the variables found to be significant as a result of the Kruskal-Wallis test differed. The Wilcoxon signed rank test was used in the dependent groups to compare the 36-hour and 7-day observation values of the numerical variables within each group. Type I error probability was taken as $\alpha = 0.05$ in all hypothesis tests.

RESULTS

Blood levels of glucose, insulin, and C peptide

Plasma glucose, C peptide, and insulin levels were similar in the S group, B group, and the B+Nut group at 36 hours and 7 days after burn induction or sham procedure (Table 1).

Morphometric analysis of the pancreatic tissue

The total number of cells in the whole pancreas tissue increased after burn injury. This increase was greater in rats fed with the fat-rich diet compared with the other groups ($P = .01$). The B group had the lowest mean number of islets per pancreatic tissue ($P < .05$) (Figure 1 and Figure 2). The increase in the number of cells per one islet (Figure 3 and Figure 4) reached statistical significance in the B group when compared with the S group and the B+Nut group ($P = .05$).

Immunohistochemical staining of caspase 3 and interleukin 1β

Caspase 3 was strongly expressed after burn trauma in the B group and the B+Nut group (Figure 5). Pancreatic tissues of all the animals in the B+Nut group showed at least a grade 1 (weak) response, whereas 28.2% of the pancreas tissues from the B group showed no immunostaining for Caspase-3 (Figure 6). No IL-1β staining was observed in 85.7% of the B group, whereas 57.2% of the B+Nut group tissue samples were stained for IL-1β (Figure 7 and Figure 8).

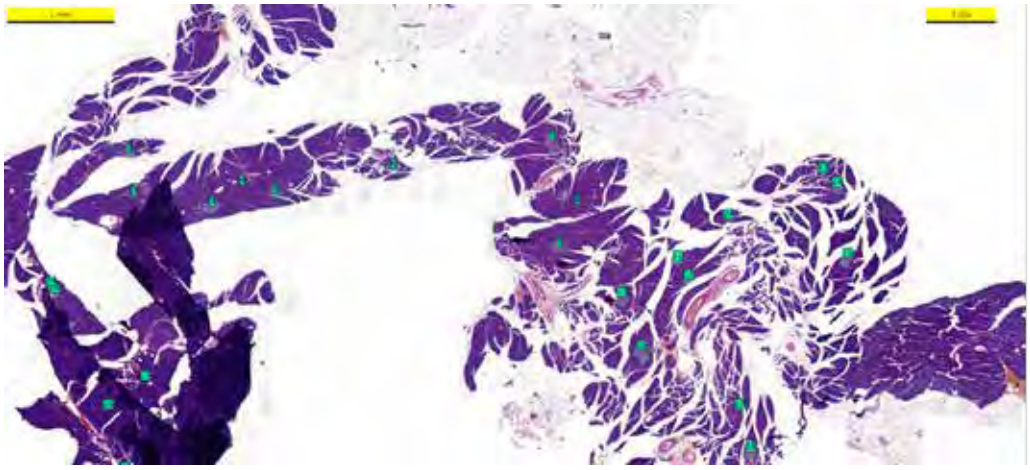
DISCUSSION

Nutrition is a key aspect of outcomes after burn injury; over the previous 4 decades, different nutritional interventions aimed at modulating the immune and

TABLE 1. Serum Level of Glucose, Insulin, C Peptide, and Interleukin 1β in Rat Treatment Groups at 36 Hours and 7 Days After Burn Induction or Sham Procedure

	Sham Group		Burn Group		Burn + Fat-Rich Diet Group	
	36 hours	7 days	36 hours	7 days	36 hours	7 days
Glucose, mg/dL	259.29 ± 117.5	281.43 ± 135.3	311.17 ± 26.1	296.33 ± 53.8	340.83 ± 73.4	270.33 ± 53.1
Insulin, pg/mL	3779.15 ± 1263.1	4191.67 ± 1213.6	3401.36 ± 645.9	4047.20 ± 924.0	3865.48 ± 628.4	3409.30 ± 437.1
C peptide, pg/mL	494.62 ± 1040.96	82.86 ± 77.7	81.66 ± 88.8	328.85 ± 170.1	376.96 ± 121.8	211.60 ± 155.4
Interleukin 1β, pg/mL	8.51 ± 7.05	4.23 ± 4.43	2.86 ± 1.72	21.92 ± 22.41	3.16 ± 1.85	3.28 ± 1.29

FIGURE 1. Representative Pancreatic Tissue From the Burn Group Showing 29 Islets in the Cut Section



The islets were marked with green before image analysis (hematoxylin and eosin staining).

FIGURE 2. Mean Number of Islets Per Pancreatic Tissue in the 3 Animal Treatment Groups

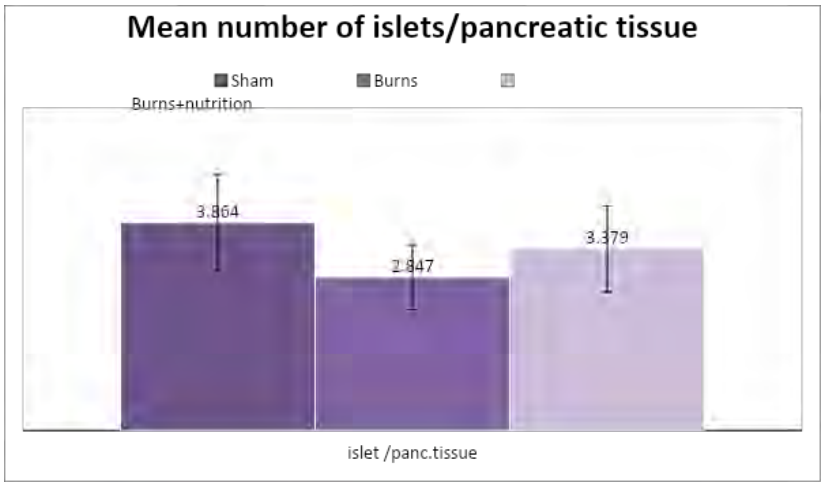
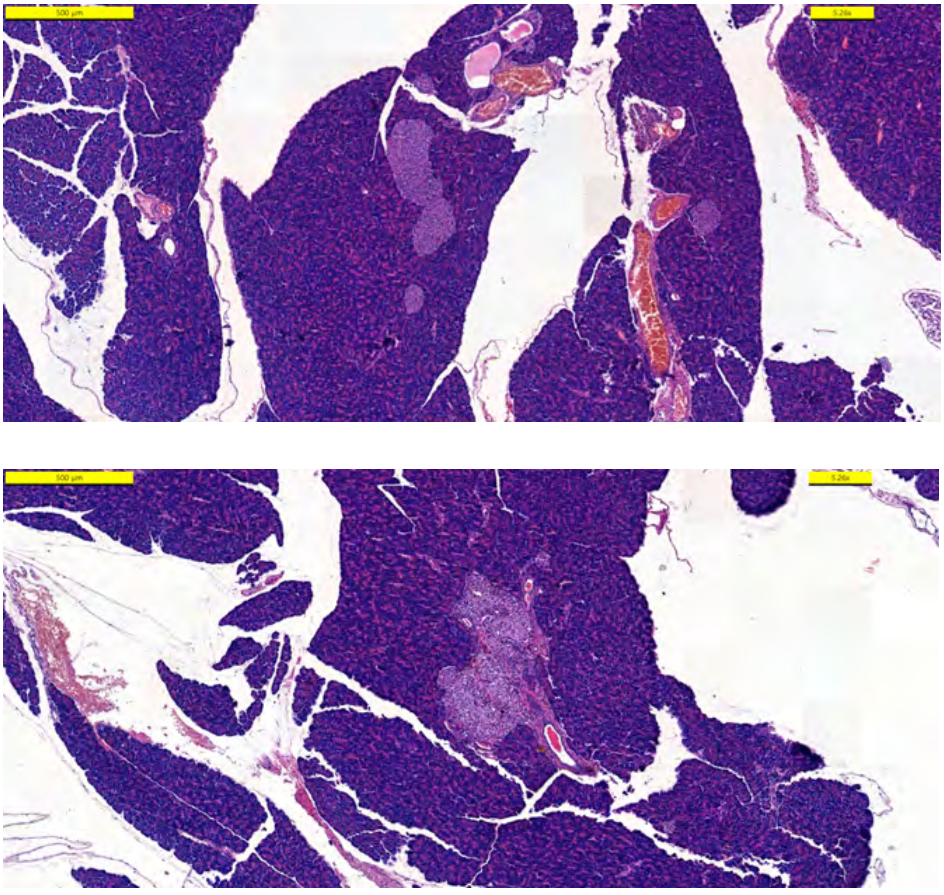


FIGURE 3. Representative Pancreatic Tissue From the Sham and Burn Groups



Images show representative pancreatic tissues from the sham group **(top)** and the burn group **(bottom)**. The average number of cells per islet is 121 in the sham group sample and 196 in the burn group sample (hematoxylin and eosin staining).

metabolic responses have been evaluated in critically ill burn patients.[12,13]

A major finding of our study was that the density of caspase staining was increased after burn trauma in both burn groups. However, the percentage of diffuse caspase staining was highest and more abundant in the animals fed the fat-rich diet; this means that pancreatic cells from animals fed with the fat-rich diet experienced a higher apoptotic activity.

FIGURE 4. Comparison of the Mean Number of Islet Cells Per Islet in the 3 Animal Treatment Groups

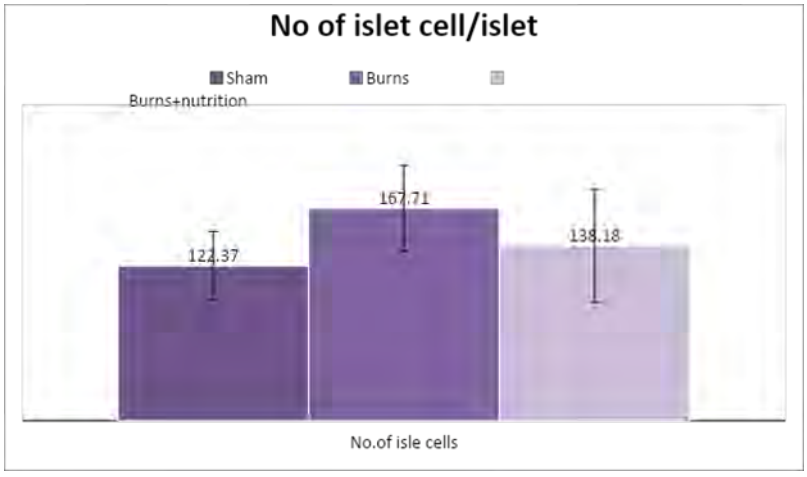


FIGURE 5. Immunohistochemistry for Caspase 3 Showing Strong Cytoplasmic Staining

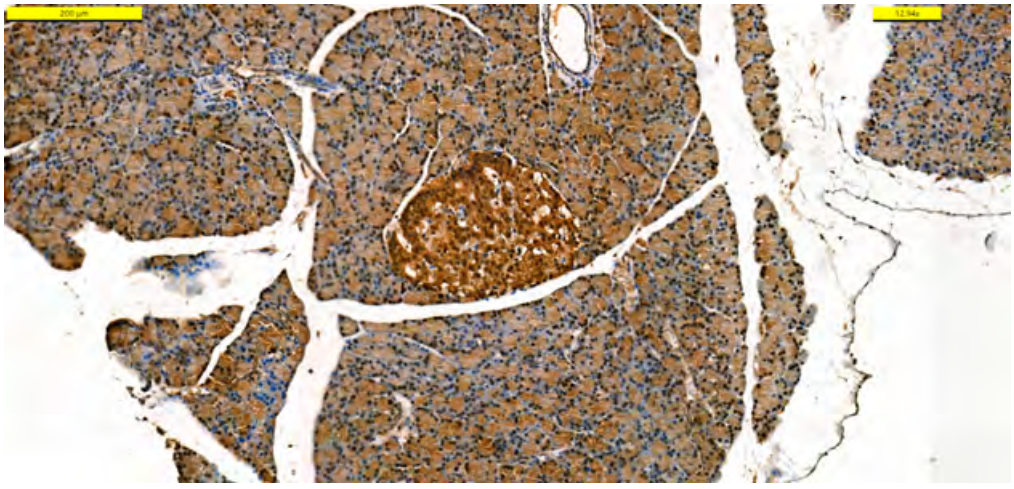
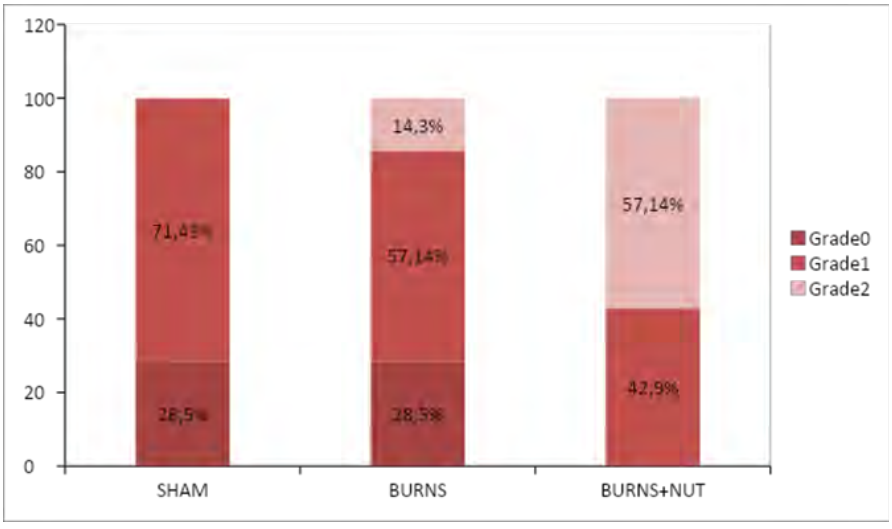


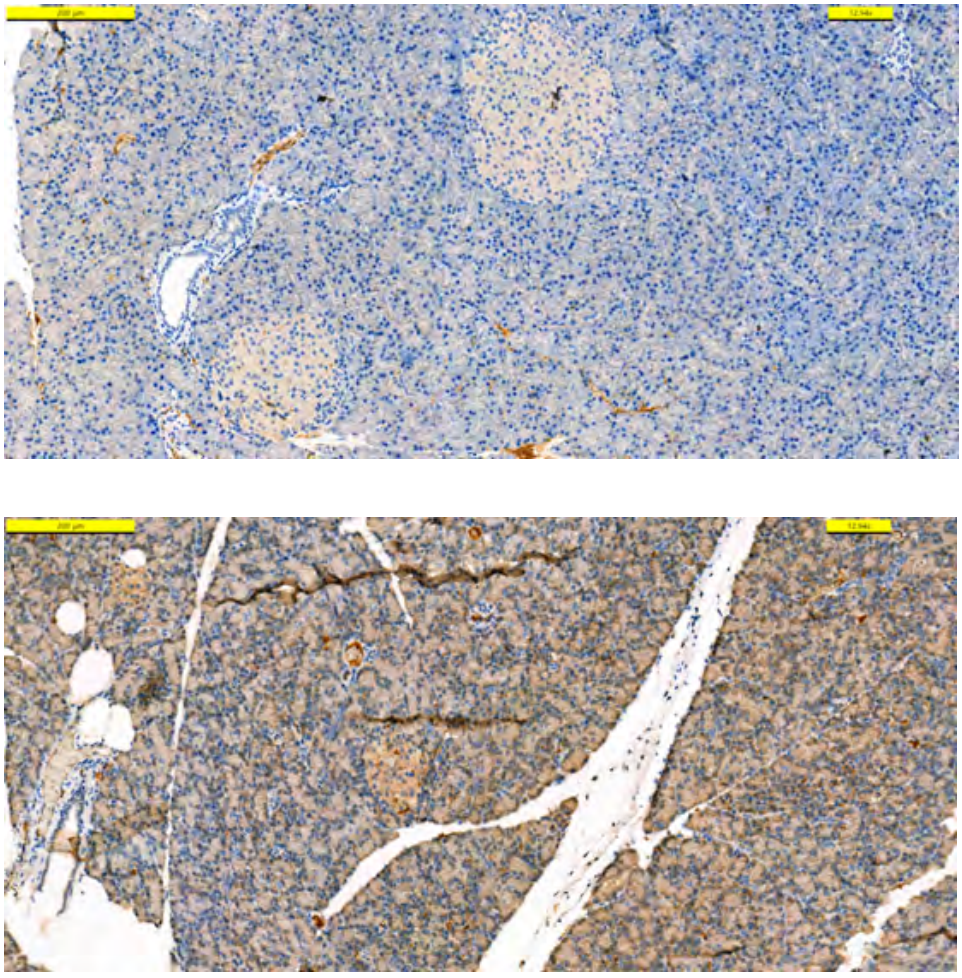
Image shows representative staining of pancreas specimen from a rat in the burn plus high-fat diet group.

FIGURE 6. Comparison of Immunohistochemistry for Caspase 3 in the 3 Treatment Groups



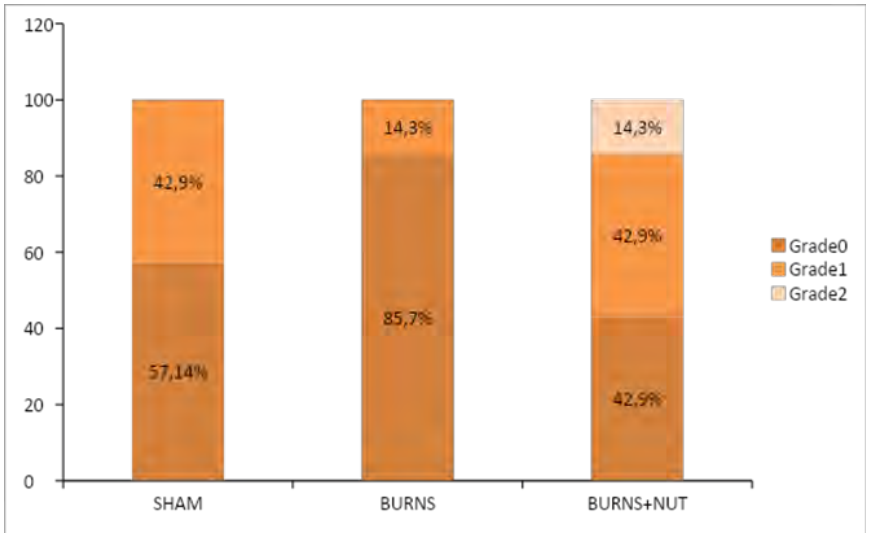
Abbreviations: Burns+Nut, animal treatment group that received burn injury plus fat-rich diet

FIGURE 7. Representative Staining of Pancreas Specimens from Animals in the Sham Group and Burn Plus Fat-Rich Diet Group



Immunohistochemistry results show no evidence of immunostaining for interleukin (IL)-1β in the sham group (**top**) and positive staining for IL-1β in more than 5 cells in islets in the burn plus fat-rich diet group (**bottom**).

FIGURE 8. Comparison of Immunohistochemistry Results for Interleukin 1β in the 3 Animal Groups



Abbreviations: Burns+Nut, animal treatment group that received burn injury plus fat-rich diet

Interleukin 1β impairs insulin secretion by activating the nuclear factor kappa B, which induces Fas expression and beta-cell apoptosis,[14-16] and the quick activation of monocytes and macrophages after burn injury induces the release of mature IL-1β and inflammasome components, including caspase 1.[6] From our findings, we suggest a fat-rich diet after burn injury may influence the interactions between caspase activity and IL-1β. Our results suggest that, in the acute phase, pancreatic apoptosis observed in the B+Nut group may be mainly because of lipotoxicity induced by the fat-rich diet rather than proinflammatory responses. Because major burn injuries trigger a dynamic chain of events (eg, burn shock, systemic inflammatory response syndrome, and hypermetabolic responses), the size and severity of burn wounds and the timing of tissue and blood samplings may have influenced the IL-1β levels; multiple factors, including other proinflammatory cytokines, may have also caused variations in the activity. Further studies are needed that focus on the effects of postburn nutrition on pancreatic IL-1β activity at different stages of severe burn injuries.

Lipotoxicity contributes to multisystem organ failure and worse clinical outcomes in obese patients and mice with pancreatitis. In our model, lipotoxicity of the burn trauma itself and fat-rich feeding after burn injury caused an augmented lipotoxic insult leading to apoptosis of pancreatic cells. In an obese population, ectopic fat deposition, including those deposited in the intrapancreatic tissue in excess, was shown to undergo lipolysis and cause local release of both saturated and unsaturated fatty acids during a major stress, with both the systemic and local lipolysis leading to lipotoxicity, which exerts deleterious effects on the pancreatic tissue.[17] Interestingly, in the postburn period, we observed an increase in the pancreatic apoptotic indices and in the number of cells in the islets in both groups of rats with burn injuries; this increase reached a statistical significance in the rats fed with the standard rat chow ($P = .05$).

Another important finding of our study was that the glycemic status did not differ in both groups of rats that received burn injuries. Plasma glucose, C peptide, and insulin levels were similar in the burned rats. Although the total percentage of cells undergoing apoptosis increased after burn trauma, it was more dramatic in animals fed the fat-rich diet; however, the number of islet cells increased after burn in both groups. If we analyze these results together with the similar glycemic status of the rats in both groups, we can conclude that the lipotoxicity of burn trauma plus the additional toxic insult induced by the fat-rich diet led to a major metabolic stress on islet cells ending with augmented apoptosis in some groups of cells and remaining as hyperfunctioning cells that were experiencing regeneration. These surviving cells tried to compensate and achieve a normal glucose homeostasis in response to the dramatic insulin resistance presented.

It was also interesting to find that the total number of cells in the whole pancreas tissue increased after burn. This increase was greater in rats fed the fat-rich diet compared with the other groups ($P = .01$). We assume that both the infiltrating inflammatory cells of major burn trauma and the reactive cells to augmented lipotoxicity may explain the excess number of cells in the pancreatic tissue in the animal group fed the high-fat diet.

CONCLUSIONS

Our findings suggest that excess nutrient conditions, especially overconsumption of saturated fat and free fatty acids, impair insulin responses in peripheral tissues, inducing a compensatory response by beta-cells that eventually can progress to beta-cell dysfunction during the acute phase of burn injury. Damage of pancreatic beta cells can also be a consequence of obesity and insulin resistance. Although burn trauma itself can lead to a significant increase in the number of cells per islets, providing excessive fat nutrients during the acute postburn phase attenuates this increase. Despite the morphophysiological pancreatic changes observed in our study animals, all animals were able to achieve a similar glycemic homeostasis.

This study describing the effects of a fat-rich diet during the acute phase of burns can form the basis of future studies to evaluate the impact of this nutritional protocol in the long term on both the pancreatic tissue as a whole and in beta-cells.

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