The Impact of Fat Rich Enteral Feeding on the Histopathological Changes of Liver in Third Degree Burns: An Experimental Study

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ABSTRACT

OBJECTIVES: This study aimed to describe the histopathological changes in the liver secondary to severe burns and to investigate the effects of a fat-rich diet during the initial 7 days post burn in a rat model.

MATERIALS & METHODS: We randomly assigned 21 Wistar-albino rats into 3 groups: sham group, burn + standard-diet group, and burn + fatty-diet group. A full-thickness burn wound was induced on the dorsa of the animals in both burn groups. Seven days after sham procedures/burn induction, hepatic tissues were examined under light microscopy; hepatocyte size, portal-triad size, and binuclear hepatocyte numbers were calculated.

RESULTS: The mean number of binuclear hepatocytes was higher in burn + standard-diet group than in the sham group. Mean hepatocyte area and mean portal-triad area were narrower in the burn + standard-diet group compared with the sham group ($P < .05$). Mean hepatocyte area and mean portal-triad area in the burn + fatty-diet group were closer to those shown in the sham animals, and binuclear hepatocytes number in the burn + fatty diet group was slightly higher than in the burn + standard diet group ($P > .05$).

CONCLUSIONS: Liver damage was mildly influenced by fat-rich enteral feeding in the 7 days postburn. Further studies must focus on dynamic feeding protocols designed according to the phases involved in the burn trauma instead of seeking a stable suitable protocol.

KEY WORDS: *Fat-rich diet, Hepatocyte, Liver damage*

INTRODUCTION

Severe burn injuries, during the acute phase, induce a strong systemic inflammatory response characterized by alterations in hormones, cytokines, and acute phase proteins, followed by a profound hypermetabolic stress response that lasts for years.[1] This chain of events affects many organs, including the liver. Although postburn hepatic responses are so far not well-defined, the liver is known to play a pivotal role in patient survival and recovery. Postmortem studies on pediatric patients with fatal burns revealed that, in complete absence of total parenteral nutrition, fatty infiltration was associated with increased bacterial translocation, liver failure, and endotoxemias in livers; other studies revealed that the damage of the liver in the acute period after burns was associated with an increased hepatic edema; necrosis and induction of hepatic apoptosis are thought to be other factors resulting in undesired changes in the liver. In support of these observations, similar results have been found in experimental murine models; burn injury induced upregulation of cyclooxygenase-2 immunoexpression and cell proliferation in livers of mice in the initial 14 days after burn injury.[2-6]

During the postburn period, glucose production increases via glycogenolysis and gluconeogenesis, lipolysis, and protein catabolism secondary to the injury. Liver damage ruins the regulation of these energy stores, subsequently affecting the distribution and disposition of various nutrients and the synthesis, transformation, and metabolism of many endogenous substrates and pollutants. Thus, the effects of nutritional support containing different amounts of nutrients on survival, mortality, and morbidity are currently subjects of investigations and debates in burn care research.[2-7] There is some evidence to suggest that diets

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containing more carbohydrates and less fat may result in better patient outcomes; however, supplying appropriate amounts of lipids, including essential fatty acids, for good wound healing and sustainable and feasible energy storage without overhydration and with a well-functioning liver is also vital.[8-10]

To protect the liver from this type of damage, instead of seeking a strict feeding regimen with low-fat ingredients, fatty product levels in enteral nutritional formulas may be configured differently according to the severity of the inflammatory response and hypermetabolism during different phases of burn trauma (eg, the acute phase, the hypermetabolic phase). In the acute phase of severe burn trauma, in which the systemic inflammatory response is prominent, a fat-rich diet may enhance the outcomes with an acceptable load to the liver. For this purpose, our present experimental study aimed to observe the histopathological changes triggered by burn injury and the effects of a fat-rich diet on the liver during the initial 7 days of severe burn trauma in a rat model.

MATERIALS AND METHODS

We obtained 21 male Wistar albino rats weighing 390 to 450 g from the Baskent University Laboratory Animal Breeding Center (Ankara, Turkey). After 1 week of standard feeding with rat chow and water ad libitum under standardized conditions for light and temperature, the study was started. We followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* for humane care at the Baskent University Laboratory Animal Center (approval was received from the Baskent University Animal Care and Ethics Committee; DA/2022/10-2).

Groups

Animals were randomly divided into 3 groups of 7 rats per group: a sham group, a burn group (with standard diet), and a burn + fatty diet group. All of the interventional procedures were performed under anesthesia.

Sham group. Seven healthy Wistar albino rats were given intraperitoneal anesthesia before dorsum was shaved. Lactated Ringer solution (2 mL/100 g) was injected intraperitoneally. Fentanyl hydrochloride was administered, and wound dressings were placed on the shaved area. The same standard diet continued under laboratory conditions for 7 days. On postprocedural day 7, liver biopsies were obtained from each animal and animals were euthanized.

Burn + standard diet group. Before rats received the burn injury, the dorsum of each animal was shaved under anesthesia. We induced a 25% total body surface burn area as described previously.[11,12] After burn induction, intraperitoneal injections of Ringer lactate solution and fentanyl were given subsequently for fluid resuscitation (2 mL/100 g) and for postprocedural analgesia. Rats were fed with the same nutritional protocol of standard rat chow until the end of the experimental period. Two animals from the burn group died 2 days after burn induction. These animals were excluded from analysis of the study group after postmortem examination. The experimental procedures were repeated for 2 new animals. Seven days after burn injury, hepatic biopsies were obtained and the animals were euthanized.

Burn + fatty diet group. The dorsum of each animal was shaved, and burn injuries were induced as previously described.[11,12] After burn induction, rats received intraperitoneal injection of Ringer lactate solution and fentanyl for the same purposes as described for other groups. Rats were fed a fat-rich diet containing 60% kcal from butter origin (Arden Arastirma Deney, Ankara, Turkey) until the end of the experimental period. Seven days after the burn injury, hepatic biopsies were obtained and the animals were euthanized.

Anesthesia and analgesia

Rats received a combined injection of ketamine (100 mg/kg ketamine hydrochloride, [Alfamine 10%]; Alfasan) and xylazine (10 mg/kg xylazine hydrochloride [Rompun 2%]; Bayer Kimya San) intraperitoneally as anesthesia. Fentanyl hydrochloride (0.02 mg/kg) was administered for postprocedural analgesia.

Burn model

A burn model was designed to induce uniform and reproducible full-thickness burns. For wound dressing, silver sulfadiazine (Silverdin) was applied before the wounds were covered with superior film with adhesive. The animals were returned to the separate dams 1 by 1 when they regained spontaneous movement and righting reflexes. The thickness of each lesion was confirmed by histopathological examination.[11,12]

Hepatic biopsies

On postprocedure day 7, the abdominal area of each animal was shaved under anesthesia, and standard aseptic techniques were applied. With an upper abdominal midline incision, the peritoneal cavity was opened. As soon as exposed, the liver and structures around the liver were inspected macroscopically and hepatic biopsies were obtained from the left side of the liver, including the left portion of the medial lobe and the left lateral lobe.

Hematoxylin-eosin and Masson Trichrome staining processes

The liver tissues of the rats were fixed in 10% phosphate-buffered formaldehyde at room temperature for 1 day. All specimens were dehydrated with a graded series of

ethyl alcohol and xylene and embedded in paraffin blocks. Sections were obtained with a conventional microtome at 4- to 5-μm thickness for hematoxylin-eosin staining, histochemistry, and Masson Trichrome staining (Atom Scientific). All histopathological observations, including inflammatory changes, fibrosis, necrosis, and fatty cysts, were performed under a light microscope (model BX43F; Olympus) by a pathologist blinded to the type of treatment, and the parameters were evaluated semiquantitatively.

Image acquisition and image analysis

The presence and number of nuclear binucleations in hepatocytes in all groups were observed in 1 high magnification field. The presence and the severity of steatosis (macrovesicular, microvesicular, or mixed) were recorded. All slides were scanned with the 3DHistech P250 Flash III scanner, and the largest hepatocyte and the largest portal triad were marked in the scanned slides. Digital imaging and hepatocyte area and portal triad area analyses of the preparations were performed in the ViraPath application (Virasoft Software Inc), with quantitative measures for each case obtained (in mm^2).

Statistical analyses

We used SPSS software (Statistical Package for the Social Sciences, version 25.0, SSPS Inc) for statistical analyses. Results for quantitative variables are given as means ± SE and evaluated with Mann-Whitney U test. Categorical data were evaluated with the Fisher exact test. Values lower than $P < .05$ were considered statistically significant.

RESULTS

We monitored the animals during the whole experimental period; other than the 2 animals that were lost from the burn + standard diet group, the experimental process was uneventful. Postmortem examination of the excluded 2 animals revealed no specific macroscopic findings. Mortality was considered to be due to burn shock conditions.

Fibrosis and necrosis

Significant fibrotic and/or necrotic histopathological changes were not observed in any of the animals among any of the 3 groups.

Fatty cyst

A fat-filled cyst was observed in only 1 animal's biopsy material (in the burn + fatty diet group).

Binuclear hepatocytes

Formation of binucleated hepatocytes was observed in all groups. A significant statistical difference was found when we compared both burn groups with the sham group ($P < .05$). The number of these cells and the regeneration activity of the hepatocytes in burn + fatty diet group were higher than in the burn + standard diet group, although not significantly ($P > .05$) (Table 1, Figure 1).

Steatosis

Macrovesicles were observed in 6 animals (3 from the sham group, 2 from the burn + fatty diet group, and 1 from the burn + standard diet group), and mixed vesicles were observed in only 1 animal from burn + fatty diet group. Seven days after injury, no significant differences were observed among livers of all groups ($P > .05$).

Hepatocyte areas and portal triad areas

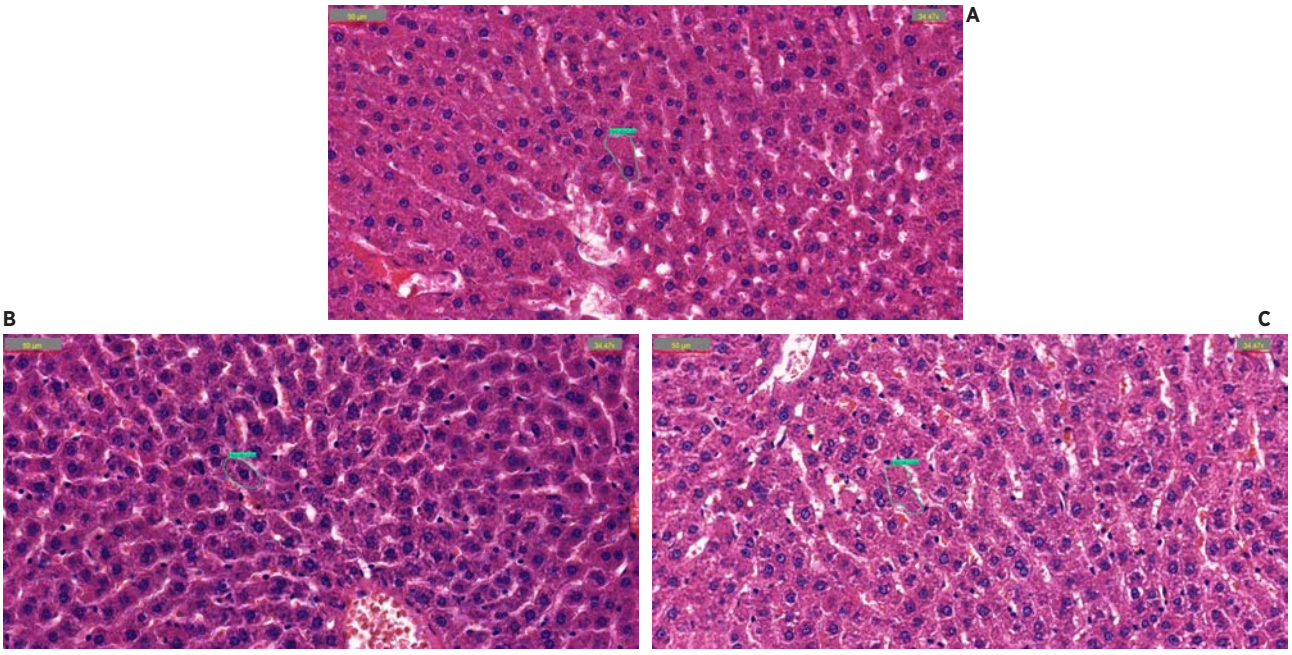
The mean hepatocyte area was significantly lower in the burn + standard diet group compared with the sham group ($P < .05$); both groups were fed standard rat chow. However, 7 days postinjury, the mean hepatocyte area in burned animals fed the fat-rich diet was larger compared with the burn + standard diet group, with a size closer to that observed in the sham group. This finding was not significant ($P > .05$). Regarding the mean portal triad areas, the mean portal triad area was narrower in the burn + standard diet group than in the sham group ($P < .05$); mean portal triad area was similar in the sham group versus the burned animals fed the fat-rich diet, this finding also was not significant ($P > .05$) (Table 1, Figure 2, Figure 3).

TABLE 1. Comparison of Rat Groups

Group	Results, mean (SD)			
	Hepatocyte Area, μm^2	Portal triad Area, μm^2	Binuclear hepatocyte count, per 1 unit area	Hepatic Steatosis, per 1000
Sham	620.4 (45.2)*	105 658.8 (3786.4)**	1.63 (1.061)***	13.75 (19.955)
Burn + standard diet	460.7 (42.6)*	31 020 (1147.8)**	4.71 (1.380)***	0.14 (0.378)
Burn + fatty diet	560.3 (70.1)	57 592.9 (1984.6)	5.14 (3.185)	5.29 (11.056)

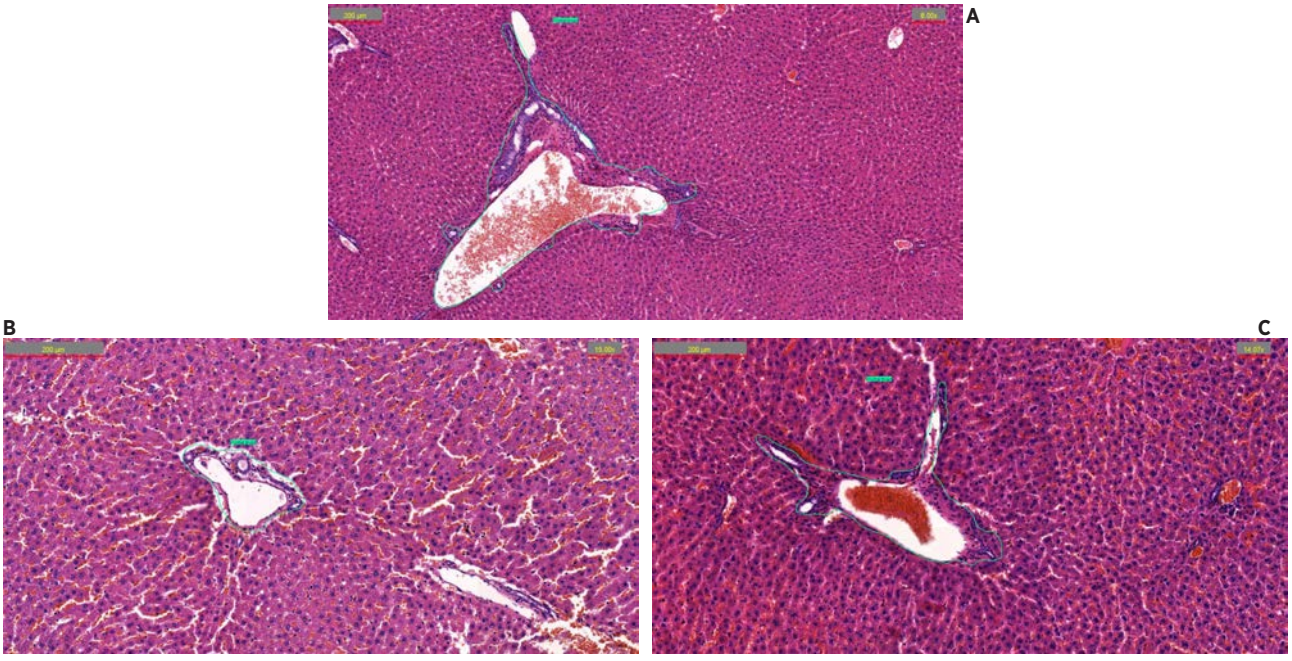
*$P = .026$, **$P = .029$, ***$P = .001$.

FIGURE 1. Photomicrographs Showing Representative Liver Tissues from All Groups: Hepatocyte



(A) Liver tissue from sham group. **(B)** Liver tissue from burn + standard diet group. **(C)** Liver tissue from burn + fatty diet group. The largest hepatocyte is marked with the green line before image was analyzed (hematoxylin and eosin stain).

FIGURE 2. Photomicrographs Showing Representative Liver Tissues from All Groups: Portal Triad

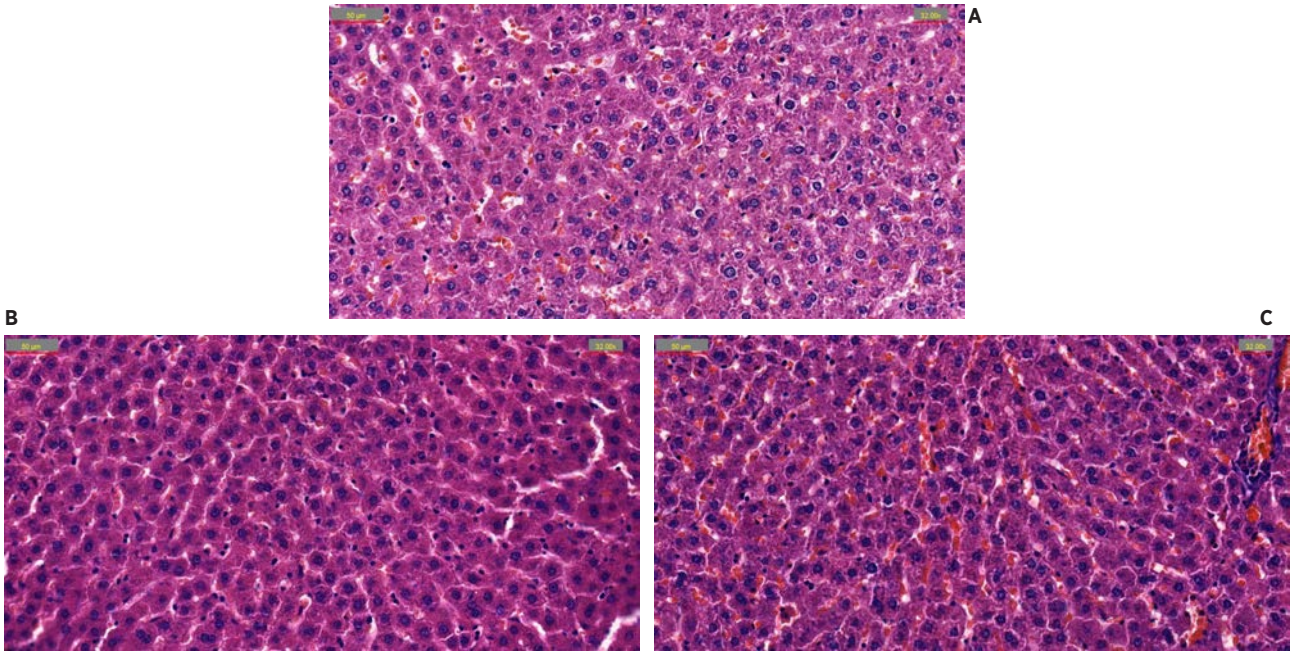


(A) Liver tissue from sham group. **(B)** Liver tissue from burn + standard diet group. **(C)** Liver tissue from burn + fatty diet group. The biggest portal triad is marked with the green line before image was analyzed (hematoxylin and eosin stain).

DISCUSSION

The liver plays an essential role in the postburn period because of its important contributions to the immune, circulatory, inflammatory, and metabolic responses, as well as in the production of acute phase proteins. Thus, hepatic damage is one of the most important factors in patient outcomes after severe burn injuries and must be prevented

FIGURE 3. Photomicrographs Showing Representative Liver Tissues from All Groups: Binucleated Hepatocyte



(A) Liver tissue from sham group. **(B)** Liver tissue from burn + standard diet group. **(C)** Liver tissue from burn + fatty diet group. The biggest binucleated hepatocyte is marked with the green line before image was analyzed (hematoxylin and eosin stain).

or minimized. Immediate interstitial hepatic edema due to the systemic inflammatory response has been described after a severe thermal injury. The presence of edema is associated with hepatomegaly and a significant decrease in hepatic proteins within the first 3 hours after injury in mice.[6] Hepatocyte hypertrophy and edema are expected immediately after the injury, and hepatocyte destruction with increased levels of alanine aminotransferase and aspartate aminotransferase is expected at the end of the first 24 hours postburn.[3,13,14] Our present preliminary study focused on the next period after immediate injury (the initial week), and the results confirmed the occurrence of histopathological changes in the liver of rats at 7 days postburn, suggesting that early enteral fat-rich nutritional support can ameliorate these changes. Our findings may provide initial clues about the benefits of dynamic nutritional support adjusted to the needs of each phase of the burn trauma.

Surprisingly, in our model, the largest mean portal area was found in the sham group; we saw a significant reduction in the portal areas in both burn groups. This result may support the suggestion by Barrow and colleagues, who found that the increase in liver size was not related to edema.[15] Changes in the sources and the mechanisms throughout post burn 7 days seemed to reduce the hepatic edema observed on the injury day. These changes may be triggered by various factors. Additionally, the portal area sizes of the burn + fatty diet group were closer to the size of the sham animals. This result may indicate the benefits of a fatty diet for reducing the acute hepatic damage.

Liver polyploidy occurs during repair in response to burn injury from various stresses, and binucleated cells in the adult liver indicate compensatory hepatic cell proliferation that is related to necroptosis and apoptosis,[6,16] with hepatocyte areas found to increase in the initial 14 days after burn injury in mice.[5] In light of this information, the relevance of our findings about the counts of binucleated hepatocytes and hepatocyte areas should be discussed together to evaluate the nature of regenerative growth observed in the burn groups with and without a fat-rich diet. In the present study, hepatic regeneration was demonstrated with a string formation of binucleated hepatocytes in both groups that underwent burn injury, compared with that shown in sham animals, and no fibrotic changes were observed in either group. This result confirmed the compensatory regeneration of the livers after burn injury in our model. Moreover (although not significant), the higher numbers of binuclear hepatocytes observed in the burn + fatty diet group may indicate the benefits of a fat-rich diet for a better regeneration response after burn injury. However, in contrast with the previous study in mice,[5] our findings at the end of 7 days revealed a significant shrinkage of the hepatocyte areas in the burn group compared with the sham group. The reason for this inconsistent result

may be due to the different animal species, different burn induction methods with different burn sizes, and especially different timings of hepatic biopsies in both studies.

The shrinkage of the hepatocytes in the burn + fatty diet group was milder than that observed in the burn group fed standard rat chow, and it was more similar to the sham animals. These findings support our suggestion that, in the early phase of burn trauma, systemic inflammatory responses may conduct the liver damage more dominantly and result in hepatocyte hypertrophy and edema; however, by the end of the first week, the liver may benefit from a fatty-rich diet. Various influencing factors may change the whole scene by triggering apoptosis and/or necroptosis within a pathophysiological chain of events and result in reduction of hepatic edema.

Fatty acids are needed to reduce the damage from burn-induced impairment of fatty acid metabolism and persistent mitochondrial dysfunction.[17] However, the long-term effects of a fat-rich diet is presumably detrimental to the liver.[2,7-9] Appropriate nutritional management of people with severe burn injuries is necessary to ensure an optimal outcome. Several studies have demonstrated how a steady high-carbohydrate plus low-fat diet can result in promising and good patient outcomes with less liver damage and how a prolonged fat-rich diet can lead to hepatic steatosis after severe burn injuries.[2,7-9] In our investigations on hepatic steatosis, no significant differences were found in the histopathological investigations among all groups. These findings may suggest that the concentrations of the amount of fatty nutrients given were appropriate (at least harmless) for the initial 7 days postburn and may be used for further studies concerning the effects of a fat-rich diet during this period. Further studies are needed on developing enteral feeding protocols with different concentrations of ingredients (fat, carbohydrate, and proteins) tailored to the burn-specific requirements during each phase of burn injury and adapted to the mechanisms by which patients are fed.

CONCLUSIONS

Our findings confirmed that liver damage is usually present along with acute inflammation after a burn injury, and dynamic treatment and supportive modalities (including postburn surgical interventions) can change the severity of the hepatic injury. Our results suggest that liver damage after burn injury is influenced by enteral feeding modalities; instead of seeking a stable and suitable feeding protocol, studies must focus on dynamic protocols designed according to the phases and related complications involved in burn trauma.

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