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Fasting increases the severity of acute pancreatitis in a mouse model: implications for preoperative interventions to reduce complications of pancreatic surgery

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HIGHLIGHTS

- Acute pancreatitis following pancreatic surgery can lead to serious complications.
- The authors investigated whether prolonged fasting affects the severity of acute pancreatitis in an experimental model.
- Prolonged fasting exacerbated pancreatic enzyme levels increased inflammatory cytokines, liver oxidative stress and pancreatic necrosis.

ABSTRACT – Background – Acute pancreatitis following surgical or endoscopic procedures on the pancreas can compromise the outcome and lead to severe complications and even death. The aim of this study was to determine whether prolonged fasting affects the severity of acute pancreatitis (AP). **Methods** – Male mice were divided into 4 groups: Group CF (n=5) control animals that fasted for 24 hours; Group CNF (n=5) control animals that did not fast; Group APF (n=7) that fasted for 24 hours and underwent induction of acute pancreatitis (AP) and Group APNF (n=7) that did not fast and underwent AP. Eight hours after AP blood was collected for evaluation of cytokines: IL-1β, IL-6, IL-10, TNF-α and MCP-1. Liver tissue was collected for determination of Malondialdehyde, pancreatic tissue for determination of enzyme content and lung tissue for determination of myeloperoxidase. **Results –** Significant increase in pancreatic amylase content was observed in group CF and increased serum levels of IL -6, Il-10 and MCP-1 were in group APF. Liver malondialdehyde was also increased in APF animals. APF group showed much more necrosis of the pancreatic acinar cells. **Conclusion –** In the present study, we observed an increase in the severity of acute pancreatitis with prolonged fasting in a severe acute pancreatitis model. These results suggest that in clinical practice, the preoperative fasting time should be shortened before pancreatic procedures.

Keywords – Fasting; acute pancreatitis; pancreatic surgery; postoperative complications; ERCP.

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INTRODUCTION

Most patients with postoperative acute pancreatitis have a benign course. However, in some patients, pancreatic inflammation may compromise the outcome of pancreatic procedures and lead to increased complications and even death[1].

Several prophylactic measures have been proposed to reduce the severity of postoperative and postendoscopic manipulations of the pancreas[2]. There is no study on the effect of duration of preoperative fasting on the severity of acute pancreatitis (AP).

A previous study showed that the reduction of pancreatic enzymes decreased the severity of AP[3]. This study suggested that the severity of the disease was related to the level of pancreatic enzymes. We therefore hypothesized that prolonged fasting is associated with decreased excretion and thus increased pancreatic enzyme levels might increase the severity of acute pancreatitis compared with no fasting or a short fasting period.

The aim of this study was to determine whether prolonged fasting affects the severity of AP. In clinical practice, the results of this experimental study could influence the duration of preoperative fasting before pancreatic procedures, including endoscopic manipulations of the pancreas.

METHODS

This study was performed at the Laboratory of Medical Investigations (LIM -51) of the University of São Paulo, Brazil and approved by the Ethical Committee for Animal Experimentation under the number 1471/2020.

Animals

Male C57black/6 mice (6 to 8 weeks old, Bioterium of the University of São Paulo) weighing 20–25 g was used. The animals were kept in polycarbonate cages under the following conditions: Temperature 23±2ºC, humidity 54±9%, light cycle 7:00–19:00 and tap water *ad libitum*.

Experimental design

The animals were divided into 4 groups: Group control fasting (CF) (n=5) control animals that had fasted for 24 hours and had free access to water; Group control non fasting (CNF) (n=5) control animals that had not fasted and had free access to food and water; Group acute pancreatitis fasting (APF) (n=7) that had fasted for 24 hours and underwent laparotomy and induction of acute pancreatitis; and Group acute pancreatitis non fasting (APNF) (n=7) that had not fasted and underwent laparotomy and induction of acute pancreatitis.

Control animals (CF and CNF) were only used to determinate the baseline amylase and lipase content. Acute pancreatitis was induced in the remaining groups APF and APNF by the technique developed in our laboratory and published previously[4]. Anesthesia was induced by intramuscular injection of ketamine 50 mg/kg and xylazine 20 mg/kg. A midline laparotomy was performed, the duodenal loop was identified, and a 1.5% Na-taurocholate solution (Sigma-Aldrich, St Louis, Missouri, USA) was retrogradely infused into the pancreatic duct at a rate of 0.015 mL/min using an infusion pump (Harvard Apparatus USA). Animals were examined 8 hours after induction of acute pancreatitis. After anesthesia, laparotomy was performed, and blood was drawn for determination of amylase and lipase and for determination of inflammatory cytokines.

Amylase and lipase determination

The content of amylase and lipase in serum and pancreatic tissue was determined (Bioclin - Quibasa, REF K003=1 e K025-1). The content of amylase and lipase in pancreatic tissue was expressed as units per mg of protein and determined by BCA (Pierce BCA Kit #23225).

Measurements of cytokines

Cytokines levels were determined using the Milliplex Map MCYTOMAG-70k kit (Merck KGaA, Darmstadt, Germany). Interleukin 1 beta (IL1-β), Interleukin 6 (IL-6), Interleukin 10 (IL-10), tumor necrosis factor alpha (TNF-α) and monocyte chemotactic protein-1 (MCP-1) were measured.

Tissue determination

Liver tissue was collected for the determination of thiobarbituric acid reactive substances (TBARs), malondialdehyde (MDA) and lipid peroxidation. MDA

formation was used as an indicator of lipid peroxidation in the liver and estimated as TBARS. Liver tissue (100 mg/mL) was homogenized in 1.15% KCl buffer and centrifuged at 14,000 g for 20 min. An aliquot of the supernatant was then added to a reaction mixture of 1.5 mL 0.8% thiobarbituric acid, 200 µL 8.1% (v/v) sodium dodecyl sulfate, 1.5 mL 20% acetic acid (pH 3.5), and 600 µL distilled water. The mixture was then heated at 90°C for 45 min. After cooling to room temperature, the samples were purified by centrifugation (10,000 g for 10 min) and absorbance was measured at 532 nm using malondialdehyde bis (dimethylacetyl) as an external standard. Lipid peroxide content was expressed as nmol MDA per mg protein.

The presence of neutrophils in lung tissue was determined by MPO activity. Lung tissue was collected, and pulmonary myeloperoxidase (MPO) activity was determined according to a previously published method[5].

At the time of sacrifice (after 8 hours), after blood collection, lung fragments were collected for analysis of mieloperoxidase (MPO) activity. Samples of 300 mg wet lung tissue were homogenized using a Polytron homogenizer (Polytron PT -2100 homogenizer, Kinematica AG, Lucerne, Switzerland) for 60 s in 1 mL of sodium phosphate buffer, pH 6.2, containing 0.5 g/dL hexadecyltrimethylammonium bromide and 5 mM ethylenediaminetetraacetic acid. The homogenized samples were then sonicated at 40 Hz for 60 s and centrifuged at 3,000 g for 30 min at 4°C. MPO activity in the supernatant was determined by measuring the change in absorbance at 460 nm (A460) resulting from the metabolism of hydrogen peroxide in the presence of O-dianisidine. MPO content was expressed as units of MPO activity per mg of protein and determined using the Bradford assay (BioRad cat# 5000006).

Pancreas histology

Pancreatic tissue was harvested 8 hours after AP induction, fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy. Histologic evaluation of the pancreatic sections was performed by the same pathologist in a blinded fashion. The severity of acinar and fat necrosis was analyzed in accordance with Schmidt et al.[6] The slides were evaluated with respect to five histopathological parameters, namely edema, inflammatory infiltration, ductal dilation, hemorrhage, and acinar necrosis. The changes were quantified according to a scoring system. The scores of each parameter for each specimen were summed and a histopathologic score for each specimen was determined[7].

Statistical analysis

Data are expressed as average ± standard error and were analyzed using Student's *t*-test or Kruskal-Wallis test followed by Dunn's test (GraphPad Prism, San Diego, CA, USA). A *P*-value <0.05 was considered significant.

RESULTS

Analysis of amylase and lipase content in the control groups showed a significantly higher content (TABLE 1) in the 24 hours fasted control group CF when compared with the non-fasted control group CNF (FIGURE 1). Lipase plasma levels were not significantly different between both control groups (control animals that had fasted for 24 hours and had free access to water CF and control animals that had not fasted and had free access to food and water CNF) (TABLE 2).

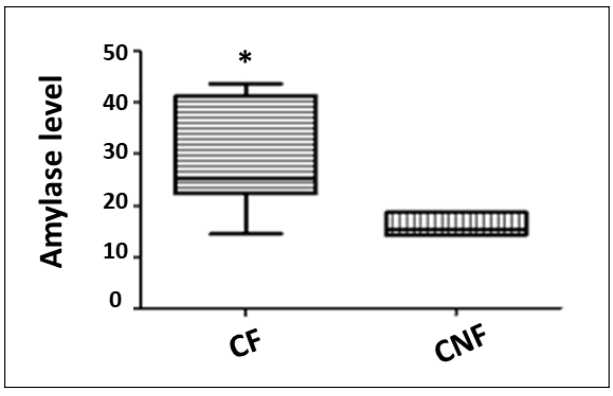

FIGURE 1. Amylase content (U/mg protein) in mouse pancreas. Control 24 hours fasted group (CF) presented significantly higher (*P*<0.05) level of amylase upon sacrifice than non-fasted control group (CNF).
CF: control fasting; CNF: control non fasting.

TABLE1. Amylase and Lipase content in mouse pancreas (U/mg protein).

	CF	CNF
Amylase	29.75±5.41	16.24±1.01*
Lipase	0.45±0.08	0.22±0.08**

CF: control fasting; CNF: control non fasting.
Data Average ± Standard error. *t*-test. **P*=0.0392; ***P*=0.0353.

TABLE 2. Amylase and Lipase content in mouse plasma (U/L).

	CF	CNF	APF	APNF
Amylase	2183.94±341.29	1401.46±186.19*	6233.58±647.79	5297.18±328.28
Lipase	40.91±1.82	42.03±2.13	338.14±85.69**	208.59±84.23

CF: control fasting; CNF: control non fasting; APF: acute pancreatitis fasting; APNF: acute pancreatitis non fasting.
Data Average ± Standard error. Analysed (Kruskal-Wallis), followed by Dunn's test. *P=0.0014 CNF versus APF and CNF versus APNF, **P=0.0010 CF versus APF and CNF versus APF.

The results of cytokines determination in the acute pancreatitis groups with or without fasting (TABLE 3) showed a marked and significantly (P<0.05) elevation of IL-6, IL-10 and MCP-1 (FIGURE 2A, 2B and 2C) in animals that had fasted for 24 hours and underwent laparotomy and induction of acute pancreatitis (APF) when compared to the animals that had not fasted and underwent laparotomy and induction of acute pancreatitis (APNF). There were no significant differences in the analysis of TNF-α and IL1-β in both groups.

TABLE 3. Cytocines level in mouse plasma (pg/mL).

	APF	APNF
TNF-α	10.30±3.56	6.55±0.69
IL-1	4.2±0.37	4.36±0.25
IL-6	28332.8±5402.75	3997.71±1315.61*
IL-10	451.68±239.39	68.28±22.97**
MCP-1	3646.00±834.46	856.37±411.96***

APF: acute pancreatitis fasting; APNF: acute pancreatitis non fasting.
Data Average ± Standard error; t-test *P=0.004; **P=0.0011;***P=0.006.

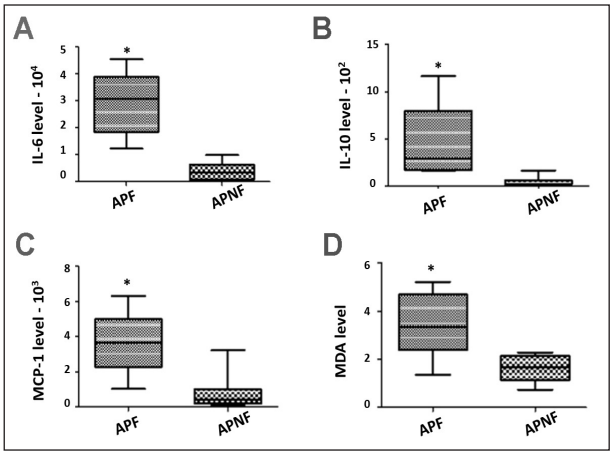

FIGURE 2. Comparison between acute pancreatitis after 24 hs fasting group (APF) and acute pancreatitis without fasting group (APNF) in terms of IL-6, IL-10, MCP-1 and Malondialdehyde (MDA) plasma levels. * P<0.05. A) This graphic shows a significant higher (P<0.05) plasma level of IL-6 (pg/mL) in the APF when compared to APNF group. B) This graphic shows a significant higher (P<0.05) plasma level of IL-10 (pg/mL) in the APF when compared to APNF group. C) This graphic shows a significant higher (P<0.05) plasma level of MCP-1 (pg/mL) in the APF when compared to APNF group. D) This graphic shows a significant higher (P<0.05) plasma level of MDA (mmoL/mg protein) in the APF when compared to APNF group. APF: acute pancreatitis fasting; APNF: acute pancreatitis non fasting.

The results of liver and lung tissues showed a significant elevation of Malondialdehyde (MDA) formation in the liver (FIGURE 2D) but no difference in myeloperoxidase activity levels in mouse lungs was observed (TABLE 4).

TABLE 4. MPO level content in mouse lung (U/mg protein) and MDA level content in mouse liver (nmoL/mg protein).

	APF	APNF
MPO	2.54±0.23	2.70±0.38
MDA	3.41±0.57	1.62±0.20*

APF: acute pancreatitis fasting; APNF: acute pancreatitis non fasting.
Data Average ± Standard error. t-test;*P=0.001.

At the time of sacrifice, acute pancreatitis fasting, and non-fasting groups showed not differently statistically score of edema, inflammation, perivascular infiltrate, hemorrhage and fat necrosis. However, 24 hour fasting group showed much more necrosis of the pancreatic acinar cells (P=0.0012) (FIGURE 3).

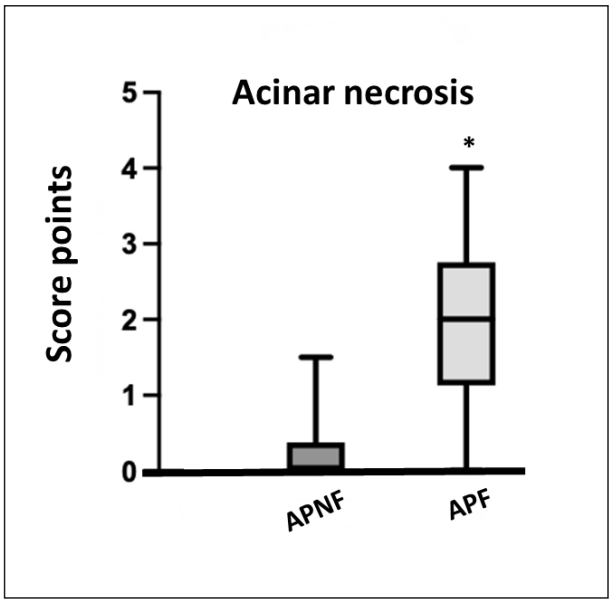

FIGURE 3. Acinar cell necrosis score. Prolonged fasted group (APF) presented significantly higher (P=0.0012) score of pancreatic acinar cell necrosis upon sacrifice than non-fasted group (APNF). *P=0.0012.
APF: acute pancreatitis fasting; APNF: acute pancreatitis non fasting.

DISCUSSION

Acute pancreatitis is a disease with an enormous potential for complications. Despite a larger number of clinical and experimental studies on factors that may influence the outcome of the disease, the influence of nutritional status on the pathogenesis of the disease is still controversial[8]. The importance of this topic is related to the potential use of this knowledge in preparing patients for pancreatic surgery or endoscopic manipulations of the pancreas, including endoscopic ultrasound-guided biopsies, because acute pancreatitis that may follow can be severe[1]. A previous study has shown the influence of the amount of enzymes in pancreatic tissue on the severity of acute pancreatitis[3]. Therefore, it is conceivable that fasting, which decreases enzyme secretion and thus increases pancreatic enzyme content, negatively affects the outcome of acute pancreatitis. A previous study also demonstrated an increase in protein content per milligram of DNA in the pancreatic tissue of rats fasted for 24 hours compared with non-fasted animals[9]. In the present study, we found an increased amount of amylase in the pancreatic tissue of 24 hours fasted animals (FIGURE 1).

In a previous report, fasting was observed to exacerbate acute experimental pancreatitis by occluding the common bile duct[10]. However, in another study, fasting was found to decrease the severity of acute pancreatitis induced by cerulein[11], and in another study, no differences in the severity of acute pancreatitis were observed in fasted animals compared with non-fasted animals[12]. In the present study, serum amylase and lipase levels were higher in fasting animals with acute pancreatitis (APF), but without statistical significance. No differences were found in serum levels of TNFα and IL -1β, but higher significant serum levels of IL -6, IL -10, and MCP-1 were found in animals that had fasted for 24 hours and underwent laparotomy and induction of acute pancreatitis (APF) when compared to the animals that had not fasted and underwent laparotomy and induction of acute pancreatitis (APNF) (FIGURE 2). Acinar necrosis score was also significantly higher in the prolonged fasting group. The elevation of these proinflammatory and anti-inflammatory cytokines is an early event in severe acute pancreatitis[13].

Higher levels of MDA were also detected in the liver tissue of fasting animals with acute pancreatitis (APF), indicating greater disease severity in these animals (FIGURE 2D). These results suggest greater severity of acute pancreatitis in fasting animals (APF). No differences were observed in MPO in the lungs. The absence of increased MPO levels in the lungs of fasting animals may be due to the fact that the animals were sacrificed early (8 hours) after the onset of acute pancreatitis.

The different results observed in previous studies might be related to the different methods used to induce pancreatitis. The structural or functional changes of the pancreas induced by fasting[8] and also an increased amount of pancreatic enzymes could lead to the increased severity of acute pancreatitis observed in fasted animals (APF) in the present study.

Despite much evidence to the contrary, many surgeons and anesthesiologists worldwide still consider a preoperative fast of 6–8 hours to be essential, relying on old concepts. In contrast, modern guidelines from various international anesthesia societies recommend more flexible fasting times. Moreover, the actual duration of preoperative fasting is usually much longer than the prescribed fasting time. Because of delays in the operating room and changes in the surgical schedule, patients often fast for 12 hours or longer[14]. These experimental results, along with evidence of the safety of oral carbohydrate-rich fluid intake before surgery, may provide the basis for shortening the preoperative fasting period[14].

CONCLUSION

In the present study, we observed an increase in the severity of acute pancreatitis with prolonged fasting in a severe acute pancreatitis model. These results suggest that in clinical practice, the preoperative fasting time should be shortened before pancreatic procedures.

Authors' contribution

Souza ML and Ariga S experimental work and review of the manuscript. Barbeiro DF: Laboratory analysis and review of the manuscript. Machado MA: Writing, discussion and review of the manuscript. Machado MC: Idealization, intellectual plan-

ning and review of the manuscript. Souza HP: Intellectual planning and review of the manuscript.

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Souza ML, Ariga S, Barbeiro DF, Machado MA, Machado MC, Souza HP. Jejum aumenta a gravidade da pancreatite aguda em camundongos: implicações para intervenções pré-operatórias para reduzir as complicações da cirurgia pancreática. Arq Gastroenterol. 2024

RESUMO - **Contexto** – A pancreatite aguda após procedimentos cirúrgicos ou endoscópicos no pâncreas pode comprometer o resultado e levar a complicações graves e até mesmo à morte. O objetivo deste estudo foi determinar se o jejum prolongado afeta a gravidade da pancreatite aguda (PA). **Métodos** – Camundongos machos foram divididos em 4 grupos: Grupo CF (n=5) animais de controle que jejuaram por 24 horas; Grupo CNF (n=5) animais de controle que não jejuaram; Grupo APF (n=7) que jejuaram por 24 horas e foram submetidos à indução de PA e Grupo APNF (n=7) que não jejuaram e foram submetidos a PA. Oito horas após a PA, o sangue foi coletado para avaliação de citocinas: IL-1β, IL-6, IL-10, TNF-α e MCP-1. O tecido hepático foi coletado para a determinação do malondialdeído, o tecido pancreático para a determinação do conteúdo enzimático e o tecido pulmonar para a determinação da mieloperoxidase. **Resultados** – Foi observado um aumento significativo no conteúdo de amilase pancreática no grupo CF e um aumento nos níveis séricos de IL-6, Il-10 e MCP-1 no grupo APF. O malondialdeído hepático também aumentou nos animais APF. O grupo APF apresentou muito mais necrose das células acinares pancreáticas. **Conclusão** – No presente estudo, observamos um aumento na gravidade da pancreatite aguda com o jejum prolongado em um modelo de pancreatite aguda grave. Esses resultados sugerem que, na prática clínica, o tempo de jejum pré-operatório deve ser reduzido antes dos procedimentos pancreáticos.

Palavras-chave – Jejum; pancreatite aguda; cirurgia pancreática; complicações pós-operatórias; CPRE.

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