EFFECTS OF HINDLIMB ISCHEMIA-REPERFUSION INJURY ON SERUM AMYLASE, LIPASE, INSULIN LEVELS, AND PANCREATIC HISTOPATHOLOGY IN A WISTAR RAT MODEL

[1] Widanto, [2] Kevin Horas, [3] Achmad Efendi

[1] The Surgical Specialist Doctor Education Program, Faculty of Medicine of Brawijaya University, Saiful Anwar Regional General Hospital, East Java Province, Indonesia

[2] Pediatric Division, Department of Surgery, Saiful Anwar Regional Hospital, Malang, Indonesia

[3] Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, East Java, Indonesia

* Widanto widanto@ub.ac.id, Kevin Horas kevinhoras@gmail.com, Achmad Efendi a_efendi@ub.ac.id

Corresponding Author: Kevin Horas

The Surgical Specialist Doctor Education Program, Faculty of Medicine of Brawijaya University, Saiful Anwar Regional General Hospital, East Java Province, Indonesia, GPT II Building, 2nd Floor, Malang, East Java, 65112, Indonesia

Phone: +6281354842196, Fax: +62-333100, E-mail: kevinhoras@gmail.com

ABSTRACT

Introduction: Ischemia-reperfusion injury (IRI) can cause remote organ damage through oxidative stress and systemic inflammatory responses. This study aimed to evaluate the effects of hindlimb IRI on serum amylase, lipase, insulin levels, and pancreatic histopathology in Wistar rats.

Methods: Thirty-three male Wistar rats were divided into three groups: control (sham), 120-min ischemia (bilateral femoral artery occlusion), and ischemia-reperfusion (120-min occlusion + 30-min reperfusion). Serum amylase, lipase, and insulin levels were measured. Pancreatic tissue was assessed histopathologically using a semiquantitative scoring system (0-15) for edema, leukocyte infiltration, vacuolization, necrosis, and hemorrhage.

Results: No significant differences were found in amylase ($p=0.798$), lipase ($p=0.426$), or insulin ($p=0.208$) levels across groups. However, pancreatic histopathological damage scores showed highly significant differences ($p<0.001$). The ischemia group had the highest score (4.10±1.20), the control group the lowest (0±0), and the ischemia-reperfusion group showed partial improvement (2.90±0.99). Correlation analysis revealed no meaningful relationship between serum enzyme levels and histopathological scores.

Discussion: Hindlimb ischemia induced subclinical pancreatic damage not reflected by elevated serum enzymes. The partial protective effect of reperfusion suggests possible ischemic preconditioning mechanisms. The dissociation between biochemical markers and histopathology indicates that pancreatic injury can occur without serum enzyme elevation.

Conclusion: 120-min hindlimb ischemia causes significant pancreatic histopathological damage without altering serum amylase, lipase, or insulin levels. 30-min reperfusion exerts a partial protective effect. Serum biochemical markers do not accurately reflect the degree of pancreatic injury in this model.

Keywords: ischemia-reperfusion injury; pancreas; amylase; lipase; insulin; histopathology

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INTRODUCTION

Ischemia-reperfusion injury (IRI) occurs when blood flow to an organ or tissue is temporarily interrupted, followed by reoxygenation upon restoration of perfusion. Although reperfusion is essential to restore oxygen and nutrient supply, it frequently triggers a cascade of deleterious biological reactions that cause significant cellular damage. A central mechanism underlying IRI is the excessive generation of reactive oxygen species (ROS), which overwhelms endogenous antioxidant defences and induces oxidative stress at the cellular level (Granger

& Kvietys, 2015; Rosiana et al., 2019; Zarbock et al., 2014).

Oxidative stress exerts destructive effects on multiple cellular components. Within mitochondria, it impairs oxidative phosphorylation, the primary pathway for adenosine triphosphate (ATP) synthesis, leading to energy failure. This disruption promotes intracellular calcium overload and activation of membrane phospholipases, further propagating cellular injury (Kowalczyk et al., 2021). Beyond the locally affected tissue, ROS generated during IRI can mediate remote organ damage through leukocyte activation, chemotaxis, leukocyte-endothelial interactions, complement system activation, and the release of proinflammatory mediators. These events disturb vascular homeostasis and can culminate in widespread organ injury (Collard & Gelman, 2001; Hussein et al., 2014).

Clinically, IRI is frequently encountered during surgical procedures that require aortic cross-clamping of the thoracic or thoracoabdominal aorta. Aortic clamping induces marked haemodynamic alterations proximal to the clamp, displacing blood volume and increasing myocardial oxygen demand, which may precipitate ventricular decompensation and failure. Moreover, cross-clamping contributes to pulmonary complications through complex, incompletely understood mechanisms (Cuzick et al., 2011). The pancreas is among the organs vulnerable to injury following abdominal aortic repairs; acute necrotising pancreatitis has been reported as a serious complication. Additional mechanisms include atherosclerotic plaque disruption during surgical dissection or direct clip application, resulting in paradoxical retrograde microembolisation into pancreatic arteries, causing acute focal ischaemia and necrosis that can evolve into diffuse acute pancreatitis (Roncati et al., 2020).

Acute pancreatitis is a critical condition characterised by acute pancreatic inflammation. Numerous studies have documented acute pancreatitis as a complication of various surgical procedures. A 2023 meta-analysis demonstrated that acute pancreatitis commonly occurs after pancreaticoduodenectomy (Z. Wu et al., 2023). Although it is most frequently associated with abdominal surgery, acute pancreatitis has also been reported following total knee arthroplasty and high tibial osteotomy (S. Liu et al., 2023; W. Liu et al., 2021; O'Connor & Asaad, 2019; Seah et al., 2013). Acute pancreatitis is a potentially life-threatening, reversible inflammatory process with a wide spectrum of severity. The revised Atlanta classification stratifies acute pancreatitis into mild, moderate, and severe forms. Over 80% of cases are mild, while the remaining 20% are severe (Meher et al., 2015). Severe pancreatitis is associated with high morbidity and mortality, often complicated by abscess formation, pseudocysts, and organ failure (Iqbal et al., 2015). Mortality rates rise substantially in elderly patients and those with comorbidities such as low cardiac output, peripheral arterial disease, chronic obstructive pulmonary disease, diabetes mellitus, obesity, and chronic renal failure. Early diagnosis and management significantly reduce both morbidity and mortality (Yalvaç et al., 2018).

The diagnosis of acute pancreatitis typically combines clinical findings, laboratory investigations, and imaging techniques. The revised Atlanta classification defines three diagnostic criteria: characteristic abdominal pain, serum lipase (or amylase) activity at least three times the upper limit of normal, and characteristic findings on contrast-enhanced computed tomography, magnetic resonance imaging, or transabdominal ultrasonography. Serum lipase is a more sensitive and specific biomarker than amylase (Meher et al., 2015). Ischaemic pancreatic injury most commonly manifests as acute pancreatitis. Key histopathological features reflect the degree of damage and range from interstitial oedema, leukocyte infiltration, and vacuolisation to fat necrosis, pancreatic parenchymal necrosis, and focal haemorrhage (Abogresha et al., 2016; V. Kumar et al., 2018; Warzecha et al., 2017). Thus, pancreatic histopathology serves as a direct method to identify and grade pancreatic damage caused by ischaemic insults.

To date, no single gold standard exists for the diagnosis of acute pancreatitis. This gap highlights the need for deeper investigation into the remote effects of IRI on the pancreas and the potential association with changes in amylase, lipase, insulin levels, and pancreatic tissue architecture. The present study was designed to address this knowledge gap by exploring the effect of reperfusion following femoral artery occlusion on serum amylase, lipase, insulin concentrations, and pancreatic histopathology in Wistar rats (*Rattus norvegicus*). We employed a hindlimb IRI model to determine whether a peripheral ischaemic insult can induce measurable biochemical and structural pancreatic alterations, thereby contributing to the understanding of remote pancreatic consequences of IRI and providing insights that may aid in predicting the severity of acute pancreatitis associated with ischaemia-reperfusion states.

METHODS

Animals and Experimental Design

Male Wistar rats (*Rattus norvegicus*), aged 3–4 months and weighing 180–200 g, were obtained from the Animal Laboratory of the Faculty of Medicine, Universitas Brawijaya. All animals were

housed individually in standard cages (30 × 20 × 15 cm) under a 12-h light/dark cycle with standard rodent chow (BR-1) and distilled water ad libitum. After a 7-day acclimatization period, 33 healthy rats were randomly assigned to three groups using a simple random sampling method. The sample size was determined using the resource equation method, requiring a minimum of 9 animals per group; to allow for possible dropouts, the number was increased by 20% to 11 rats per group. The experimental protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya (Ethical Approval No. 25/EC/KEPK–PPDS/01/2025) and conducted in accordance with the Declaration of Helsinki. A true experimental post-test-only design was employed, comprising a sham-operated control group (Group 1), an ischemia group subjected to 120 min of bilateral femoral artery occlusion (Group 2), and an ischemia–reperfusion group subjected to 120 min of occlusion followed by 30 min of reperfusion (Group 3).

Surgical Procedures and Sample Collection

All rats were fasted for 3 h before the procedure. Anesthesia was induced with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and confirmed by the loss of the righting reflex. In Groups 2 and 3, bilateral hindlimb ischemia was induced by tightly applying orthodontic rubber bands around the proximal thighs until the femoral pulse was no longer palpable, as previously described (Veite-Schmahl et al., 2017). Occlusion was maintained for 120 min; in Group 3, the bands were then removed to permit 30 min of reperfusion. The sham-operated rats underwent identical anesthetic and handling procedures without band application. At the end of each protocol, a laparotomy was performed, and blood was collected from the inferior vena cava. Samples were centrifuged at 3,000 × g for 5 min at 4°C, and the serum was stored at −20°C until analysis. The pancreas was then carefully harvested for histopathological examination.

Biochemical Analyses

Serum amylase, lipase, and insulin concentrations were measured using an Olympus AU600 automatic biochemical analyzer in accordance with the manufacturer's instructions. All assays were performed in duplicate.

Pancreatic Histopathology

Pancreatic tissues were fixed in 10% neutral buffered formalin, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin (H&E). A pathologist blinded to the group allocation evaluated pancreatic injury using a semiquantitative scoring system that assessed five parameters: edema (0 = absent, 1 = interlobar edema, 2 = moderate interlobar and intralobular edema, 3 = severe interlobar and intralobular edema); leukocyte infiltration (0 = absent, 1 = sparse perivascular, 2 = moderate perivascular with sparse diffuse, 3 = abundant diffuse infiltration); acinar cell vacuolization (0 = absent, 1 = <25% of acinar cells, 2 = 25–50%, 3 = >50%); acinar cell necrosis (0 = absent, 1 = <15%, 2 = 15–35%, 3 = >35%); and hemorrhage (0 = absent, 1 = 1–2 foci/slide, 2 = 3–5 foci/slide, 3 = >5 foci/slide) (Abogresha et al., 2016; Hussein et al., 2014). A total pancreatic damage score (0–15) was calculated by summing the scores of the individual parameters.

Statistical Analysis

Data were analyzed using SPSS software. Normality was checked with the Shapiro–Wilk test, and homogeneity of variances was assessed by Levene's test. Normally distributed data were compared using one-way analysis of variance (ANOVA) with appropriate post-hoc tests, whereas non-parametric data were analyzed with the Kruskal–Wallis test. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1. Comparison of amylase levels, lipase levels, insulin levels, and pancreatic tissue histopathology in each treatment group

Variable	Group 1 (O1) (Control) (Mean ± SD)	Group 2 (O2) (Occlusion) (Mean ± SD)	Group 3 (O3) (Occlusion + Reperfusion) (Mean ± SD)
Amylase	0.93 ± 0.42	0.96 ± 0.48	1.15 ± 0.35
Lipase	0.85 ± 0.54	0.96 ± 0.55	0.71 ± 0.46
Insulin	4.15 ± 0.99	3.65 ± 1.04	4.30 ± 0.60
HPP Score	0 ± 0	4.10 ± 1.20	2.90 ± 0.99

The analysis showed that overall there was a highly significant difference in the response variable Y among the three treatment groups. Although the One Way ANOVA test yielded $F(2,27) = 55.01$ with $p < 0.001$, the assumption of homogeneity of variances was not met because the variance in group 1 (control) was zero; therefore, the Classic ANOVA results should only be used as a reference and cannot serve as

the primary basis for conclusions. As a more valid alternative, the Kruskal-Wallis test, which does not require homogeneity of variances or normality of data, was conducted, yielding a statistic of $H = 21.69$ with $p < 0.001$, confirming the presence of significant differences in the distribution of Y among the groups. To determine which groups differed significantly, two-sided Mann-Whitney tests were performed for each pair, and the results showed that all group pairs were significantly different, with group 1 (control) differing very significantly from group 2 (occlusion) and group 3 (occlusion + reperfusion), each with $U = 100$ and $p < 0.001$, while group 2 also differed significantly from group 3, albeit at a lower level of significance ($U = 20$, $p < 0.05$), confirming that all three groups had statistically distinct distributions of Y values. Descriptive data indicated that the HPP Score variable best reflected the differences among groups, as the control group (O1) had a mean HPP Score of 0 ± 0, the occlusion group (O2) had the highest mean (4.10 ± 1.20), and the occlusion + reperfusion group (O3) was in between (2.90 ± 0.99), suggesting that occlusion caused a marked increase in HPP scores and reperfusion provided a partial improvement without restoring normal conditions, while for the other variables such as amylase, lipase, and insulin, the patterns of differences among groups were inconsistent, indicating that the significant differences found in the Kruskal-Wallis and Mann-Whitney tests were most likely driven by the contribution of the HPP Score variable.

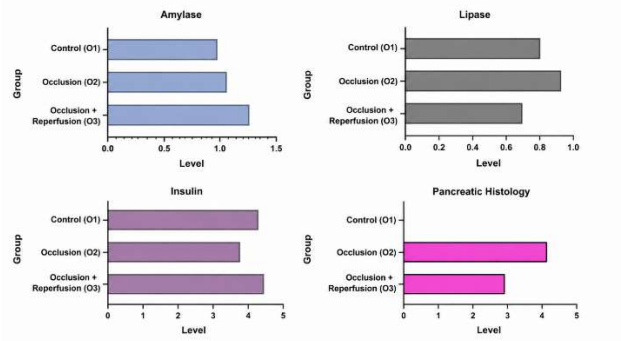

Figure 1. Effects of pancreatic ischemia and reperfusion on amylase, lipase, insulin, and pancreatic histology. Three experimental groups were compared: Control (O1), Occlusion (O2), and Occlusion + Reperfusion (O3). A. Amylase levels (arbitrary units) measured at four time/severity levels (0.0, 0.5, 1.0, 1.5). B. Lipase levels (arbitrary units) measured at six levels (0.0, 0.2, 0.4, 0.6, 0.8, 1.0). C. Insulin levels (arbitrary units) measured at six levels (0, 1, 2, 3, 4, 5). D. Pancreatic histology scores (0–5, where higher scores indicate greater histopathological damage) assessed at six severity levels (0, 1, 2, 3, 4, 5). Data are presented as [insert appropriate measure, e.g., mean ± SEM]. O2 vs. O1, O3 vs. O2; significance levels are indicated as [insert if applicable, e.g., *p < 0.05].

Based on the data presented, occlusion and reperfusion treatment did not have a significant effect on serum amylase and lipase levels. For the amylase parameter, although there was a trend of gradual increase from the control group (0.93 ± 0.42), occlusion (0.96 ± 0.48), to occlusion and reperfusion (1.15 ± 0.35), the One Way ANOVA results showed no statistically significant difference ($p = 0.798$). The Tukey HSD post-hoc test also confirmed that all three groups belonged to the same homogeneous subset. A similar pattern was observed for lipase levels, where descriptively there was fluctuation with the highest value in the occlusion group, but statistical testing showed that these differences were not significant ($p = 0.426$). Thus, neither the occlusion procedure nor its combination with reperfusion caused significant changes in serum pancreatic enzyme levels Figure1 & table 1.

Analysis of insulin levels showed a declining trend from the control group to the treatment groups, with the highest insulin level recorded in the control group (4) and the lowest in the occlusion + reperfusion group (1). However, statistical test results indicated that the difference in insulin level distribution among the three groups was not statistically significant ($p = 0.208$). This suggests that although descriptively there appears to be a pattern of decreasing insulin levels with occlusion and reperfusion treatment, the substantial data variation within groups rendered these differences insufficiently robust to reach the threshold of statistical significance, leading to acceptance of the null hypothesis stating that insulin level distribution is the same across all treatment groups.

In contrast to the serum biochemical parameters, the pancreatic histopathology analysis revealed highly significant differences among the treatment groups ($p = 0.000$). The control group displayed normal pancreatic structure with a score of 0.00, whereas significant histological damage was observed in the 120-minute ischemia group with the highest score of 4.10 ± 1.197. Administration of 30-minute reperfusion significantly reduced the degree of pancreatic damage to 2.90 ± 0.994. The Tukey HSD post-hoc test confirmed that all intergroup comparisons were significantly different ($p \leq 0.016$). These findings demonstrate that limb ischemia can induce remote organ injury in the pancreas, characterized by edema, PMN cell infiltration, vacuolization, and acinar cell necrosis; however, restoration of blood flow through early reperfusion exerts a protective effect on pancreatic tissue structure.

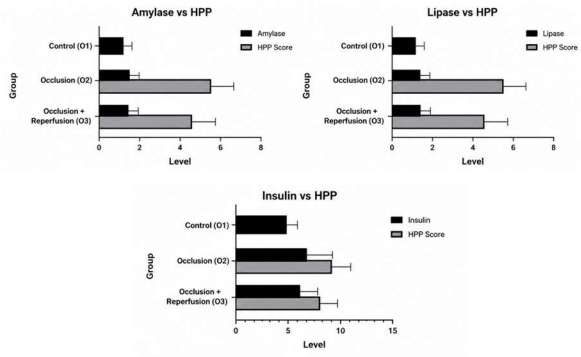

Figure 2 Relationship of pancreatic enzymes and insulin to high pancreatic pressure (HPP) across experimental groups. Three groups were compared: Control (O1), Occlusion (O2), and Occlusion + Reperfusion (O3). **A.** Amylase levels vs. HPP measured at five pressure levels (0, 2, 4, 6, 8). **B.** Lipase levels vs. HPP measured at the same five pressure levels (0, 2, 4, 6, 8). **C.** Insulin levels vs. HPP measured at five pressure levels (0, 2, 4, 6, 8). Data are presented as [insert measure, e.g., mean ± SEM]. O2 and O3 groups were compared with controls (O1); O3 also compared with O2 where applicable. Significance is indicated as [insert if applicable, e.g., *p < 0.05].

A comparative analysis of biochemical markers and histopathological scores across treatment groups revealed discordant patterns between serum parameters and pancreatic tissue damage. Serum amylase levels demonstrated a gradual, non-significant increase from control (0.93 ± 0.42) through occlusion (0.96 ± 0.48) to occlusion-reperfusion (1.15 ± 0.35) groups (p = 0.200), whereas the total histopathology score (HPS) exhibited a dramatic and significant elevation from control (0.00 ± 0.00) to occlusion (4.10 ± 1.20), followed by a modest decline in the occlusion-reperfusion group (2.90 ± 0.99; p < 0.001). Notably, the trajectory of amylase and HPS were not parallel—the highest HPS occurred when amylase was moderate (occlusion), while HPS decreased when amylase peaked (occlusion-reperfusion). Spearman correlation analysis confirmed the absence of a meaningful linear relationship between amylase levels and HPS, yielding a very weak correlation coefficient (rho = 0.151) that was not statistically significant (p = 0.427), with a 95% confidence interval crossing zero (-0.232 to 0.493). Regression analysis similarly failed to establish a significant association (B = 1.048; p = 0.251). These findings indicate that serum amylase levels do not reliably reflect the degree of pancreatic histopathological injury in this experimental model (figure 2).

Examination of lipase levels in relation to histopathological scores yielded comparable conclusions despite both parameters demonstrating significant intergroup differences individually. Serum lipase varied significantly across groups (p = 0.002), with values of 0.85 ± 0.54 in controls, a slight increase to 0.96 ± 0.55 in the occlusion group, and a decline to 0.71 ± 0.46 in the occlusion-reperfusion group, while HPS followed a distinct pattern of elevation and partial recovery (p < 0.001). Despite the parallel group-level changes, Spearman correlation analysis between individual lipase levels and HPS revealed a weak correlation (rho = 0.175) that did not reach statistical significance (p = 0.355), with the 95% confidence interval encompassing zero (-0.208 to 0.512). Regression analysis similarly found no meaningful association (B = 0.748; p = 0.307). The absence of significant correlation, despite both variables changing across groups, is attributable to substantial individual variability within groups, as evidenced by the large standard deviations in lipase measurements (up to ±0.55), and the lack of consistent linear correspondence between the two variables at the individual subject level. Consequently, serum lipase levels do not serve as a reliable surrogate marker for the extent of pancreatic histological damage.

The relationship between insulin levels and histopathological scores demonstrated a suggestive inverse pattern that ultimately failed to achieve statistical significance. Insulin levels differed significantly among groups (p = 0.018), declining from control values (4.15 ± 0.99) to their nadir in the occlusion group (3.65 ± 1.04) and recovering in the occlusion-reperfusion group (4.30 ± 0.60), thereby exhibiting a trend opposite to that of HPS, which peaked during occlusion and partially recovered upon reperfusion. This visually apparent inverse relationship was not confirmed statistically, as Spearman correlation analysis yielded a weak negative coefficient (rho = -0.227) with a non-significant p-value (p = 0.228) and a 95% confidence interval that crossed zero (-0.551 to 0.156). Regression analysis similarly showed no significant association (B = -0.419; p = 0.311). The failure to detect a significant correlation, despite the consistent inverse trend at the group level, can be attributed to considerable within-group variability (insulin standard deviations ranging from 0.60 to 1.04), limited sample size potentially constraining statistical power, and the fact that group-level trends do not necessarily translate into consistent linear relationships at the individual data point level. Thus, while a biological inverse association between insulin and pancreatic injury is plausible, the current data do not provide sufficient statistical evidence to establish a meaningful relationship between serum insulin levels and histopathological scores.

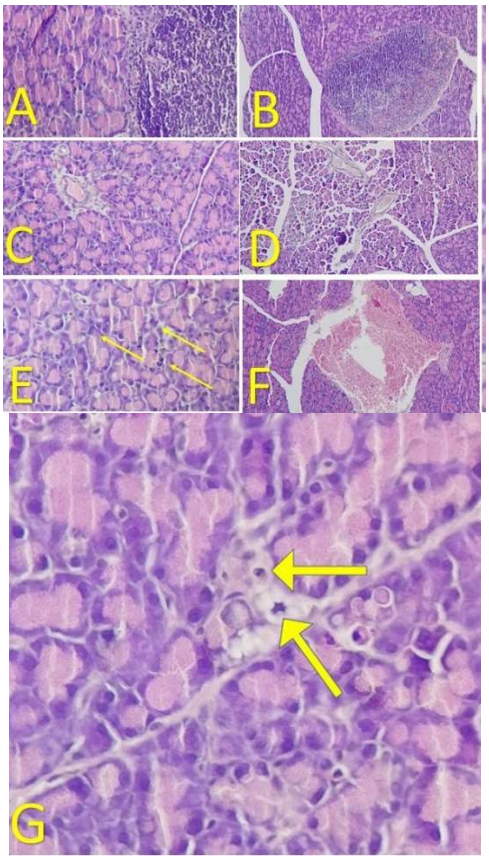

Figure 3. Histopathological findings in pancreatic tissue (hematoxylin and eosin stain).
(A) Intralobar lymphoid aggregate (400x magnification). (B) Interlobar lymphoid aggregate (100x). (C) Normal pancreatic histology from the negative control group (400x). Pathological changes include (D) interstitial edema (100x), (E) focal acinar cell necrosis (400x), (F) parenchymal hemorrhage (100x), and (G) cytoplasmic vacuolization of acinar cells (400x).

DISCUSSION

The present study demonstrates that 120-min bilateral femoral artery occlusion, with or without subsequent reperfusion, does not significantly alter serum amylase, lipase, or insulin levels in healthy Wistar rats. However, histopathological analysis revealed a significant increase in pancreatic injury scores, particularly in the ischemia-only group. This divergence between unchanged biochemical markers and distinct structural damage indicates that the pancreas can sustain subclinical remote injury following hindlimb ischemia. The dissociation aligns with the concept of a "silent pancreas," wherein acinar and islet cell damage, evident microscopically as edema, leukocyte infiltration, vacuolization, and necrosis, precedes or occurs independently of a diagnostic rise in circulating enzymes (Abogresha et al., 2016; Hussein et al., 2014). This suggests that serum amylase and lipase may be late markers of pancreatic injury that require a critical mass of necrotic acinar cells to become elevated, whereas histopathological changes capture earlier and more subtle damage (Nassar & Qunibi, 2019). The findings reinforce the notion that remote organ injury in the pancreas is a time- and threshold-dependent phenomenon.

The most striking observation was that pancreatic damage was significantly greater in the ischemia-only group than in the ischemia–reperfusion group, a seemingly paradoxical result. Mechanistically, prolonged hindlimb ischemia triggers a systemic release of damage-associated molecular patterns (DAMPs) from dying myocytes, which activate liver Kupffer cells and resident pancreatic macrophages, initiating a robust chemokine-mediated neutrophil infiltration into the pancreas (Yassin et al., 2002; H. Chen et al., 2025). Simultaneously, systemic activation of complement and coagulation cascades induces disseminated microthrombus formation within the pancreatic microvasculature, leading to secondary focal ischemia and patchy necrosis even in the absence of a local reperfusion event (Gui et al., 2023). In the ischemia–reperfusion group, restoration of flow, despite its associated oxidative burst, appears to partially clear these microthrombi and limit the duration of pancreatic hypoperfusion, thereby reducing the overall histological injury. Additionally, ischemic preconditioning may be at play: the brief reperfusion phase could activate pro-survival kinases, upregulate heat shock proteins, and enhance endogenous antioxidant defenses, rendering the pancreas more tolerant to the oxidative insult of subsequent reperfusion (Yang et al., 2019; Ouahmi et al., 2026). This protective effect likely underlies the counterintuitively lower damage score and the lowest lipase level observed in the ischemia–reperfusion group.

The behavior of serum lipase deserves special attention. The lowest mean lipase concentration was found in the ischemia–reperfusion group, whereas the ischemia-only group showed the highest, although these differences did not reach statistical significance ($p=0.557$). This trend, together with the histopathological data, suggests that the combination of ischemia and reperfusion may activate adaptive mechanisms that preserve cellular membrane integrity or enhance lipase clearance. Previous studies in pancreatic ischemia have documented ischemic preconditioning, where brief, intermittent episodes of ischemia protect against subsequent prolonged ischemic insults by modulating pro-survival signaling pathways (Dembiński et al., 2003; Hogan et al., 2012).

The disparity between amylase and lipase responses—amylase tended to be highest in the ischemia–reperfusion group—highlights that these two acinar cell enzymes are released through distinct dynamics and may reflect different aspects of cellular injury, with lipase's longer half-life potentially making it a more integrated marker of the injury course (Ismail & Bhayana, 2017). Nevertheless, the large inter-individual variability, evidenced by high standard deviations in all enzyme measurements, likely masked potentially significant differences and reflects genuine biological heterogeneity in the pancreatic response to remote ischemic stress.

Insulin levels displayed a non-significant downward trend in the ischemia-only group (3.646 μU/mL vs. 4.151 μU/mL in controls), consistent with in vitro evidence that beta-cells are highly vulnerable to anoxia/reoxygenation, which depresses ATP production and impairs insulin secretion (Ouahmi et al., 2026). The absence of statistical significance, again attributable to considerable variability, suggests that the magnitude of beta-cell dysfunction induced by 120 min of limb ischemia is modest and potentially compensated for by the remaining functional beta-cell mass in healthy animals. However, this trend is clinically relevant because even subtle chronic impairment of insulin secretion, combined with repeated ischemic insults or underlying metabolic syndrome, may progress to diabetes secondary to pancreatic disease, a condition well documented in patients with chronic pancreatitis and vascular comorbidities (Ciochina et al., 2022; Li et al., 2018). The lack of correlation between insulin and exocrine enzymes (Pearson $r < 0.14$ for all pairwise comparisons) further indicates that exocrine and endocrine compartments respond to remote ischemia through independent pathophysiological pathways. Acinar cell enzyme leakage is largely a consequence of membrane disruption, while beta-cell dysfunction primarily reflects metabolic failure and activation of the unfolded protein response; thus, a normal amylase or lipase level does not rule out concurrent islet cell stress.

The substantial variability observed across all biochemical parameters underscores that the pancreatic response to remote ischemia is not uniform but is modulated by intrinsic factors such as basal antioxidant capacity, metabolic rate, and genetic background. This heterogeneity has important methodological implications: future studies investigating remote pancreatic injury must employ larger sample sizes or paired designs to overcome this variability and detect subtle effects. Moreover, the failure of 120-min femoral occlusion to elicit a significant systemic enzyme elevation does not negate the pancreas's vulnerability; rather, it suggests that the threshold for remote pancreatic injury is higher than for organs such as the lungs or kidneys, which are affected more rapidly after limb ischemia–reperfusion (Kalogeris et al., 2012). The pancreas possesses a rich collateral arterial supply from the pancreaticoduodenal and splenic arteries, and splanchnic autoregulation may preserve pancreatic perfusion despite systemic inflammatory changes, explaining its relative resilience to a short period of extremity ischemia (McDermott et al., 2013). Only when the ischemic stimulus is prolonged or compounded by shock does frank pancreatitis with elevated enzymes become manifest, as in the setting of direct mesenteric ischemia (Wilson & Imrie, 1986).

Several limitations of this study must be acknowledged. The observation period was relatively short (up to 120 min ischemia plus 30 min reperfusion), and blood sampling was performed at a single time point immediately after the protocol, which may have missed later peaks in pancreatic enzymes. Only healthy young male rats were used; the presence of comorbidities such as diabetes, atherosclerosis, or advanced age could significantly lower the ischemic threshold and unmask more pronounced pancreatic injury. Furthermore, the semiquantitative histopathology scoring, while comprehensive, is subject to observer variability, although blinding of the pathologist minimized this bias. The study did not assess systemic inflammatory mediators (e.g., IL-6, TNF-α) or markers of oxidative stress, which could have provided mechanistic insight into the pathways linking limb ischemia to pancreatic damage. The use of a hindlimb ischemia model, although clinically relevant to orthopedic and vascular surgery, differs from the more severe mesenteric or aortic cross-clamping models in the degree of systemic involvement; thus, extrapolation to those clinical scenarios requires caution.

CONCLUSION

In conclusion, this study provides compelling evidence that 120 min of femoral artery occlusion induces significant, albeit subclinical, pancreatic histopathological injury in rats without concomitant elevation of serum amylase, lipase, or insulin. The paradoxical attenuation of damage in the ischemia–reperfusion group supports the existence of endogenous protective mechanisms, possibly ischemic preconditioning, that mitigate remote organ injury. These findings expand the understanding of remote pancreatic injury by demonstrating that structural damage can precede biochemical abnormalities and that the pancreas exhibits a threshold-dependent response to distant ischemic insults. Future investigations should explore longer occlusion times, incorporate repeated ischemia–reperfusion cycles, and

employ a broader panel of molecular markers to capture the full spectrum of pancreatic involvement. Such studies will be essential to translate these observations into clinical strategies for monitoring and protecting pancreatic function in patients undergoing prolonged orthopedic or vascular procedures.

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