**Name of Journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 24455**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

**Steatotic livers are susceptible to normothermic ischemia-reperfusion injury from mitochondrial Complex-I dysfunction**

Chu MJJ *et al*. Complex I dysfunction in steatotic livers

Michael JJ Chu, Rakesh Premkumar, Anthony JR Hickey, Yannan Jiang, Brett Delahunt, Anthony RJ Phillips, Adam SJR Bartlett

**Michael JJ Chu, Rakesh Premkumar,Anthony RJ Phillips, Adam SJR Bartlett,** Department of Surgery, University of Auckland, Auckland 1142, New Zealand

**Anthony JR Hickey, Anthony RJ Phillips,** School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand

**Anthony JR Hickey, Anthony RJ Phillips, Adam SJR Bartlett,** Maurice Wilkins Centre for Biodiscovery, University of Auckland, Auckland 1142, New Zealand

**Yannan Jiang,** Department of Statistics, University of Auckland, Auckland 1142, New Zealand

**Brett Delahunt,** Department of Pathology and Molecular Medicine, Wellington School of Medicine, University of Otago, Wellington 8140, New Zealand

**Anthony RJ Phillips, Adam SJR Bartlett,**New Zealand Liver Transplant Unit, Auckland City Hospital, Auckland 1142, New Zealand

**Author contributions:** Chu MJJ, Premkumar R and Hickey AJR equally contributed to this paper; Chu MJJ designed the study, carried out experiments, performed data analysis, drafted manuscript and revised the manuscript; Premkumar R designed the study, carried out animal surgery and revised the manuscript; Hickey AJR designed the study, analysed the data and revised the manuscript; Jiang Y performed statistical analysis and revised the manuscript; Delahunt B performed histological analysis and revised the manuscript; Phillips ARJ designed the study, analysed the data and revised the manuscript; and Bartlett ASJR designed the study and revised the manuscript.

**Supported by** the University of Auckland Faculty Research Development Fund.

**Institutional animal care and use committee statement:** All procedures were reviewed and approved by the University of Auckland Animal Ethics Committee (R965).

**Conflict-of-interest statement:** The authors do not have any conflict of interest to declare.

**Data sharing statement:**No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to:Michael JJ Chu, PhD, Candidate/General Surgical Resident,** Department of Surgery, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. michaeljjc@gmail.com

**Telephone:**+64-2-1345320

**Fax:** +64-9-3779656

**Received:** January 23, 2016

**Peer-review started:** January 25, 2016

**First decision:** February 18, 2016

**Revised:** March 5, 2016

**Accepted:** March 18, 2016

**Article in press:**

**Published online:**

### **Abstract**

### **Aim**: To assess effects of ischemic preconditioning (IPC, 10-min ischemia/10-min reperfusion) on steatotic liver mitochondrial function after normothermic ischemia-reperfusion injury (IRI).

### **Methods**: Sixty male Sprague-Dawley rats were fed 8-wk with either control chow or high-fat/high-sucrose diet inducing > 60% mixed steatosis.Three groups (*n =* 10/group) for each dietary state were tested: (1) IRI group underwent 60 min partial hepatic ischemia and 4 h reperfusion; and (2) IPC group underwent IPC prior to same standard IRI, iii) Sham underwent the same surgery without IRI or IPC.Hepatic mitochondrial function were analysed by oxygraphs.Mitochondrial Complex-I, Complex-II enzyme activity, serum alanine transferase (ALT) and histological injury were measured.

### **Results**: Steatotic-IRI livers had a greater increase in ALT (2476 ± 166 *vs* 1457 ± 103 IU/L, *P* < 0.01) and histological injury following IRI compared to the lean liver group.Steatotic-IRI demonstrated lower Complex-I activity at baseline [78.4 ± 2.5 *vs* 116.4 ± 6.0 nmol/(min**.**mg protein), *P* < 0.001] and following IRI [28.0 ± 6.2 *vs* 104.3 ± 12.6 nmol/(min**.**mg protein), *P* < 0.001].Steatotic-IRI also demonstrated impaired Complex-I function post-IRI compared to the lean liver IRI group.Complex-II activity was unaffected by hepatic steatosis or IRI.Lean liver mitochondrial function was unchanged following IRI.IPC normalized ALT and histological injury in steatotic livers but had no effect on overall steatotic liver mitochondrial function or individual mitochondrial complex enzyme activities.

### **Conclusion**: Warm IRI impairs steatotic liver Complex-I activity and function.The protective effects of IPC in steatotic livers may not be mediated through mitochondria.

**Key words**: Mitochondrial respiration; Fatty liver; Liver ischemia; Oxidative phosphorylation; Liver injury; Hepatic steatosis; Ischemic preconditioning

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We report the first study on detailed mitochondrial function analysis of dietary-induced hepatic steatosis, which was not choline-deficient, during warm ischemia and after ischemia-reperfusion injury. We also evaluated mitochondrial complex I and II activities as well as the impact of ischemic preconditioning on mitochondrial function.This study demonstrates that steatotic livers have decreased Complex-I activity at baseline and Complex-I function is further impaired after warm ischemia-reperfusion injury.Ischemic preconditioning was unable to attenuate the harmful effect of ischemia-reperfusion on mitochondrial function.

Chu MJJ, Premkumar R, Hickey AJR, Jiang Y, Delahunt B, Phillips ARJ, Bartlett ASJR. Steatotic livers are susceptible to normothermic ischemia-reperfusion injury from mitochondrial Complex-I dysfunction. *World J Gastroenterol* 2016; In press

**Introduction**

Hepatic steatosis is the most common liver disease observed in clinical liver biopsies[[1](#_ENREF_1)] and autopsy-based estimates on studies have placed the prevalence of hepatic steatosis to be between 15%-30% in the Western world[[1](#_ENREF_1)].Consequently, there is an increasing number of patients with hepatic steatosis encountered during liver surgery.Hepatic steatosis has been associated with a 2-3 fold increase in post-operative complication rates following liver resection[[2](#_ENREF_2),[3](#_ENREF_3)].It has been proposed that steatotic livers are more susceptible to ischemia-reperfusion injury (IRI), which impairs liver regeneration and is a major cause of liver damage leading to worse outcomes[[3](#_ENREF_3)].

The exact mechanism for the increased susceptibility of steatotic livers to IRI is not fully understood.Steatotic livers have been shown to have decreased recovery of adenosine triphosphate (ATP) concentrations following IRI[[4](#_ENREF_4)].One of the proposed underlying mechanisms behind the decreased ATP recovery and increased steatotic liver susceptibility to IRI is mitochondrial dysfunction[[5](#_ENREF_5)].Mitochondria are responsible for producing the bulk of cellular ATP, and are therefore fundamental for cellular viability[[6](#_ENREF_6)].Impaired mitochondrial function (MF) disrupts normal cellular bioenergetics which leads to cell death[[7](#_ENREF_7)].

To attenuate the deleterious effect of IRI, ischemic preconditioning (IPC) of the liver has been used[[8](#_ENREF_8)].IPC involves a brief period of ischemia followed by reperfusion (generally, 10 min ischemia and 10 min reperfusion) prior to a period of sustained ischemic insult[[9](#_ENREF_9),[10](#_ENREF_10)].IPC has been reported to improve post-IRI liver injury in experimental[[9](#_ENREF_9)] and clinical[[10](#_ENREF_10)] steatotic livers.IPC has also been reported to improve ATP levels in steatotic livers[[11](#_ENREF_11)].While the mechanism by which IPC improves hepatic outcome following IRI is unknown, it has been postulated that IPC modulates and somehow preserves MF[[9](#_ENREF_9)].The aim of this study was to evaluate the impact of IPC on the MF of rat livers with steatosis after being subjected to warm (normothermic) IRI.Mitochondrial bioenergetics and liver injury markers were evaluated.

**Materials and methods**

***Animals***

All reagents were purchased from Sigma-Aldrich (New South Wales, Australia) unless otherwise specified.All experiments were performed in 11-wk old male Sprague-Dawley rats.The animal protocol was designed to minimize discomfort to the animals.The animals were enrolled when 3-wk old and removed from the mother at this age.They were then randomized to receive standard chow (lean animals; Teklad TB 2018; Harlan, Madison, WI; 18% kcal fat, 58% kcal carbohydrate) or a high-fat/high-sucrose diet (steatotic animals; Rodent Diet D03021303; Research Diets, Inc., New Brunswick, NJ, United States; 45 kcal% fat, 25% kcal sucrose; Table 1)[[12](#_ENREF_12)].The animals were kept under a 12-h light/dark cycle (50%-70% humidity, 22 ± 2 °C) with *ad libitum* access to food and water.Bodyweight and blood glucose were measured weekly.Rats were fasted for 6 h prior to surgery to mimic pre-operative fasting.All surgical procedures were started between 12:00-1:00 pm.All experiments were approved by the University of Auckland Animal Ethics Committee (R965).

***Experimental design and surgical procedures***

Sixty animals were randomized into one of 6 groups (*n =* 10 each): (1) Lean + Sham (Lean-Sham); (2) Lean + IRI (Lean-IRI); (3) Lean + IRI + IPC (Lean-IPC); (4) Steatotic + Sham (Steatotic-Sham); (5) Steatotic+IRI (Steatotic-IRI); and (6) Steatotic + IRI + IPC (Steatotic-IPC).

A model of partial (70%) hepatic ischemia was used that prevented mesenteric venous congestion by permitting portal decompression through the right and caudate lobes[[9](#_ENREF_9)].Rats were anesthetized with isofluorane inhalation.Following tracheostomy, anesthesia was maintained (1%-2% isofluorane) through a pressure-controlled ventilator (Kent Scientific Corporation, Torrington, United States).Core body temperature was maintained (37-38 oC) by a thermostatically-controlled warming plate.Fluid administration via the right femoral vein and mean arterial pressure monitoring via the right carotid artery were undertaken with a radio-opaque 22G catheter and 2F solid-state pressure transducer (SPR-320 pressure catheter; Millar Instruments Inc., Houston, United States), respectively.

Following a transverse laparotomy, the hepatic artery and portal vein to the left and median lobes were occluded for 60 min with a microvascular clip.Removal of the clip initiated the 240 min of reperfusion.Rats receiving IPC received 10 min of ischemia and 10 min of reperfusion prior to 60 min of ischemia.In the sham group, the rats were anesthetized and a laparotomy was performed for 5.5 h without induction of ischemia.

The placement of a tracheostomy (reflecting endotracheal intubation) and prolonged anaesthesia mimic clinical liver resection whereby patients are anaesthetized continuously.We did not perform short intervals of anaesthesia and repeated mini-laparotomies for our protocol as it does not reflect clinical practice.

***Tissue collections***

Liver samples were obtained by “cheese-wire” ligating the liver with 4-0 silk tie.This technique caused minimal bleeding from the cut surface of the liver and allowed repeated sampling from each rat.Liver samples were obtained at various time-points: “A”: baseline (immediately following laparotomy), “B”: 10 min (after 10 min ischemia), “C”: 20 min (end of IPC), “D”: 80 min (after 60 min ischemia), and “E”: 320 min (end of 240 min reperfusion phase).Liver samples were obtained for histology, MF and enzymatic analysis.The amount of liver tissue removed did not exceed 20% of total hepatic mass.At the end of the procedure, serum (5-ml) was collected from the inferior vena cava.

***Histology***

Histology was performed on time-points A and E to assess severity of the hepatic steatosis and IRI, respectively.Formalin-fixed and paraffin-embedded liver samples were stained with haematoxylin and eosin (HE).A consultant specialist histopathologist (BD) blinded to the group assessed the severity of steatosis with a published clinical grading system[[13](#_ENREF_13)].Severity of the IRI was assessed using a 4-point grading system previously described[[14](#_ENREF_14)].

***Assessment of hepatocyte injury***

The severity of hepatic injury was assessed by serum levels of alanine aminotransferase (ALT) and was analyzed using a Roche Cobas 8000 modular analyzer (c702 module).

***Homogenized tissue preparation for mitochondrial function analysis***

Liver samples were immediately placed in ice-cold (about 4 oC) mitochondrial respiration media (Table 2).The samples were then removed from the media, blotted, weighed (20-30 mg) and placed in a 2-ml flat-bottom scintillation vial with 500 µl of mitochondrial respiration media.The sample was homogenized for 5 seconds with an Omni TH homogenizer (Omni International, Kennesaw, Georgia, United States) before analysis.Homogenates were utilized as they are more physiological and decrease the risk of any organelle selection bias inherent to the process of mitochondrial isolation whereby more healthy organelles are preferentially selected during isolation[[15](#_ENREF_15)].Our protocol also permits shorter processing time and rapid measurement of the function of the entire mitochondrial population within the tissue to provide an immediate measure of mass specific flux.

***Mitochondrial respiration assays***

Mitochondrial respiration of liver homogenate was measured at each time-point.Respiration was measured in 2-mL chambers using an OROBOROS Oxygraph 2K (Anton Paar, Graz, Austria), at 37 °C in mitochondrial respiration media, with a calculated saturated oxygen concentration of 190 nmol O2 per mL at 100 kPa barometric pressure, and oxygen flux calculated using the DatLab 5 analysis software.Liver homogenate (50 μL) was added to each chamber and the remainder stored at -80°C for later analysis.To account for potential variations in mitochondrial mass, citrate synthase (CS)-normalized[[16](#_ENREF_16)] oxygen flux (pmol O2.s-1.U CS-1) was calculated.

A multiple substrate-inhibitor titration protocol was used to explore relative contributions of complex I (CI), complex II (CII) and combined CI+CII in the electron transport system (ETS).Respiration states were defined according to Gnaiger[[17](#_ENREF_17)], where leak respiration and oxidative phosphorylation (OXPHOS) were the flux measured before and after addition of adenosine diphosphate (ADP), respectively.The assay protocol steps are described in Table 3.The integrity of tissue preparations and comparison of coupling efficiencies were made from the respiratory control ratio (RCR).

***Citrate synthase and total protein measurement***

Liver homogenate, as used in mitochondrial respiration assay, was analysed for CS activity and total protein content.CS activity was measured as a surrogate for mitochondrial mass[[16](#_ENREF_16)].Frozen (-80 °C) liver homogenate was thawed and CS activities were determined following Srere and modified to microtitre-plate[[18](#_ENREF_18)].Protein content was determined by the Biuret test with bovine serum albumin as standard.

***Complex I & Complex II activities***

Liver homogenate from time-points A, D and E were used to measure individual CI and CII enzyme activities using the NADH-oxidation and dichlorophenolindophenol-oxidation method, respectively[[19](#_ENREF_19)].Frozen liver homogenate was thawed, centrifuged for 10 min at 600g (4 °C) and the supernatant used for analysis.Assays were conducted using a 96-well format and results normalized to total protein content determined by the Biuret method.In this report “CI function” refers to assessments undertaken in the oxygraph and “CI activity” refers to the isolated activity studies just described.

***Statistical and data analysis***

All study data were recorded in an EXCEL database.Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) and SAS version 9.2 (SAS institute, Cary, NC).Statistical tests were set at a 5% significance level (two-sided).Student’s *t*-tests were conducted on body weight and random blood glucose level between obese and lean rats.Difference in outcome measure between the groups of interest was tested using the analysis of covariance regression, adjusting for baseline value, bodyweight and blood glucose measured before the procedures as appropriate.Repeated measures mixed model was used to evaluate the treatment differences at different time-points, controlling for the correlation of data collected on the same animal.The results are presented as mean ± SE of mean, with associated *p*-value.

**Results**

***Rats fed high-fat/high-sucrose diet were obese***

Sprague-Dawley rats fed high-fat/high-sucrose diet for 8 weeks showed increased body weight (507 ± 10 *vs* 437 ± 6 g, *P* < 0.0001, Figure 1A) and random blood glucose level (6.1 ± 0.1 *vs* 4.6 ± 0.1 mmol/L, *P <* 0.0001, Figure 1B) relative to age-matched lean rats.

***Obese rat livers had severe mixed steatosis***

Obese rat livers had gross macroscopic fat accumulation.All lean rat livers had normal baseline underlying tissue architecture with only some mild (8% ± 1%) microvesicular steatosis when evaluated by H&E staining (Figure 1C).Obese rat livers had severe (65% ± 3%) baseline mixed steatosis with prominent macrovesicular steatosis features evident (Figure 1D).There were no signs of fibrosis or inflammation in any of the groups consistent with hepatic steatosis.

***Steatotic livers had increased liver injury following ischemia-reperfusion***

Effect of steatosis, IRI and IPC on liver injury biomarkers, tissue injury scores and histology are shown in Figure 2 and Figure 3, respectively.Both Lean-Sham and Steatotic-Sham livers did not have any biochemical (Figure 2A) or histological evidence of injury induced by the sham surgery (Figure 2B, 3A and 3B).Conversely IRI was associated with increased serum ALT (Figure 2A) and worse liver histology injury scores in both Steatotic-IRI and Lean-IRI rats compared to Steatotic-Sham and Lean-Sham rats, respectively (Figure 2B).These same injury markers were also found significantly higher in Steatotic-IRI rats compared to Lean-IRI rats (Figure 2).IPC led to improvement in the serum ALT in Steatotic-IPC and Lean-IPC rats compared to Steatotic-IRI and Lean-IRI rats, respectively (Figure 2A).IPC also led to a decrease in injury score in Steatotic-IPC rats compared to Steatotic-IRI rats (Figure 2B).These results indicate that IPC was able to attenuate liver injury in steatotic livers.

***Baseline mitochondrial function in steatotic livers were similar to lean livers***

The baseline mitochondrial functions were similar between steatotic and lean rat livers. Baseline (T=0) samples from steatotic rat livers (*n =* 30) had similar MF to lean rat livers (*n =* 30) (Figure 4A-F).These results indicate that steatotic liver mitochondria were initially functioning adequately *in vivo*.

***Sham-operated rat liver mitochondrial function remained stable***

There were no changes in MF in both Lean-Sham and Steatotic-Sham livers with a stable CI-OXPHOS, CII-OXPHOS and RCR throughout all time-points (Figure 4A-F).These data indicate that the act of repeated liver sampling from each rat did not in itself significantly influence the underlying MF.

***Prolonged ischemia led to impaired mitochondrial function***

At the end of 60 min of ischemia, both lean and steatotic livers demonstrated impaired MF with significantly lower CI-OXPHOS (about 30%-40%), CII-OXPHOS (45%-60%) and RCR (about 60%-80%) compared to pre-ischemic levels or corresponding Sham livers (Figure 4A-F).There was no observable difference in MF between Lean-IRI and Steatotic-IRI livers at the end of ischemia.These findings indicate impaired MF occurs to the same extent in all groups immediately following 60 min of ischemic insult.

***Reperfusion injury led to decreased Complex I mediated respiration in steatotic livers***

After 60 min of ischemia and following 240 min of reperfusion, MF in Lean-IRI returned to pre-ischemic levels and was comparable to Lean-Sham livers (Figure 4A, C, E).In Steatotic-IRI livers, CI-OXPHOS flux and RCR was significantly lower compared to baseline levels or Steatotic-Sham livers (Figure 4B, F) whereas CII-OXPHOS flux rates returned to pre-ischemic levels and was comparable to Steatotic-Sham livers (Figure 4D).CI-OXPHOS flux rates and RCR in Steatotic-IRI livers was observed to be 57% and 54% relative to Lean-IRI livers post-reperfusion (*P <* 0.01).These results indicate that unlike lean livers, steatotic liver CI function was impaired by IRI leading to decreased RCR.

***Ischemic preconditioning had no significant effect on hepatic mitochondrial function***

MF in both types of livers subjected to IPC was similar to livers subjected to IRI only (Figure 4A-F).Lean-IPC and Steatotic-IPC livers demonstrated a similar pattern of CI-OXPHOS, CII-OXPHOS and RCR at each of the sampling time-points as Lean-IRI and Steatotic-IRI livers, respectively (Figure 4A-F).Post-reperfusion, CI-OXPHOS and RCR in Steatotic-IPC livers remained impaired while CII-OXPHOS was comparable to pre-ischemic levels.These results indicate that despite the improvement in injury markers (ALT and histology) in IPC livers (Figure 2 and 3), IPC did not influence underlying MF over this timeframe.

***Citrate synthase activity was unaffected by ischemia-reperfusion injury***

Citrate synthase (CS) activity in lean and steatotic livers was stable throughout the experiment and was not affected by IRI or IPC (Figure 5A, B).There was also no difference in CS activity between lean and steatotic livers at all time-points measured.

***Decreased Complex I but not Complex II enzymatic activity following reperfusion in steatotic livers***

Baseline CI activity was significantly lower in steatotic livers compared to lean livers [78.4 ± 2.5 *vs* 116.4 ± 6.0 nmol/(min**.**mg protein), *P <* 0.001] while baseline CII activity was similar between the 2 groups [104.9 ± 3.3 *vs* 116.8 ± 6.1 nmol/(min**.**mg protein), *P* = 0.08, Figure 5C-D].Following 60 min of ischemia, both types of liver demonstrated significantly lower CI activity (Figure 5C, D) compared to pre-ischemic or sham livers.Steatotic liver CI activity was also observed to be lower post-ischemia compared to lean livers.Following reperfusion, CI activity returned to pre-ischemic levels in lean livers but remained significantly lower in steatotic livers by approximately 65% (Figure 5D).CII activity (Figure 5E, F) was stable throughout the procedure and there was no difference in CII activity between steatotic and lean livers.IPC did not have any significant beneficial effect on CI and CII activity in both types of livers.These activity results showed that IRI led to decreased CI activity in steatotic livers, and also that IPC was not able to influence CI or CII activities; all of which was consistent with the earlier oxygraph functional analysis (above).

**Discussion**

In this study, we used Sprague-Dawley rats with diet-induced hepatic steatosis.Steatotic livers demonstrated increased parenchymal injury following IRI compared to normal lean livers, as indicated by their raised serum ALT and histology injury scores.Steatotic livers had lower baseline CI activity but similar baseline CS and CII activity compared to lean livers.The steatotic livers were also observed to have decreased CI activity and function following IRI, which unlike lean livers, showed no recovery of either activity or function even after prolonged reperfusion times.This indicated CI as a particularly vulnerable site of IRI-induced damage in steatotic livers.Our results demonstrated that IPC was effective in decreasing liver injury in both lean and steatotic livers according to ALT levels.However, this protective effect was not translated to measures of MF or CI activity.In summary these data demonstrate that steatotic livers developed significant underlying mitochondrial impairment that was worsened by IRI but not able to be recovered by IPC.

In this study we developed a novel methodology for the repeated procurement of liver samples from the same animal over time, which has not been published before.This technique offers decreased animal numbers but also presents statistical advantages by enabling repeated measures analyses to look for study effects.The theoretical disadvantage of the progressive hepatectomy samples altering the status of the subsequent samples did no eventuate.In particular when the Sham groups were examined, there was no significant change found in serum ALT, histological scores or MF from the progressive sampling approach.

***Mitochondrial function and complex enzyme activities***

Steatotic livers had impaired MF, which is thought to contribute to the increased steatotic liver susceptibility to IRI[[5](#_ENREF_5)].CS activity was similar across groups and time-points, indicating that MF differences are from mitochondria functional or activity differences and not a difference in mitochondrial mass in this study.

A key dysfunction was found in CI which is a large protein (about 1 MDa) comprising 45-47 subunits and is embedded in the mitochondrial inner membrane to form an essential component to the mitochondrial ETS[[20](#_ENREF_20)].Impaired CI function has a substantial effect on ATP generation and contributes to a wide range of pathologies[[21](#_ENREF_21)].Our MF findings are consistent with a previous study that reported finding similar baseline CI function between steatotic and control livers; and lower post-reperfusion CI function in isolated mitochondria from steatotic livers[[5](#_ENREF_5)].We have now extended this to show that the individual CI activity in tissue homogenates was also affected in steatotic livers post-IRI.Steatotic liver CI has previously been shown to be susceptible to oxidative damage from decreased mitochondrial antioxidants[[5](#_ENREF_5)].Further exacerbating this, CI can be a major site of reactive oxygen species production[[22](#_ENREF_22)] and steatotic livers produce more reactive oxygen species *in vivo* than lean livers[[5](#_ENREF_5)].Oxygen reperfusion post-ischemia also leads to greater superoxide generation in steatotic livers relative to lean livers[[23](#_ENREF_23)].The lower CI activity observed in steatotic livers may be due to damage from IRI or potentially as a physiological response from steatotic livers as it attempts to limit oxidative mitochondrial damage; and may be contributing to the decreased tolerance of steatotic livers to IRI.

Complex II, is the only mitochondrial membrane-bound enzyme that is also involved in the citric acid cycle, as it oxidizes succinate and transfers electrons to co-enzyme Q[[24](#_ENREF_24)].CII function has been reported to be similar at baseline and post-IRI between steatotic and lean rat livers[[5](#_ENREF_5)], and our results corroborate this finding.CII abnormalities are infrequently reported in the literature[[19](#_ENREF_19)] and its function in steatotic livers seldom reported.We observed that CII activity post-ischemia and post-reperfusion was similar between steatotic and lean livers which have not been previously described in this context.Given that CII activity and function appears to be unaffected by IRI, this suggests that CII is more resistant to damage.CII may even contribute to superoxide production through an apparent reverse electron flow to CI instead[[24](#_ENREF_24)], although this is somewhat controversial as it appears to defy thermodynamics and redox potentials[[21](#_ENREF_21)].Despite intact CII function, CII-driven flux is less effective in ATP synthesis (coupled to OXPHOS at Complex III and Complex IV) compared to CI-driven flux (coupled to OXPHOS at CI, Complex III and Complex IV), and this should impair reconstitution of ATP pools on reperfusion.

***Effect of ischemic preconditioning***

MF recovery following IRI is thought to be essential as it generates the majority of cellular ATP[[6](#_ENREF_6)].Inadequate MF post-IRI would lead to decreased or delayed ATP generation during the critical period of reperfusion and could impair liver recovery.In this study we hypothesized that IPC would protect MF from IRI-induced damage as part of the mechanism for how IPC protects the steatotic liver in this setting.IPC was shown in our study to be partially protective against normothermic IRI (biochemical and histological indices) in both lean and steatotic livers, consistent with other studies[[9](#_ENREF_9),[11](#_ENREF_11)].Our findings of improved liver transaminases in preconditioned steatotic livers have not been previously investigated in diet-based models of obesity but were consistent with the limited clinical data on the effect of IPC on biochemical markers from steatotic livers subjected to normothermic IRI during liver resection[[10](#_ENREF_10),[25](#_ENREF_25)].

However our results also indicated that despite some improvement in conventional liver injury markers, IPC did not improve MF or key enzyme activities over the duration of this study.This was in contrast to a previous study in choline-deficient rats that showed improvement in conventional liver injury markers and MF in preconditioned steatotic livers post-IRI[[9](#_ENREF_9)].Importantly, there were substantial differences in our study design to that of the only previous experimental study.In that study, Rolo *et al*[[9](#_ENREF_9)] performed MF analysis on isolated mitochondria at 25oC and demonstrated lower RCR in both lean and steatotic livers post-IRI, which were normalized by IPC in both groups of livers.However, mitochondrial respiration and particularly State 4 respiration is sensitive to assay temperature[[26](#_ENREF_26)], and the results may not be truly representative of physiological MF at 37 oC.Furthermore, the process of mitochondrial isolation results in the loss of fragile and/or damaged mitochondrial sub-populations.Here we decided to use tissue homogenates as a means to lessen the potential for any mitochondrial selection bias[[15](#_ENREF_15)].Additionally, animals fed the choline-deficient diet used by Rolo *et al* show weight loss, which is contrary to the clinical setting of most patients with hepatic steatosis being obese[[27](#_ENREF_27)].We suggest that in our current study the combination of using a dietary model, tissue homogenates and undertaking MF analysis at 37oC now represents the first advancement to a more physiological and clinically relevant MF analysis of the interaction of steatosis, IRI and IPC.It is of note that most other previous studies on IPC in steatotic livers were performed in genetic models of hepatic steatosis[[28](#_ENREF_28)].However, the underlying mutations in these models are not prevalent in clinical hepatic steatosis pathophysiology and the high-fat/high-carbohydrate used in our study more closely resembles the clinical setting[[27](#_ENREF_27)].

The mechanism of IPC has been extensively reviewed elsewhere[[29](#_ENREF_29)], but the direct impact of IPC on mitochondria is not so well characterised.In experimental lean rat livers subjected to IRI, ATP recovery was unaffected by IRI but in steatotic livers ATP recovery was found to be impaired post-IRI compared to lean livers[[30](#_ENREF_30)].In other studies, IPC was reported to preserve ATP recovery in lean livers post-IRI[[29](#_ENREF_29)] while other studies have suggested that IPC was also effective in improving ATP recovery in steatotic livers post-IRI[[11](#_ENREF_11)].For our study, we investigated MF and complex activities.We chose a 10’+10’ IPC protocol as this was similar to the first clinical protocol described by Clavien *et al*[[10](#_ENREF_10)].In their study, IPC led to an improvement in biochemical and histological markers of injuries.This finding was then replicated in a further prospective trial[[25](#_ENREF_25)] and demonstrated that IPC improved ATP levels in younger patient’s post-reperfusion.However, IPC was associated with no improvement in older patients but instead a further decrease in ATP levels post-reperfusion when compared to control livers.Our results showed no improvement in MF with IPC, and although we were not able to measure ATP in this study, our findings suggests the protective effect of IPC was not likely to be mediated through increased mitochondrial ATP production in this model.This suggests that there may be other mechanisms underlying the effect of IPC on hepatic ATP recovery.These may include decreased cellular metabolism in preconditioned livers leading to conservation of ATP or reduced microcirculatory dysfunction.Alternatively some of the IPC benefit maybe from increased production of nitric oxide and opening of ATP-dependant potassium channels in preconditioned livers with a subsequent decrease in energy consumption[[29](#_ENREF_29)].

The lack of full protection and liver function recovery observed by us and others in the clinical and animal studies from IPC may reflect the persistent underlying mitochondrial dysfunction demonstrated in our present study[[3](#_ENREF_3)].This observation supports future investigation of other IPC protocols or combinatorial use with mitochondrial-targeted therapies, as these may provide further clinical improvements.However, it could also potentially be influenced by our animal model as our model differ from those published in the literature[[9](#_ENREF_9)].For completeness, liver weights should have been measured but as samples were obtained in “piece-meal” fashion, this was made impossible.

As we witness the rising prevalence of the metabolic syndrome in society, hepatic steatosis has become the most common hepatic abnormality[[1](#_ENREF_1)].It is increasingly important to find ways to improve outcomes from steatotic liver surgery as this is rapidly becoming the status of the routine patient.In this study, we investigated the impact of IPC on MF in a steatosis setting.We demonstrated that IRI was associated with increased liver injury in steatotic livers.Although the precise mechanisms underlying the increased susceptibility of steatotic liver to IRI remain unclear, we have shown for the first time using a clinically relevant diet model and MF analysis at physiological temperatures, that there was an inherent decreased CI activity in steatotic livers, which worsened following IRI.Our results also showed that the IPC protocol used in our study, while improving liver biomarkers and histology, did not influence MF directly.If we are to further improve the clinical benefit of IPC on livers with steatosis, then testing alternative IPC protocols or adjunct mitochondrial therapies may also be needed.

**Comments**

***Background***

Steatotic livers are encountered with increasing frequency in liver surgery.It has been associated with poor outcome following warm ischemia-reperfusion injury.One possible proposed mechanism was mitochondrial dysfunction.However, the relationship between hepatic steatosis and mitochondrial dysfunction in warm ischemia-reperfusion injury has not been clearly defined.Ischemic preconditioning has been touted as a possible therapeutic option for attenuating the harmful effect of ischemia-reperfusion injury.

***Research frontier***

Mechanisms of injury in steatotic livers are poorly understood and needs further understanding to improve patient outcome.

***Innovations and breakthrough***

This is the first study investigating mitochondrial function, mitochondrial Complex I and Complex II activities; and the impact of ischemic preconditioning on mitochondrial function in warm ischemia-reperfusion injury, in a dietary-induced model of hepatic steatosis.

***Applications***

Steatotic livers have decreased baseline Complex I activity but after reperfusion injury, Complex I function and activity was impaired in steatotic livers compared to lean livers.Ischemic preconditioning was unable to influence mitochondrial function in this setting.

***Terminology***

Mitochondrial Complex I is paramount for intact mitochondrial function and impairment of Complex I leads to impaired ATP (cellular energy currency) production, and consequently cell death.

***Peer-review***

It is well known that hepatic steatosis increased susceptibility to ischemia-reperfusion (I/R) injury and this effect is linked to mitochondrial dysfunction. This study explored alterations of mitochondrial Complexes I and II in lean and high-fat, high sucrose diet–induced steatotic rat livers after 1 h-warm ischemia plus 4 h reperfusion. The authors showed that there was a significant decrease in Complex I in steatotic livers compared to lean livers but there was no difference in Complex II between these two groups. IPC decreased alanine aminotransferase release and histological changes after I/R but did not blunt decreases in Complex I in steatotic livers. This study obtained some interesting data.

**REFERENCES**

1 **McCormack L**, Dutkowski P, El-Badry AM, Clavien PA. Liver transplantation using fatty livers: always feasible? *J Hepatol* 2011; **54**: 1055-1062 [PMID: 21145846 DOI: 10.1016/j.jhep.2010.11.004]

2 **Behrns KE**, Tsiotos GG, DeSouza NF, Krishna MK, Ludwig J, Nagorney DM. Hepatic steatosis as a potential risk factor for major hepatic resection. *J Gastrointest Surg* 1998; **2**: 292-298 [PMID: 9841987 DOI: 10.1016/S1091-255X(98)80025-5]

3 **Veteläinen R**, van Vliet A, Gouma DJ, van Gulik TM. Steatosis as a risk factor in liver surgery. *Ann Surg* 2007; **245**: 20-30 [PMID: 17197961 DOI: 10.1097/01.sla.0000225113.88433.cf]

4 **Selzner M**, Rüdiger HA, Sindram D, Madden J, Clavien PA. Mechanisms of ischemic injury are different in the steatotic and normal rat liver. *Hepatology* 2000; **32**: 1280-1288 [PMID: 11093735 DOI: 10.1053/jhep.2000.20528]

5 **Caraceni P**, Domenicali M, Vendemiale G, Grattagliano I, Pertosa A, Nardo B, Morselli-Labate AM, Trevisani F, Palasciano G, Altomare E, Bernardi M. The reduced tolerance of rat fatty liver to ischemia reperfusion is associated with mitochondrial oxidative injury. *J Surg Res* 2005; **124**: 160-168 [PMID: 15820243 DOI: 10.1016/j.jss.2004.10.007]

6 **Mitchell P**. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol Rev Camb Philos Soc* 1966; **41**: 445-502 [PMID: 5329743 DOI: 10.1111/j.1469-185X.1966.tb01501.x]

7 **Hand SC**, Menze MA. Mitochondria in energy-limited states: mechanisms that blunt the signaling of cell death. *J Exp Biol* 2008; **211**: 1829-1840 [PMID: 18515712 DOI: 10.1242/jeb.000299]

8 **Lloris-Carsí JM**, Cejalvo D, Toledo-Pereyra LH, Calvo MA, Suzuki S. Preconditioning: effect upon lesion modulation in warm liver ischemia. *Transplant Proc* 1993; **25**: 3303-3304 [PMID: 8266552]

9 **Rolo AP**, Teodoro JS, Peralta C, Rosello-Catafau J, Palmeira CM. Prevention of I/R injury in fatty livers by ischemic preconditioning is associated with increased mitochondrial tolerance: the key role of ATPsynthase and mitochondrial permeability transition. *Transpl Int* 2009; **22**: 1081-1090 [PMID: 19619169 DOI: 10.1111/j.1432-2277.2009.00916.x]

10 **Clavien PA**, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg* 2000; **232**: 155-162 [PMID: 10903590 DOI: 10.1097/00000658-200008000-00001]

11 **Selzner N**, Selzner M, Jochum W, Clavien PA. Ischemic preconditioning protects the steatotic mouse liver against reperfusion injury: an ATP dependent mechanism. *J Hepatol* 2003; **39**: 55-61 [PMID: 12821044 DOI: 10.1016/S0168-8278(03)00147-8]

12 **Smith GC**, Vickers MH, Shepherd PR. Olanzapine effects on body composition, food preference, glucose metabolism and insulin sensitivity in the rat. *Arch Physiol Biochem* 2011; **117**: 241-249 [PMID: 21671852 DOI: 10.3109/13813455.2011.576681]

13 **D'Alessandro AM**, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen D, Belzer FO. The predictive value of donor liver biopsies for the development of primary nonfunction after orthotopic liver transplantation. *Transplantation* 1991; **51**: 157-163 [PMID: 1987685 DOI: 10.1097/00007890-199101000-00024]

14 **Abu-Amara M**, Yang SY, Quaglia A, Rowley P, Tapuria N, Seifalian AM, Fuller BJ, Davidson BR. Effect of remote ischemic preconditioning on liver ischemia/reperfusion injury using a new mouse model. *Liver Transpl* 2011; **17**: 70-82 [PMID: 21254347 DOI: 10.1002/lt.22204]

15 **Mittal A**, Hickey AJ, Chai CC, Loveday BP, Thompson N, Dare A, Delahunt B, Cooper GJ, Windsor JA, Phillips AR. Early organ-specific mitochondrial dysfunction of jejunum and lung found in rats with experimental acute pancreatitis. *HPB (Oxford)* 2011; **13**: 332-341 [PMID: 21492333 DOI: 10.1111/j.1477-2574.2010.00290.x]

16 **Larsen S**, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, Schroder HD, Boushel R, Helge JW, Dela F, Hey-Mogensen M. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol* 2012; **590**: 3349-3360 [PMID: 22586215 DOI: 10.1113/jphysiol.2012.230185]

17 **Gnaiger E**. Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck, 2014: 80

18 **Hickey AJ**, Chai CC, Choong SY, de Freitas Costa S, Skea GL, Phillips AR, Cooper GJ. Impaired ATP turnover and ADP supply depress cardiac mitochondrial respiration and elevate superoxide in nonfailing spontaneously hypertensive rat hearts. *Am J Physiol Cell Physiol* 2009; **297**: C766-C774 [PMID: 19553568 DOI: 10.1152/ajpcell.00111.2009]

19 **Kirby DM**, Thorburn DR, Turnbull DM, Taylor RW. Biochemical assays of respiratory chain complex activity. *Methods Cell Biol* 2007; **80**: 93-119 [PMID: 17445690 DOI: 10.1016/S0091-679X(06)80004-X]

20 **Hickey AJ**, Jüllig M, Aitken J, Loomes K, Hauber ME, Phillips AR. Birds and longevity: does flight driven aerobicity provide an oxidative sink? *Ageing Res Rev* 2012; **11**: 242-253 [PMID: 22198369 DOI: 10.1016/j.arr.2011.12.002]

21 **Duchen MR**. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol Aspects Med* 2004; **25**: 365-451 [PMID: 15302203 DOI: 10.1016/j.mam.2004.03.001]

22 **Paradies G**, Petrosillo G, Pistolese M, Ruggiero FM. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. *Gene* 2002; **286**: 135-141 [PMID: 11943469 DOI: 10.1016/S0378-1119(01)00814-9]

23 **Nardo B**, Caraceni P, Pasini P, Domenicali M, Catena F, Cavallari G, Santoni B, Maiolini E, Grattagliano I, Vendemiale G, Trevisani F, Roda A, Bernardi M, Cavallari A. Increased generation of reactive oxygen species in isolated rat fatty liver during postischemic reoxygenation. *Transplantation* 2001; **71**: 1816-1820 [PMID: 11455263 DOI: 10.1097/00007890-200106270-00018]

24 **Murphy MP**. How mitochondria produce reactive oxygen species. *Biochem J* 2009; **417**: 1-13 [PMID: 19061483 DOI: 10.1042/BJ20081386]

25 **Clavien PA**, Selzner M, Rüdiger HA, Graf R, Kadry Z, Rousson V, Jochum W. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann Surg* 2003; **238**: 843-50; discussion 851-2 [PMID: 14631221 DOI: 10.1097/01.sla.0000098620.27623.7d]

26 **Lemieux H**, Garedew A, Blier P, Tardif JC, Gnaiger E. Temperature effects on the control and capacity of mitochondrial respiration in permeabilized fibers of the mouse heart. *Biochim Biophys Acta* 2006; **1757**: 201-202

27 **Panchal SK**, Brown L. Rodent models for metabolic syndrome research. *J Biomed Biotechnol* 2011; **2011**: 351982 [PMID: 21253582 DOI: 10.1155/2011/351982]

28 **Chu MJ**, Vather R, Hickey AJ, Phillips AR, Bartlett AS. Impact of ischaemic preconditioning on experimental steatotic livers following hepatic ischaemia-reperfusion injury: a systematic review. *HPB (Oxford)* 2015; **17**: 1-10 [PMID: 24712641 DOI: 10.1111/hpb.12258]

29 **Montalvo-Jave EE**, Piña E, Montalvo-Arenas C, Urrutia R, Benavente-Chenhalls L, Peña-Sanchez J, Geller DA. Role of ischemic preconditioning in liver surgery and hepatic transplantation. *J Gastrointest Surg* 2009; **13**: 2074-2083 [PMID: 19404711 DOI: 10.1007/s11605-009-0878-7]

30 **Hui AM**, Kawasaki S, Makuuchi M, Nakayama J, Ikegami T, Miyagawa S. Liver injury following normothermic ischemia in steatotic rat liver. *Hepatology* 1994; **20**: 1287-1293 [PMID: 7927264 DOI: 10.1002/hep.1840200528]

**P-Reviewer:** Nagarajan P, Zhong Z **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

**Table 1 Content of high-fat/high-sucrose diet (D03021303; Research Diets, Inc., NJ, United States)**

|  |  |  |
| --- | --- | --- |
|  | **Gram%** | **Kcal%** |
| **Protein** | 23.7 | 20 |
| **Carbohydrate** | 41.4 | 35 |
| **Fat** | 23.6 | 45 |
| **Total** |  | 100 |
| **Kcal / g** | 4.73 |  |
|  |  |  |
| **Ingredient** |  |  |
| Casein, 80 Mesh | 200 | 800 |
| L-Cystine | 3 | 12 |
| Corn Starch | 50 | 200 |
| Maltodextrin 10 | 45.6 | 182 |
| Sucrose | 250 | 1000 |
| Cellulose, BW200 | 50 | 0 |
| Soybean Oil | 25 | 225 |
| Lard | 177.5 | 1598 |
| Mineral Mix S10026 | 10 | 0 |
| DiCalcium Phosphate | 13 | 0 |
| Calcium Carbonate | 5.5 | 0 |
| Potassium Citrate. 1 H20 | 16.5 | 0 |
| Vitamin Mix V10001 | 10 | 40 |
| Choline Bitartrate | 2 | 0 |
| FD&C Blue Dye #1 | 0.05 | 0 |
| **Total** | **858.15** | **4057** |

**Table 2 Mitochondrial respiration media**

|  |  |
| --- | --- |
| **Chemical** | **Final concentration (mmol/L)** |
| EGTA | 0.5 |
| MgCl2 | 3 |
| K-Lactobionate | 60 |
| Taurine | 20 |
| KH2PO4 | 10 |
| Sucrose | 110 |
| Bovine serum albumin | 1 mg/mL |
| HEPES | 20 (pH 7.0 at 37 °C) |

**Table 3 Mitochondrial respiration assay protocol**

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagents added** | **Final concentration in oxygraph chamber (mmol/L)** | **Action of reagent** | **Measurement output** |
| Step 1   * Glutamate * Malate * Pyruvate | * 10 * 5 * 10 | CI substrates | CI leak respiration  (CILeak) |
| Step 2   * ADP | * 1.25 | Substrate for ATP generation | CI oxidative phosphorylation |
| Step 3   * Succinate | * 10 | CII substrate | CI+CII oxidative phosphorylation1 |
| Step 4   * Rotenone | * 0.001 | CI inhibitor | Isolate flux to CII  [CII(rot)] |
| Step 5   * Oligomycin | * 0.0025 | ATP-Synthase inhibitor | CI+CII leak respiration  (CI,IILeak) |
| Step 6   * FCCP | * 0.0015 | Mitochondrial uncoupler | ETS capacity |
| Step 7   * Antimycin A | * 0.005 | CIII inhibitor | Residual oxidase consumption |

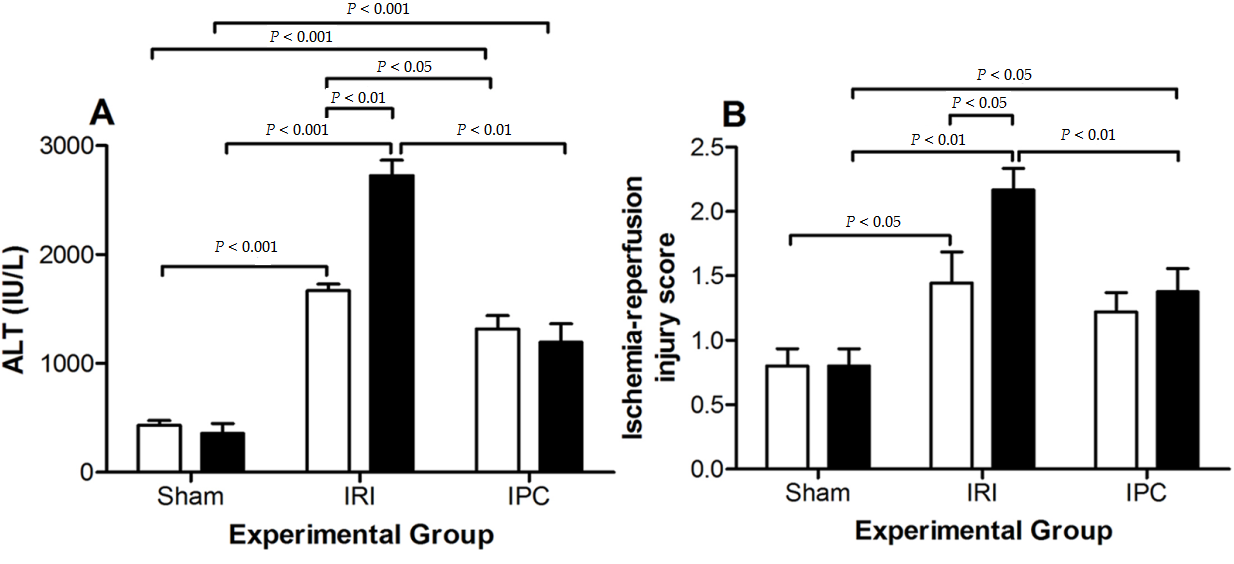
1The individual contribution of CII to oxidative phosphorylation (CII-OXPHOS) can also be derived (CI+II-OXPHOS minus CI-OXPHOS). ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; CI: Complex I; CI-OXPHOS: Complex I oxidative phosphorylation; CI+II-OXPHOS: Complex I + Complex II oxidative phosphorylation; CI,IILeak: Complex I + Complex II leak respiration; CILeak: Complex I leak respiration; CII: Complex II;CIII: Complex III; ETS: Electron transfer system; FCCP: Carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone; Rot: Rotenone.

**C:\Users\Administrator\Desktop\24455\24455\24455-Figures\24455-Figure 1.tif**

*P* < 0.0001

*P* < 0.0001

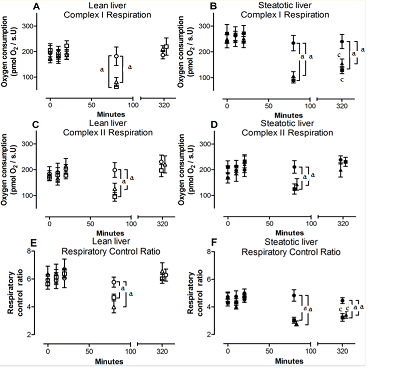
**Figure 1 Bodyweight, random blood glucose and baseline histology of high-fat/high-sucrose-fed (obese) and lean Sprague-Dawley rats**.Obese Sprague-Dawley rats were significantly heavier (A) with higher random blood glucose (B) than age-matched lean rats.Baseline liver tissue sections were stained for haemotoxylin and eosin (x 40 magnification).Representative slides are displayed and obese rats (D) showed severe mixed hepatic steatosis while lean rat livers (C) showed mild microvesicular steatosis.Data are shown as mean ± SE (*n =* 30 rat/group).Statistical analyses were performed using Students t-tests for body weight and random blood glucose.



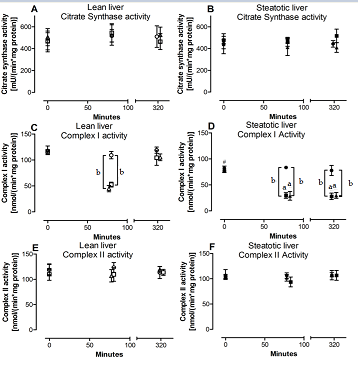
**Figure 2 Serum alanine aminotransferase levels and histology injury score following ischemia-reperfusion.**Serum alanine aminotransferase (ALT) (A) and histology injury score (B) following reperfusion were significantly higher in rats subjected to IRI compared to sham rats.Both injury markers were significantly higher in obese rats compared to lean rats.IPC decreased ALT levels in both lean and obese rats; and decreased injury score in obese rats compared to the corresponding IRI groups.Data are shown as mean ± SEM (*n =* 10 rat/group; lean rats, open bar; obese rats, closed bar).IRI: Ischemia-reperfusion injury; IPC: Ischemic preconditioning.

C:\Users\Administrator\Desktop\24455\24455\24455-Figures\24455-Figure 3.tif

**Figure 3 Liver histology following ischemia-reperfusion**.No evidence of injury was observed in Lean-Sham (A) and Steatotic-Sham (B) livers.Lean-IRI livers (C) had mild injury while Steatotic-IRI livers (D) had moderate-severe injury.Both Lean-IPC (E) and Steatotic-IPC livers (F) were observed to have mild injury following reperfusion.Representative slides are shown (*n =* 10 rat/group). IRI: Ischemia-reperfusion injury; IPC: Ischemic preconditioning.



**Figure 4 Mitochondrial function of lean and steatotic livers subjected to sham, ischemia-reperfusion injury with or without ischemic preconditioning.**Baseline MF was similar between lean and steatotic livers in all outcome measures.Lean-Sham and Steatotic-Sham have stable CI-OXPHOS (A, B), CII-OXPHOS (C, D) and RCR (E, F) throughout the procedure.CI-OXPHOS (Fig A, B), CII-OXPHOS (Fig C, D) and RCR (E, F) were significantly lower following 60 min of ischemia in Lean-IRI, Lean-IPC, Steatotic- IRI and Steatotic-IPC livers compared to the corresponding sham group.Following reperfusion, CI-OXPHOS (B) and RCR (F) was significantly lower in Steatotic-IRI and Steatotic-IPC livers compared to Steatotic-Sham or lean livers while CII-OXPHOS (D) returned to pre-ischemic levels comparable to Steatotic-Sham or lean livers (D). Data are shown as mean ± SE (*n =* 10 rat/group; Lean-Sham, open circle; Lean-IRI, open square; Lean-IPC, open triangle; Steatotic-Sham, closed circle; Steatotic-IRI, closed square; Steatotic-IPC, closed triangle).a*P <* 0.05 *vs* Lean-IRI; c*P <* 0.05 *vs* Lean-IPC (end of reperfusion). IRI: Ischemia-reperfusion injury; IPC: Ischemic preconditioning.



**Figure 5Citrate synthase, Complex I and Complex II activity at baseline, following ischemia and post-reperfusion in lean and steatotic livers**.CS activity was similar between lean (A) and steatotic livers (B) throughout the procedure and was not affected by IRI or IPC.Baseline CI enzyme activity in steatotic livers were lower than lean livers (C, D).After ischemia, CI enzyme activity was significantly lower in lean and steatotic livers compared to sham livers.Additionally, CI enzyme activity (D) was lower in steatotic livers post-ischemia compared to lean livers.After reperfusion, CI activity remained lower in Steatotic-IRI and Steatotic-IPC livers (D) compared to Steatotic-Sham or lean livers.CII activity (E, F) remained stable throughout the procedure and was similar between both types of livers.IPC did not have a significant effect on CI or CII activity in both types of livers.Data are expressed as mean ± se (*n =* 10 rat/group; Lean-Sham, open circle; Lean-IRI, open square; Lean-IPC, open triangle; Steatotic-Sham, closed circle; Steatotic-IRI, closed square; Steatotic-IPC, closed triangle). a*P <* 0.05, b*P <* 0.001 *vs* time- and group-matched lean livers. CS: Citrate synthase; CI: Complex I; CII: Complex II; IRI: Ischemia-reperfusion injury; IPC: Ischemic preconditioning.