The effects of physical prehabilitation: Improved liver regeneration and mitochondrial function after ALPPS operation in a rodent model

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Abstract

**Background:** To identify the role of physical prehabilitation (PP) in liver regeneration, mitochondrial function, biogenesis, and inflammatory response was investigated after associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) in a rodent model.

**Methods:** Male Wistar rats (n = 60) underwent ALPPS. Animals were divided (n = 30) to the physical prehabilitation group (PP) and sedentary group (S). The animals were exsanguinated before (0 hour) and 24, 48, 72, or 168 hours after the operation. Regeneration rate and proliferation index were assessed. Mitochondrial function, biogenesis, and inflammatory response were evaluated.

**Results:** Regeneration rate and Ki67 index were significantly increased in the PP group compared to the S group (P < .001). Due to the changes in oxidative capacity and ATP production rate, the P/O ratio of PP group compared to the S group was significantly increased (P < .05). PP group was characterized by accelerated mitochondrial biogenesis and less intense inflammatory response compared to the S group.

**Conclusions:** To our knowledge, this is the first demonstration of the beneficial effects of PP on liver regeneration, mitochondrial function, biogenesis, and the inflammatory response after ALPPS.

**KEYWORDS**
animals, Exercise, hepatectomy, liver regeneration, mitochondria

Abbreviations: PP group, Associating Liver Partition and Portal vein ligation for Staged hepatectomy and physical prehabilitation group; S group, Associating Liver Partition and Portal vein ligation for Staged hepatectomy without physical prehabilitation.

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1 | INTRODUCTION

Over the last few decades, outcomes of liver resections have improved due to the development of surgical technique, critical care, and the expansion of the resectability criteria. As a result of these improvements, at the present time, the only remaining restrictive factor of major hepatectomy is insufficient future liver remnant (FLR) volume, which can cause life-threatening post-hepatectomy liver failure (PHLF). \(^1\) Portal vein embolization (PVE) is currently the gold standard to achieve sufficient FLR volume before curative liver resection. \(^2\) However, this interventional technique also has its own disadvantages, as the long waiting time combined with the possible tumor spread and progression could lead to the failure of the second-stage liver resection. \(^3,4\) In order to overcome these obstacles, a novel procedure, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), has been developed, which could not only reduce the time between the two steps of hepatectomy, but could also significantly increase the regeneration capacity of the remaining liver tissue compared to PVE. \(^5\)

Unfortunately, a high 90-day mortality (12%) was observed following ALPPS, which raised skepticism. \(^5\) Since then, due to better patient selection and the refinement of the surgical technique, morbidity and mortality rates decreased. \(^6,7\) Nevertheless, ALPPS still carries significant risks of mortality and morbidity. Although the reasons behind this phenomenon are still convoluted, it has been shown that accelerated regeneration might form immature liver units, which results in a gap between the enhanced liver volume and the actual functioning liver volume. \(^8,9\) Based on our previous findings, one explanation for the lagging functional recovery of the liver after ALPPS could be the impaired mitochondrial biogenesis. As we investigated the above-mentioned mitochondrial changes in ALPPS compared to its concordant control model, portal vein ligation (PVL), we found that both mitochondrial function and biogenesis are increased in the PVL group, meanwhile they remained impaired in the ALPPS group, which—along with the rapid energy-demanding cell proliferation—leads to an energetic disbalance. We demonstrated that an overwhelming and prolonged inflammatory response following ALPPS interfered with the stress-induced, peroxisome proliferator-activated receptor γ co-activator 1-α-coordinated (PGC1-α), mitochondrial biogenesis pathway. In the early phase of liver regeneration—called bioenergetic stabilization—this resulted in the accumulation of immature and malfunctioning mitochondria in hepatocytes. \(^10\)

Therefore, as the impaired mitochondrial function following surgery seems to be a trait of ALPPS, in our present study we aimed to improve the mitochondrial biogenesis and function to achieve lower mortality and better liver regeneration rates following ALPPS. A novel method to enhance mitochondrial biogenesis during liver regeneration could be physical prehabilitation (PP). Previous studies in myocardiocytes have shown evidence of exercise-induced mitochondrial biogenesis. \(^11\) In these studies, PP increased not only mitochondrial biogenesis but mitochondrial function as well through different signal pathways. \(^12,13\) Naturally, as a result of PP, the general condition of the patient also improved, as better respiratory and circulatory functions are associated with physical exercise, which greatly help in the overall recovery. \(^14\) However, our theory is that PP also has an effect on the mitochondrial biogenesis and function of hepatocytes, which is supported by Fletcher et al \(^15\) who documented improved mitochondrial respiration in rodents due to different kinds of training modalities. Thus, to identify the role of PP in liver regeneration, here we investigated for the first time how it affects mitochondrial biogenesis and function in hepatocytes after ALPPS operation in a rodent model.

2 | METHODS

2.1 | Animals

All experiments were performed in accordance with the principles of the Guide for the Care and Use of Laboratory Animals guidelines (eighth edition, NIH Publication, 2011), and the protocol was approved by the Scientific and Ethical Board of Animal Experimentation of the National Department of Food-chain Safety (approval number: PE/EA/830-7/2017). The experiments were reported according to the ARRIVE criteria. \(^16\) Male Wistar rats (n = 60), aged 6 weeks, weighing 140 g (Central Animal Facility, Semmelweis University, Budapest, Hungary) were used in the experiment. The animals were kept in a temperature (20-22°C) and humidity (40%-70%) controlled environment with a 12-hour day-night cycle and free access to food and water (for animal allocation, Table S1).

2.2 | Physical prehabilitation protocol

Thirty rats were assigned to the physical prehabilitation group (PP). The rats in the PP group underwent 6 weeks of exercise (five times/week) for one hour on a treadmill. During a preliminary study, we analyzed the rats’ running capacity without the signs of fatigue for 60 minutes from which the maximum tolerable running speed was determined (20 m/min). From the first week, the rats were accustomed to running, starting from 8 m/min, afterwards the running speed was increased by 0.23 m/min each day until it reached the 80% of the maximum tolerable running speed
(16 m/min) by the sixth week. Animals of the sedentary group (S) \( n = 30 \) were placed in the animal housing department for the same amount of time and did not receive physical preconditioning.

### 2.3 Operative procedure

ALPPS operations were performed as described previously by our group \( ^{10} \). Briefly, after the ligation of the portal branches leading to the right lateral, left part of the median, left lateral, and caudate lobes, transection is performed between the left and right part of the median lobe, alongside the transition line. Electrocautery is carefully utilized to seal the liver wounds. Antibiotic treatment \( (10 \text{ mg}/\text{body weight kilograms} \ [\text{bwkg}] \text{ metronidazole intraperitoneally}) \) and analgesia \( (1 \text{ mg}/\text{bwkg} \text{ nalbuphine subcutaneously, repeated once 24 hours later}) \) were used postoperatively.

### 2.4 Sacrifice and sample extraction

The animals were sacrificed without operations \( \text{(postoperative day 0} \ [\text{POD 0}]) \) and at POD 1, 2, 3, and 7 of ALPPS by cardiopuncture after intraperitoneal \( \text{(i.p.) injection of 75 mg/bwkg ketamine and 7.5 mg/bwkg xylazine in 1.5 mL saline.} \)

The livers of the rats were extracted in toto. Approximately 150 mg of tissue of the right median lobe was snap frozen in liquid nitrogen and another 150 mg was fixed in 4% buffered formaldehyde for histology. The remainder of the right median lobe was used for mitochondrial functional tests. Samples were homogenized in a homogenization buffer and centrifuged two times for 10 min. After washing three times, the pellet was resuspended in 200 \( \mu \text{L} \) homogenization medium.

### 2.5 Regeneration rate calculation and histological assessments

The liver lobes were measured separately using an analytical scale \( \text{(AG245, Mettler Toledo®, Switzerland). Regeneration ratio was defined by the following formula: \((\text{lobe weight/bwkg at time of death}) / (\text{mean lobe weight/bwkg at 0 hour}) \times 100 \% \).} \)

Four micrometer thick slides were made from formalin-fixed, paraffin-embedded tissue specimens. Ki-67 immunohistochemistry was performed using Abcam Anti-Ki-67 antibody \( \text{(Abcam, UK). The histological slides were scanned by a Pannoramic P1000 slide scanner system (3DHistech, Hungary). The captured images were evaluated using the QuPath software.} \)

Ki67 index was calculated by the following formula: number of marked cells/total number of cells of the whole slide \( \% \).

### 2.6 Measurement of the mitochondrial function

#### 2.6.1 Mitochondrial oxygen consumption

As previously described \( ^{10} \), \( \text{O}_2 \) consumption levels of the NADH-dehydrogenase \( \text{C I} \) and succinate dehydrogenase \( \text{CII} \) were measured by an Oxygraph-2K high-resolution respirometry system \( \text{(Oroboros Instruments, Innsbruck, Austria). Sensor calibration was performed at air saturation and in anoxic medium. Basal (mitochondria and respiratory substrate) and the stimulated (mitochondria + respiratory substrate + ADP) \text{O}_2 \) consumption levels were measured. Glutamate plus malate was used to stimulate complex I and succinate to stimulate complex II function.}

#### 2.6.2 Measurement of the mitochondrial ATP production and P/O ratio calculation

The mitochondrial ATP output levels were measured as described previously \( ^{10} \). The measurement was based on coupled glucokinase reactions, in which NADP + reduces to spectrophotometrically detectable NADPH as an end product while the produced ATP is used up. Absorbance levels were measured at 340 nm by a Jasco V650 UV/VIS double-beam spectrophotometer \( \text{(ABL&E Jasco). Similarly to the \text{O}_2 \) consumption measurements, basal and induced ATP production was assessed. Phosphate/oxygen (P/O) ratio was calculated using the following formula: \( \text{induced ATP production / (oxygen consumption} \times 2 \).}

### 2.7 Western blots

Total liver tissue isolates were made from 35mg of tissue using a Bead Beater homogenizer \( \text{(Next Advance). Twenty-five} \mu \text{g of protein were electrophoresed on 8%-15\%v/v polyacrylamide SDS-PAGE gels. Proteins were blotted onto polyvinylidene-difluoride (PVDF) membranes. Blocking was performed in a 3% skimmed milk solution, then were incubated overnight at 4\(^{\circ}\)C with primary antibodies (for antibody list Table S2). Bound primary antibodies were detected with HRP-conjugated secondary antibodies \( \text{(Advansa, UK). For visualization, membranes were treated with chemiluminescent substrate (Clarity, Bio-Rad), and protein bands were detected by a Genie Imager. The bands were quantified by ImageJ software and normalized to the} \)
total protein load. We have investigated the levels of mitochondrial proteins NRF-1 (nuclear respiratory factor 1), NRF-2 (nuclear respiratory factor 2), PGC1-α (Peroxisome proliferator-activated receptor gamma co-activator 1-α), Cytochrome-c, and OXPHOS (Oxidative Phosphorylation System). Inflammatory factors such as NF-κB P65 (nuclear factor kappa-light-chain-enhancer of activated B cells P65), IL-1B (interleukin 1-beta), and IL-1RA (interleukin-1-receptor-antagonist) were measured to analyze the inflammatory response.

2.8 | Statistical analysis

Statistical analysis of the collected data was performed with the R software (4.0.2) environment, and the results were visualized with ggpubR (0.4.0) using JupiterLab. Homogeneity tests were performed with Shapiro tests. Analysis of variance was calculated by two-way ANOVA, differences between individual measurement points were calculated by pairwise t-tests with Bonferroni’s p-adjustment (Bonferroni's post hoc). Differences were deemed significant if \( P < .05 \), \( n = 5-6 \) at each measured time point/group.

A power calculation was performed for parameters, based on our previous study (ATP production of complex I at the POD 2). In order to determine an important 20% difference between the arms using a two-sided, two-sample equal-variance t-test at a 5% level of significance with 80% power, 4.3 animals per arm were required. Assuming a dropout rate of 20%, we aimed to enroll six animals per group.

3 | RESULTS

3.1 | PP has a beneficial effect on cell proliferation and liver regeneration

The regeneration rate of the S group increased significantly by POD 7 compared to POD 0 value that resulted in a two times growth (234.24% ± 22.22 y%). Compared to the S group, from POD 2, the regeneration rate of the PP group significantly increased. The volume of regenerated liver in the PP group reached four times its initial value by the end of the experiment (449.47% ± 0.86%) (Figure 1A). Supporting this, Ki-67 proliferation rate was significantly higher in the S group reaching its highest value at POD 2 and decreasing to its initial value by POD 7. In the PP group, the cell proliferation showed similar tendency, however, by POD 3, the number of proliferating cells in the PP group was significantly higher compared to the S group (Figure 1B-C).

![FIGURE 1 Changes in regeneration rate and proliferation. Regeneration rate (A) and Ki-67 index (B and C) at the time point before surgery (POD 0), and at POD 1, 2, 3, and 7 after associating liver partition and portal vein ligation for staged hepatectomy (S) vs associating liver partition and portal vein ligation for staged hepatectomy plus prehabilitation (PP) (n = 6). *\( P < .050 \), **\( P < .010 \), ***\( P < .001 \) vs S; ###\( P < .001 \) vs corresponding controls (POD 0); (Statistics: two-way ANOVA, with Bonferroni’s post hoc test).]
3.2 PP enhances ATP production rate, effectiveness of oxygen consumption, and P/O ratio

In the S group, the basal oxygen consumption of complex I and II increased temporarily, reaching its highest value on POD 1-2 and decreasing to its initial value by POD 7. Contrary to the S group, in the PP group, a different tendency could be observed. The basal oxygen consumption of complex I and II reached its highest value later, by POD 2-3, which resulted in a significant difference between the two groups (Figure 2A-B).

The induced oxygen consumption of complex I and II increased significantly in the S group compared to their initial value from POD 1 to POD 2, while there was no significant difference in the PP group compared to POD 0, nor between the two groups (Figure 2C-D).

The basal ATP production rate of complex I and II in the S group did not show significant change during the experiment. In contrary, we found significantly higher basal ATP production rate in the PP group after POD 1 compared to its initial value. The basal ATP production of PP animals was increased throughout the whole experiment compared to the S group (Figure 3A-B). Regarding induced ATP production, a decreasing tendency could be observed in the S group with the lowest point at POD 2-3, while induced ATP production remained around its initial value in the PP group throughout the experiment, therefore it was significantly higher than in the S group at almost every time point (Figure 3C-D).

In order to assess oxygen and substrate utilization, P/O ratio (Phosphate/Oxygen ratio) was measured. In the S group, both complex I and II P/O ratios showed a significant decrease in the first 3 postoperative days. Meanwhile, the P/O ratio did not change considerably in the PP group compared to its initial value. Thus, the P/O ratio of the PP group compared to the S group was significantly increased on POD 2 (Figure 3E-F).

3.3 PP improves mitochondrial biogenesis in the regenerating liver

Liver tissue lysate PGC1-α levels did not show significant elevations in the S group compared to their initial value, while in the PP group, a significant increase could be observed from POD 1 to POD 2 compared to the POD 0 value. Therefore, there was also a significant increase in PGC1-α levels in the PP group compared to the S group (Figure 4A).

NRF-1 and NRF-2 levels were temporarily increased in the S group, the peak could be measured at POD 2-3. The PP group followed a similar tendency, however, from POD 1 to POD 2, the levels of NRF-1 and NRF-2 were exceedingly higher in the PP group compared to the S group, presenting significantly increased values (Figure 4B-C).

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**FIGURE 2** Changes in oxygen consumption levels. Basal (A and B) and substrate (complex I: glutamate-malate complex II: succinate) induced (C and D) oxygen consumption at the time point before surgery (POD 0), and at POD 1, 2, 3, and 7 after associating liver partition and portal vein ligation for staged hepatectomy (S) vs associating liver partition and portal vein ligation for staged hepatectomy plus prehabilitation (PP) (n = 6). *P < .050, **P < .010, ***P < .001 vs S; ##P < .010, ###P < .001 vs corresponding controls (POD 0); (Statistics: two-way ANOVA, with Bonferroni’s post hoc test)
3.4 PP improves the transcription of mitochondrial proteins

When compared to POD 0, no significant difference was found in the S group regarding the tissue lysate complex I, II, III, and IV and ATP synthase protein. In contrast, complex I protein expression in the PP group was significantly higher at POD 1 and 2 compared to the S group (Figure 5A). Complex II protein expression was also significantly higher at POD 1, 2, and 3 in the PP group compared to the S group. However, complex II protein showed significant decrease by POD 7 in the PP group compared to POD 0 (Figure 5B). Regarding complex III protein levels, PP was tendentiously higher compared to its initial value, and showed significant elevation on POD 1 compared to the S group (Figure 5C). Complex IV was significantly higher in the PP group at POD 3 compared to POD 0. Moreover, complex IV protein levels in the PP group were significantly elevated at POD 1 and 3 compared to the corresponding S values (Figure 5D). ATP synthase protein concentrations showed significant elevation in the PP group compared to their initial value and were significantly higher than the S values at POD 1 (Figure 5E).
**FARD-AGHAIE ET AL.**

3.5 **PP alters the inflammatory response after ALPPS**

Increased inflammatory response was observed in the S group compared to both its initial values and the PP group. Tissue lysate IL-1B and IL-6 levels were significantly higher at POD 2 and 3 in the S group compared to POD 0 value and the PP cohort (Figure 6A-B). The NF-κB P65 concentrations were significantly elevated at POD 1 in the S group compared to their initial value and the PP group (Figure 6C).

Accordingly, anti-inflammatory IL1-RA levels were tentatively higher in the PP group compared to POD 0 value and presented significantly higher values at POD 1, 2, and 7 compared to the S group (Figure 6D).

4 **DISCUSSION**

In this study, we investigated the effect of PP on liver regeneration, mitochondrial function and biogenesis following ALPPS operation by creating a translational rodent model. With this approach, we found that compared to the S group, both regeneration rate and Ki67 proliferation rate were significantly higher in the PP group, which shows that PP has a beneficial effect on cell proliferation and liver regeneration. We also identified various changes in the mitochondrial biogenesis and function of the regenerating liver due to PP. It has been proven that exercise improves mitochondrial function by enhancing the oxidative capacity and the effectiveness of oxygen consumption and by increasing ATP production rate. Moreover, protein levels that play a role in the mitochondrial biogenesis—PGC1-α, NRF-1, and NRF-2—were significantly elevated as well as the transcription of mitochondrial proteins (ATP synthase and complex I-IV), which indicates that PP improves mitochondrial biogenesis in the regenerating liver lobe. Furthermore, increased inflammatory response was observed in the S group compared to the PP group, suggesting PP has a beneficial effect on overwhelming postoperative inflammatory reactions, which may have a positive impact on stress-regulated mitochondrial biogenesis.

While assessing the regeneration of the liver following ALPPS, we found that the regeneration rate of the PP group significantly surpassed the S group with a liver volume increase of more than 400%. This level of growth has not been previously described in literature in small animal models (40%-318%).10,19–21 In addition, by histological assessment, we observed that Ki67 index increased significantly in the PP group at POD 3, which corroborates with our findings that PP has a significantly beneficial effect on liver regeneration and cell proliferation.

To reveal the underlying mechanism of this accelerated regeneration, we assessed the mitochondrial function. First,
oxygen consumption values were measured from isolated liver mitochondria via high-resolution respirometry. The basal oxygen consumption of the mitochondria showed an interesting phenomenon, as the oxygen consumption in the S group reached its highest value at POD 1-2, while the PP group more protractedly reached the peak-point at POD 2-3 that eventuated in a significant difference between the two groups. Next, we measured the ATP production of the isolated liver mitochondria. While in the S group, basal ATP production rate of complex I and II did not show significant change during the experiment, in the PP group, a higher basal ATP production engendered after POD 1 and was increased throughout the whole experiment. In induced ATP production, the PP group outperformed the S group, producing significantly higher values at every time point. These changes in oxygen consumption and ATP production ministered to a significant difference in the P/O ratio between S and PP group, which showed that both complex I and II had more effective energy production in the PP group, as the ATP production was higher with a lower oxygen consumption. The finding of the P/O ratio is supported by Fletcher et al, as their investigation showed evidence of improved mitochondrial respiration in rodents due to different kinds of training modalities.15

FIGURE 5 Changes in the expression of the oxidative phosphorylation chain proteins. Complex I (A), complex II (B), complex III (C), complex IV (D), and adenosine 5′-triphosphate synthase (E) total cell lysate protein concentrations at the time point before surgery (POD 0), and at POD 1, 2, 3, and 7 after associating liver partition and portal vein ligation for staged hepatectomy (S) vs associating liver partition and portal vein ligation for staged hepatectomy plus prehabilitation (PP) (n = 6). *P < .050, **P < .010, ***P < .001 vs S; ###P < .010, ####P < .001 vs corresponding controls (POD 0); (Statistics: two-way ANOVA, with Bonferroni’s post hoc test)
the skeletal muscles and in myocardiocytes; however, no such assessments were carried out in the regenerating liver so far.11–13,22

Looking deeper into the molecular mechanism of improved mitochondrial function, favorable effects of PP were discovered, as the protein concentration of the biogenesis mediators showed significant elevation in the PP group compared to the S group during the peak of cell proliferation. A molecular mechanism explaining this phenomenon could be the enhancement of the main stress-inducible mitochondrial biogenesis pathway driven by PGC1-α. This is the key co-activator of transcription factors of mitochondrial maintenance, such as NRF-1 and NRF-2. The NRFs control most of the mitochondrial protein expression (including parts of the OXPHOS chain, or transition pore proteins).23–25 It has been already shown by previous investigations on myocardiocytes that exercise induces mitochondrial biogenesis by enhancing the PGC1-α pathway11–13; however, regarding hepatocytes, there is no evidence in the literature of exercise-induced mitochondrial biogenesis. In our investigation, we found increased protein levels of PGC1-α, NRF-1, and NRF-2 in hepatocytes following PP. In this very case, the protein levels of all three pathway components were significantly higher near the peak of regeneration (POD 2-3). This caused severe, beneficial changes in the process of mitochondrial maintenance: the mitochondrial OXPHOS protein content was significantly elevated in the total tissue lysate as well as in the mitochondrial isolates, which suggests two crucial changes in the biogenetic process. Firstly, the translation of these proteins was elevated in the cytoplasm and therefore, more of these proteins were transported into the mitochondria. Secondly, the highly NRF-1 and NRF-2 controlled mitochondrial protein transport was also positively affected.20 These processes might be the keystone to elevated energy levels, and even to faster, more balanced regeneration.

The effect of PP on postoperative inflammatory reaction could also play an important role in the improved mitochondrial function of the regenerating liver. Although moderate inflammation plays an important role in the initiation of liver regeneration,26 overwhelming and prolonged inflammation may suppress mitochondrial biogenesis and maintenance through interfering with the main stress-inducible mitochondrial biogenesis pathway driven by PGC1-α.10 These studies support our previous finding, where in the ALPPS group, TNF-α and NF-κB p65 protein levels were significantly increased, and in parallel, mitochondrial activity and biogenesis were significantly impaired compared to the PVL group, which suggests that the suppression of
PGC1-α by overwhelming inflammatory mediators might be responsible for the diminished mitochondrial biogenesis following ALPPS. In our present investigation, pro-inflammatory protein levels—IL-1β and IL-6 and NF-κB P65—were significantly higher in the S group compared to the PP group. Additionally, the concentration of the potent anti-inflammatory IL1-RA was also determined, and found to be higher in the PP group, indicating a suppressed inflammatory reaction due to PP, which could be able to suppress the overwhelming and prolonged inflammatory response and consequently improve the impaired mitochondrial biogenesis and function.

Postoperative complications after liver resections could lead to prolonged hospital stay and severe impairment. These could cause a delay of postoperative chemotherapy and have a detrimental effect on the oncological outcome.²⁷,²⁸ It has been shown by previous studies that exercise and personalized prehabilitation could lower the postoperative complication rate significantly.²⁹ However, regarding hepatopancreatico-biliary surgery, only a few articles provide information about prehabilitation,¹⁴,³⁰–³³ which suggest an average of 4-6 weeks of exercise consisting of walking or light physical exercise adjusted to the patient’s initial condition. In case of colorectal surgery, based on the more extensive literature data and our previous findings,²⁹ 4 weeks of preoperative walking-breathing exercise could have a favourable effect on the outcome. Therefore, based on literature data and our findings, we propose that PP should be considered and investigated in the future as a potential enhancing factor of postoperative and oncological outcomes following ALPPS.

We acknowledge the limitations of our study. The physiological differences between rodents and humans might be severe in this animal model. However, mitochondria and mitochondrial genes are typically well-conserved among mammals.³⁴ Therefore, findings of pathway functions can be relevant in humans. Further, elaborated clinical studies are needed for confirmation. Moreover, as the main focus of the study, based on our previous findings, was the improvement of mitochondrial biogenesis and function, it must be mentioned that it did not investigate the postoperative outcomes and clinical aspects of liver function. Therefore, further studies analyzing these parameters are needed prior to clinical investigations.

We demonstrated for the first time in a rodent model that PP has a significantly beneficial effect on liver regeneration after ALPPS. By investigating the mitochondrial function, biogenesis, and the postoperative inflammatory response, we found evidence that PP significantly increases the mitochondrial function and the biogenetical pathways, while also attenuates inflammatory response. Based on the recent findings about the effects of physical exercise, we also propose that PP should be considered and further investigated as a potential improving factor of postoperative outcomes after ALLPS.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest for this article.

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