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Original Article

Endocannabinoid and hematological responses to pre- and post-therapeutic exercises in liver transplant patients

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Abstract: Endocannabinoids (eCBs) play a crucial role in regulating the pathophysiological progression of chronic liver disease through hepatic cannabinoid receptor 2 (CB2). According to the literature, various treatment options are available for liver disease patients, including transplantation and physical activity both before and after the procedure. The aim of this study is to assess the response of endocannabinoids to pre- and post-therapeutic exercises in liver transplant patients (LTx). This analytical case-control longitudinal study was conducted on patients aged 18-70 at King Fahad Specialist Hospital in Dammam, Saudi Arabia. Participants were divided into two groups: an intervention group of LTx patients (n = 26) and a control group of end-stage liver disease patients (n = 23) who were not candidates for liver transplantation (LT). Blood samples were collected before the initiation of preoperative exercises, one month before LT, and three months after LT following postoperative exercises. The median arachidonoyl ethanolamide (AEA) levels in the control group were comparatively higher after therapeutic exercises compared to before; however, the Wilcoxon signed-rank test showed no significant differences (P = 0.212). In the LTx group, the median difference in AEA between pre- and post-therapeutic exercises was marginally significant (P = 0.091). Additionally, the Wilcoxon signed-rank test revealed a highly significant increase in median 2-arachidonoylglycerol (2-AG) levels after therapeutic exercises compared to before in the LTx group (P = 0.049), while the control group showed no significant change in post- vs. pre-therapeutic exercise median 2-AG levels (P = 0.346). The study's findings revealed an increased concentration of 2-AG after therapeutic exercises in LTx patients but not in the control group, while AEA levels were elevated after therapeutic exercises in both groups. The effect of post-therapeutic exercises on hematological and biochemical markers was significant between the control and LTx groups, particularly concerning platelet count, total bilirubin, total protein, albumin/globulin ratio, international normalized ratio, and calcium levels.

Keywords: Endocannabinoid system, endocannabinoids, cannabinoid receptors, pre- and post-therapeutic exercises, liver transplantation

Introduction

The endocannabinoid system (ECS) was first identified in the late 1980s during research on cannabis, which led to the discovery of this complex cell-signaling system in the body [1]. The ECS is an intercellular communication system recognized as a neurotransmission system [2].

The liver plays a vital role in metabolizing and breaking down both endocannabinoids (eCBs) and exogenous cannabinoids, such as those found in cannabis. This metabolic process is essential for clearing cannabinoids from the body, which in turn influences their effects on various physiological processes [3]. The ECS is composed of several key components: enzymes, cannabinoid receptors, and eCBs. Enzy-

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mes play a crucial role in maintaining a delicate balance in the system, preventing excessive signaling while also contributing to the regulation of various physiological processes beyond the endocannabinoid system itself [4].

The second most important components of the ECS are cannabinoid receptors type 1 (CB1) and type 2 (CB2), which belong to the family of 7-transmembrane G-protein-coupled receptors, specifically the Gi/o class [4]. CB1 receptors are widely distributed throughout the brain, particularly in the hippocampus, basal ganglia, neocortex, and brainstem. They are also present, though to a lesser extent, in the lungs, testes, skeletal muscles, liver, pancreas, and adipose tissues. CB1 receptors are involved in regulating immune responses, metabolism, inflammation, and various physiological functions specific to each tissue type. CB2 receptors are predominantly located outside the nervous system, in tissues such as the liver, cardiovascular system, skeletal muscles, gastrointestinal tract, and immune system. CB2 receptors are also expressed in immune cells, including progenitor cells, T and B lymphocytes, and macrophages, as well as in lymph nodes and the thymus. Owing to their more restricted distribution, CB2 receptors are primarily found in immune-related cells and certain peripheral tissues, including the liver and adipose tissue. Therefore, activation of CB2 triggers immunomodulatory effects [5].

The most studied eCBs are arachidonoyl ethanolamide (anandamide) (AEA) and 2-arachidonoylglycerol (2-AG) [4]. The metabolite 2-AG is derived from diacylglycerol and functions as a full agonist with moderate affinity for cannabinoid receptors. It is also hydrolyzed by monoacylglycerol lipase [5]. AEA acts as a partial agonist of cannabinoid receptors and is produced from N-arachidonoyl phosphatidylethanolamine by the enzyme N-acyl-phosphatidylethanolamine phospholipase D-like esterase [6]. Several studies have shown that exercise increases levels of eCBs, including anandamide, which acts as a vasodilator and enhances blood flow during physical activity [7, 8]. Furthermore, both eCBs and external cannabinoids have bronchodilatory properties, indicating that the ECS may play a role in enhancing respiratory function during exercise [8]. Elevated levels of eCBs in the bloodstream have been commonly associated with aerobic exercise [9]. Researchers reviewed studies examining the effects of exercise on endocannabinoid levels and their impact on various physiological and psychological factors. Evidence shows that moderate exercise intensity increases blood levels of AEA and 2-AG, leading to reduced anxiety, decreased pain, and improved well-being. Regular or daily exercise helps maintain ECS balance and manage stress levels [10].

eCBs play a crucial role in regulating the pathophysiological mechanisms, particularly in the progression of chronic liver disease, through their action on hepatic CB2 receptors. Therefore, CB2-selective agonists may offer therapeutic benefits for liver disease treatment [3]. CB2 receptors are primarily found on immune cells in the liver, where they play a role in modulating innate immunity and may influence the progression of chronic liver disease [11].

There is a growing focus on the benefits of engaging in physical activity both before and after the liver transplantation (LT) [12]. Williams et al. (2018) noted that there was a limited body of research on the impact of exercise on patient outcomes before and after LT, particularly concerning its potential benefits for skeletal muscle function. Liver disease is a leading cause of premature death in the United Kingdom and is associated with skeletal muscle atrophy, functional impairment, and an increased mortality risk for patients on LT waiting lists. The waiting period for LT presents a unique opportunity to assess and implement interventions such as rehabilitation [13]. Another study showed a significant improvement in quality of life after LT. The objective was to enhance physical activity levels, improve documentation of daily activities, and positively influence the quality of life among liver transplant patients (LTx) during the postoperative period. Promoting physical activity has been demonstrated to positively impact the quality of life in liver transplant recipients [14]. Our study aimed to evaluate the impact of pre- and post-therapeutic exercises on eCBs, specifically AEA and 2-AG, in LT candidates by examining the potential correlation between changes in the ECS and exercise.

Subjects and methods

This analytical case-control longitudinal study was performed at King Fahad Specialist Hos-

pital in Dammam (KFSH-D), Saudi Arabia. The study was performed after receiving approval from the institutional review board (IRB) (No.: PTD0002). Patients provided written informed consent and were given detailed information about the procedures, as well as the potential positive and negative aspects of the research. The study period lasted for two years from the beginning of the Ethics Committee approval on November 1, 2021.

A convenience sample was used to divide 49 adult patients into two groups: 26 underwent LT, while the remaining 23 served as the control group. Participants of both genders ranged in age from 18 to 70 years. At KFSH-D, the groups were categorized based on the stage of liver disease: LTx or end-stage liver disease patients (ESLD). The study excluded individuals with severe medical conditions that could pose risks during exercise, such as advanced cardiovascular or respiratory disease, uncontrolled arrhythmias, acute pulmonary embolism, uncontrolled bleeding disorders, or active infections. Additionally, participants with considerable cognitive impairments or physical limitations that would hinder their ability to understand, follow the exercise program, or engage in physical activities were excluded from the study. The evaluation was based on data collected before the implementation of preoperative exercises, which were administered one month before LT [15] and three months after LT after applying postoperative exercises [16].

Pre- and postoperative exercises

A therapeutic exercise program was administered to the participants in three phases, starting with the first phase and progressing through to the third, in accordance with the Swiss Program design for physical activity before and after LT [12].

Phase 1: preoperative exercises

Before the operation, participants received physical therapy exercises for one month. At our center, preoperative education and exercise were integrated into the pre-transplant care for both groups, who were also provided with regular follow-up and supervised physical therapy sessions [17].

The exercises performed three times a week included: (1) stretching exercises to enhance range of motion and flexibility in muscles and joints; (2) strengthening exercises to increase muscular strength, aiding in faster recovery from surgery; and (3) cardiovascular activity (walking) to enhance cardiovascular fitness and overall health, which is essential for preoperative preparation. Participants were instructed on how to incorporate walking into a home-based exercise program (HBEP) tailored to graded activity concepts and their specific rehabilitation needs. To ensure ongoing adherence and to arrange follow-up sessions, participants received weekly follow-up calls.

Phase 2: early postoperative exercises

For two weeks, daily therapeutic exercises were performed in the intensive care unit (ICU) and then in the transplant unit [12, 16]. Pulmonary physical therapy was the first treatment approach, using coughing and forced expiratory techniques [16]. An incentive spirometer was used to encourage deep breathing. The patient breathed as deeply as possible through a tube connected to a plastic chamber held in the hand, causing three balls in the chamber to rise with each breath. This exercise was performed five to ten times in a row per hour while the patient was awake. The next step involved gradually introducing mobilization and stretching activities for the patient's limbs. The patient started with limb exercises while lying supine, progressed to sitting, then standing and eventually walking exercises. It is crucial to ensure that each exercise is carefully monitored.

Phase 3: late postoperative exercises

Exercises were performed three times a week for the first three months after surgery [12, 16]. The program included (1) stretching exercises, (2) strengthening exercises, and (3) cardiovascular activity (walking). Enhancing cardiovascular fitness and overall health is essential for preoperative preparation. Participants were instructed on incorporating walking into an HBEP, with attention to graded activity concepts and their individual rehabilitation needs. To support continued walking and arrange follow-up sessions, participants received weekly follow-up calls.

Data collection

Data were collected one month before LT and three months after LT for the experimental group, both before the initiation of preoperative exercises and after the postoperative exercises. For the control group, data were collected both before the start of therapeutic exercises and four months after the completion of these exercises. All patients with chronic liver diseases who were considered candidates for LT underwent clinical examinations, including measurements of height (Ht) and body weight (BW) to calculate body mass index (BMI).

Routine liver function tests (LFTs) included measurements of total bilirubin, albumin, total protein, albumin/globulin (A/G) ratio, and international normalized ratio (INR). Additionally, fasting blood glucose, HbA1c, calcium, phosphorus, alkaline phosphatase, magnesium, sodium, potassium, blood urea nitrogen (BUN), creatinine, lipid panel, hemoglobin (Hgb), and platelets (PLTs) were routinely collected from patients.

Targeted metabolomics analysis of AEA and 2-AG

Materials and methods

Chemicals and solutions: AEA, 2-AG standards, anandamide-D4-methanol (AEA-d4), and 2-arachidonoyl glycerol-d11 (2-AG-d11) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile and formic acid were obtained from Honeywell (Morristown, USA), while hexane and ethyl acetate were obtained from VWR (Radnor, USA).

Preparation of standards: To prepare the working solutions, all standards were diluted and aliquoted in acetonitrile, then stored at -20°C at a concentration of 10 µg/mL. The internal standard working solution was also prepared in acetonitrile, with final concentrations of 150 ng/mL for AEA-d4 and 1 µg/mL for 2-AG-d11.

Preparation of samples

Blood samples were collected in EDTA tubes and promptly placed on ice. They were centrifuged within 30-60 min of collection. Plasma was then separated by centrifugation, and the supernatant was transferred directly and stored at -80°C in our KFSH-D laboratory. The samples were subsequently shipped to the King Abdullah International Medical Research Center in Riyadh (KAIMRC) and stored at -80°C until analysis. Plasma samples were prepared for analysis according to a previously published protocol [18]. Briefly, 0.5 mL of plasma was mixed with deuterated internal standards. An equal volume (1:1 v/v) of ethyl acetate and cyclohexane was then added, and the mixture was vortexed for 1 min to ensure thorough mixing. The sample was centrifuged at 14,500 rpm for 5 min to achieve phase separation. The supernatant was transferred to a new Eppendorf tube and evaporated for 60 min using a SpeedVac (Eppendorf, UK). Once completely dry, the metabolites were reconstituted in 30 µL of acetonitrile and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

LC-MS/MS analysis

The LC-MS/MS analysis was performed using an AB Sciex QTRAP 5500 hybrid linear ion-trap quadrupole mass spectrometer coupled with an Agilent 1260 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) via an Applied Biosystems Turbo Ion Spray source. A total of 3 µL of the sample extract was injected into a ZORBAX ODS column (3.0 × 150 mm, 5 µm, Agilent). The LC mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), with a gradient of 55%-90% solvent B over 10 min. The injection volume was 10 µL, and the flow rate was set at 550 µL/min. The MS scan mode was set to reaction monitoring. The MS source parameters were configured as follows: Gas source 1 = 60, Gas source 2 = 40, Curtain gas = 20, N_2 collision gas = Medium, Temperature = 550°C, and ion spray voltage = 4500 V. The transitions and collision energy values for the metabolites used in this study were based on a previously published method [19]. The analytical run included eight calibration samples and plasma samples [20]. Strong linearity in plasma was revealed by the R2 values, which showed 2-AG at 0.9994 and AEA at 0.9999.

Data analysis

Statistical analysis was performed using GraphPad Prism 10.2.2.0 (GraphPad Software, San Diego, CA, USA). The Shapiro-Wilk test was

Table 1. Comparison of demographic characteristics at baseline and after therapeutic exercises, as well as between the control and liver transplant groups

Demographic characteristics	Control (n = 23)		Sig.[a]	Liver Transplant (n = 26)		Sig.[a]	Sig.[b]	Sig.[b]
	Pre-Exs	Post-Exs		Pre-Exs	Post-Exs		Pre-Exs	Post-Exs
Age (years)	49.4 ± 10.9	--	--	52.0 ± 12.7	--	--	0.449	--
Weight (kg)	77.3 ± 16.7	75.7 ± 16.4	0.128	74.1 ± 14.0	68.4 ± 12.7*	0.001	0.471	0.086
Height (m)	1.62 ± 0.11	1.61 ± 0.10	0.069	1.65 ± 0.09	1.65 ± 0.09	0.664	0.265	0.156
BMI (kg/m^2)	29.5 ± 5.9	29.1 ± 6.0	0.372	27.1 ± 4.5	25.0 ± 4.5*	0.001	0.112	0.009

*Indicates a significant difference in mean at the 5% level of significance. Exs: Therapeutic exercises. [a]Sig: Significance value for the mean difference between pre-exercise and post-exercise in both the control and liver transplant groups. [b]Sig: Significance value for the mean difference between the control group and the liver transplant group.

used to assess the normality of the data distribution, which indicated a non-normal distribution. Paired samples were expressed as a median and interquartile range [Med (IQR: Q3-Q1)] and compared using the Wilcoxon signed-rank test. Post-therapeutic exercise data between unpaired samples (control vs. LTx group) were compared using the Mann-Whitney test to determine significant differences between pre- and post-intervention datasets. Correlation coefficients were calculated using Spearman's rank correlation. A *p*-value of ≤ 0.05 was considered statistically significant. Descriptive statistics were used to summarize demographic characteristics, baseline eCB levels, and physical fitness measures. Statistical data were entered and analyzed using the Statistical Package for Social Sciences (SPSS-29.0) (IBM, USA). The frequency and percentage of categorical variables were reported. Continuous variables, including Ht, BW, age, BMI, and hematological and biochemical markers, were presented as mean ± standard deviation (SD). The data distribution was assessed for normality using the Kolmogorov-Smirnov test. Most variables followed a normal distribution. Comparisons of baseline demographics, hematological and biochemical markers, and pre- vs. post-therapeutic exercise in the control and LTx groups were performed using a paired samples t-test. The impact of post-therapeutic exercises between the control and LTx groups was examined using an unpaired t-test. Statistical significance was defined as a *p*-value of ≤ 0.05.

Results

A total of 49 patients participated in the study to evaluate the response of eCBs to pre- and post-therapeutic exercises after LT workup. Of these, 26 (53.1%) underwent LT, while 23 (46.9%) remained on the waiting list for LT as the control group. Demographic data, including age, weight, height, and BMI, showed no significant differences between the control and LTx groups at baseline. However, a significant difference in BMI was noted after therapeutic exercises (**Table 1**). The control group did not show significant changes in weight or BMI after therapeutic exercises. In contrast, the LTx group experienced significant weight loss and changes in BMI after therapeutic exercises compared to baseline data (**Table 1**).

The LC-MS/MS extracted ion chromatograms (XIC) demonstrated effective elution and separation of both AEA and 2-AG, along with their internal standards, using various transitions. The 2-AG eluted as two distinct peaks, 2-AG and 1-AG, which were separated at baseline as expected. For quantification purposes, the combined regions of both peaks were analyzed, as shown in **Figure 1**. In the control group, the median AEA levels pre- and post-therapeutic exercise were 0.9 (IQR = 2.1-0.7) and 1.4 (IQR = 2.3-0.8), respectively. However, the Wilcoxon signed-rank test indicated a non-significant difference (P = 0.2226). In contrast, the median difference in AEA levels between pre- and post-therapeutic exercise in the LTx group was marginally significant (P = 0.091), with pre-therapeutic levels at 0.8 (IQR = 1.5-0.5) and post-therapeutic levels at 1.1 (IQR = 1.6-0.9) (**Table 2**), as illustrated in **Figure 2**.

The Wilcoxon signed-rank test revealed a highly significant increase in median 2-AG levels post-therapeutic exercise compared to pre-therapeutic exercise in the LTx group (P = 0.049), with values of 71.3 (IQR = 139.7-51.8) vs. 59.2 (IQR = 103.7-34.9), respectively. In contrast,

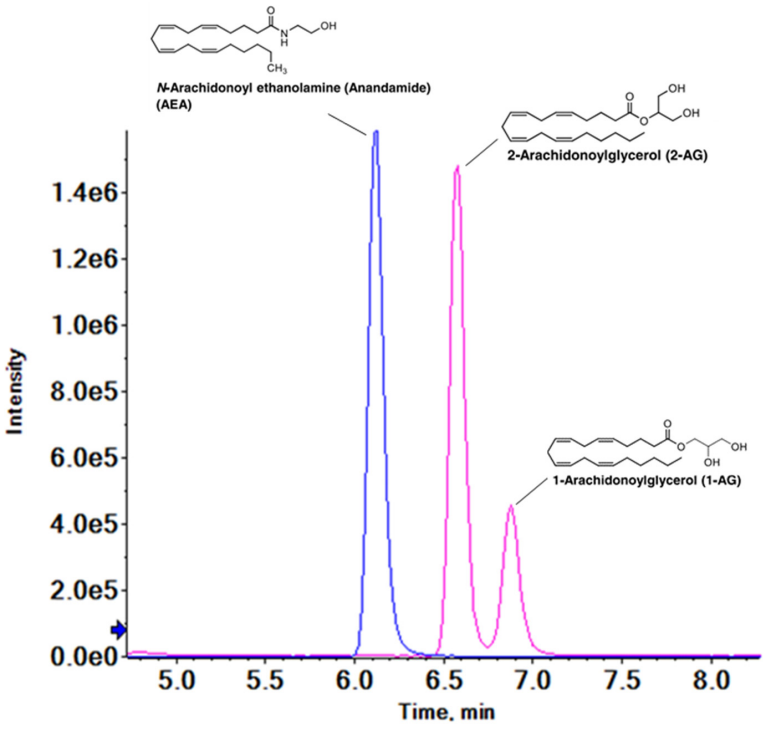

Figure 1. LC-MS/MS-extracted ion chromatograms (XIC) of AEA and 2-AG. The graph shows that AEA elutes with a single peak (blue) at its retention time, while 2-AG elutes with two peaks, corresponding to 2-AG and 1-AG, respectively.

the difference in median 2-AG levels pre- and post-therapeutic exercise in the control group was not significant (P = 0.346), with values of 40.4 (IQR = 71.5-30.0) vs. 57.3 (IQR = 141.2-26.5), respectively (**Table 2** and **Figure 3**). Additionally, the post-therapeutic exercise median 2-AG in the LTx group was significantly higher compared to the control group (71.3 vs. 40.4, P = 0.015).

However, the difference in post-therapeutic exercise median AEA levels between the LTx and control groups was not significant (1.1 vs. 1.4, P = 0.341), as detailed in **Table 2**. The scatter plot of post-therapeutic exercise 2-AG and AEA parameters in the control group revealed a positive but weak correlation (r = 0.223, P = 0.306), as shown in **Figure 4**. Similar results were observed in the scatter plot for the LTx group (r = 0.231, P = 0.256), also illustrated in **Figure 4**.

In the control group, post-therapeutic exercise did not have a significant effect on hematological and biochemical markers (P > 0.05) (**Table 3**). In contrast, post-therapeutic exercise had a significant impact in the LTx group on several markers: mean platelet count (P = 0.001), total bilirubin (P = 0.001), albumin (P < 0.001), INR (P = 0.006), magnesium (P = 0.016), sodium (P = 0.001), potassium (P = 0.001), BUN (P = 0.033), and creatinine (P = 0.014) (**Table 4**).

The effect of post-therapeutic exercise on hematological and biochemical markers showed significant differences between the control and LTx groups for several measures: mean platelet count (P < 0.001), total bilirubin (P < 0.001), total protein (P = 0.049), A/G ratio (P < 0.001), INR (P < 0.001), and calcium (P = 0.025). In the control group, therapeutic exercise considerably improved some markers toward desired levels, including albumin (P < 0.001), sodium (P < 0.001), potassium (P = 0.013), BUN (P = 0.003), and creatinine (P = 0.004), as shown in **Table 5**.

The effect of pre- vs. post-therapeutic exercises on the lipid profile was non-significant in the control group. However, in the LTx group, there was a significant increase in total cholesterol after therapeutic exercise (P = 0.014). Additionally, HDL and LDL levels showed marginally significant increases in the LTx group (P = 0.054 and P = 0.063, respectively). Post-therapeutic exercise resulted in a significantly greater increase in HDL levels in the LTx group compared to the control group (1.0 ± 0.36 vs. 0.77 ± 0.41, P = 0.041). There was also a marginally significant increase in triglycerides (P = 0.052), as shown in **Table 6**.

Discussion

Our results indicated that therapeutic exercises activated the ECS, aligning with previous research in humans [21-24]. Following LT, AEA levels increased after therapeutic exercises, but no significant differences were observed between pre- and post-exercise levels. In con-

Table 2. Comparison of endocannabinoid markers after therapeutic exercises between the control and liver transplant groups

Groups	Parameters	Pre-Exs	Post-Exs	Sig.
Control (n = 23)	AEA (M ± SD)	1.37 ± 0.95	1.68 ± 1.13	-
	2-AG (M ± SD)	168.20 ± 413.57	94.50 ± 154.59	-
	AEA [Med (IQR)]	0.9 (2.1-0.7)	1.4 (2.3-0.8)	0.212
	2-AG [Med (IQR)]	57.3 (141.2-26.5)	40.4 (71.5-30.0)	0.346
Liver transplant (n = 26)	AEA (M ± SD)	0.99 ± 0.63	1.28 ± 0.58	-
	2-AG (M ± SD)	94.94 ± 105.25	140.22 ± 208.17	-
	AEA [Med (IQR)]	0.8 (1.5-0.5)	1.1 (1.6-0.9)	0.091
	2-AG [Med (IQR)]	59.2 (103.7-34.9)	71.3 (139.7-51.8)*	0.049

2AG: 2-arachidonoylglycerol, AEA: Anandamide, IQR: Interquartile range (Q3-Q1), Exs: Therapeutic exercises. *Indicates a significant difference at the 5% level of significance. Post-therapeutic exercise median 2-AG showed a significant increase in the liver transplant group (P = 0.015), while the median AEA did not show a significant change (P = 0.341).

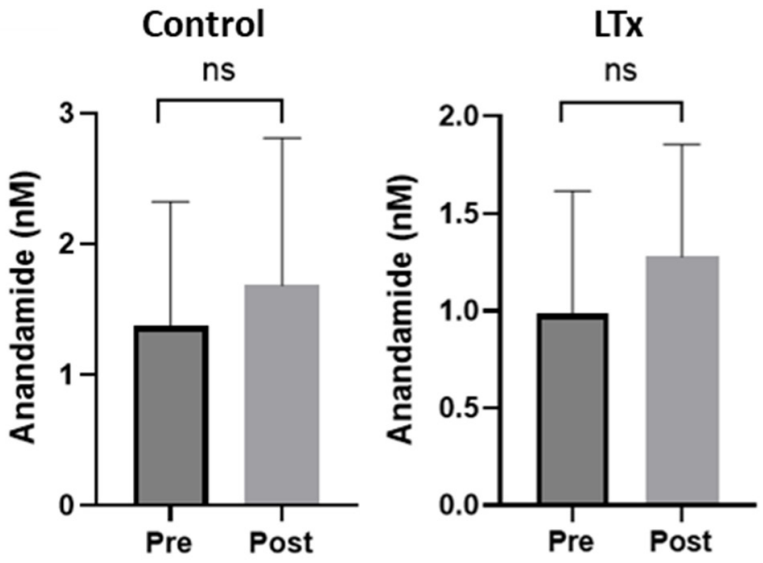

Figure 2. Box plot illustrating the concentration of anandamide (AEA) in the plasma of control and liver transplant groups. The y-axis represents AEA concentration in nM, while the x-axis shows *p*-values of 0.2226 and 0.091 for the pre- and post-therapeutic exercises, respectively. Black color indicates pre-therapeutic exercise values, and gray color represents post-therapeutic exercise values.

trast, 2-AG levels increased after therapeutic exercises, showing significant differences between pre- and post-exercise levels in the LT group. A positive correlation between pre- and post-therapeutic exercise AEA levels was found in both groups. In the control group, AEA levels remained elevated before and after therapeutic exercises and were associated with increased serum bilirubin and INR levels in ESLD. Serum bilirubin and INR levels were linearly related to AEA levels. Furthermore, no correlation was found between circulating 2-AG and any liver function tests, indicating distinct behavior of 2-AG. Before LT, AEA concentrations were elevated owing to AEA's pathological role in liver cirrhosis. Following LT, AEA levels increased after therapeutic exercises. In contrast, 2-AG was unaffected by cirrhosis or liver disorders before LT, maintaining normal concentrations. However, after LT, 2-AG levels increased following therapeutic exercises. The literature on the ECS and liver disease patients is controversial. Hepatic cirrhosis has been associated with hyperactivity of the ECS, and patients with ESLD typically have higher AEA concentrations, which may reflect the severity of liver disorder. Conversely, cirrhosis does not appear to affect 2-AG levels. LTx patients experience significant physiological changes, and therapeutic exercises may provide a proactive approach to enhancing post-transplant recovery and overall well-being.

Farrugia et al. (2023) investigated the effects of therapeutic exercise programs on patients with liver cirrhosis before and after LT. Their findings indicated that these exercises improved quality of life and reduced the 90-day hospital readmission rate, with no adverse effects. Additionally, the program enhanced physical capability and overall well-being.

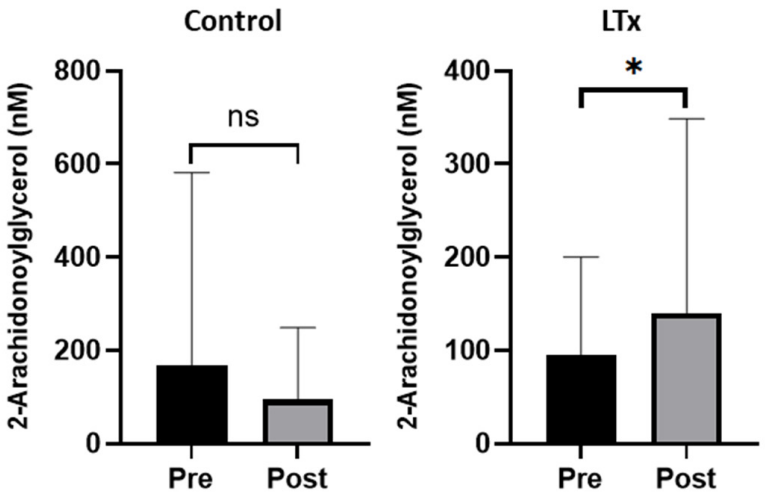

Figure 3. Concentration of 2-AG in the plasma of control and liver transplant groups. The y-axis shows 2-AG concentration in nM, with the x-axis showing *p*-values of 0.346 and 0.049 for pre- and post-therapeutic exercises, respectively. Black color indicates pre-therapeutic exercise values, while gray color represents post-therapeutic exercise values.

Maintaining physical activity before and after LT is essential for preventing frailty in these patients [25].

In our study, post-therapeutic exercises resulted in significant changes in BMI and weight loss compared to baseline in the LTx group. We also observed significant changes in biochemical and hematological markers, suggesting potential improvements in liver function and overall health for these patients. The observed changes in BMI, weight, and various markers after therapeutic exercises indicate that such interventions may be beneficial for LT recipients. Although both groups experienced improvements, the LTx group showed more pronounced effects, underscoring the greater need for exercise interventions in this population. Additionally, the significant increase in total cholesterol among LT recipients after therapeutic exercise highlights the potential for post-transplant medications to cause hyperlipidemia, emphasizing the importance of monitoring lipid profiles in these patients. Hüsing, Kabar, & Schmidt (2016) found that hyperlipidemia was a prevalent issue after LT, affecting up to 71% of patients. The development of lipid disorders in these individuals is influenced by several factors, including the use of immunosuppressive medications such as Tacrolimus, as well as additional risk factors such as diabetes mellitus, obesity, and nutritional considerations. As survival rates among liver transplant recipients improve, there is an increasing emphasis on preventing cardiovascular complications, particularly because approximately 64% of these recipients are at a high risk for such events. Addressing dyslipidemia and other modifiable cardiovascular risk factors - such as diabetes, hypertension, and smoking - has become a primary focus in the care of these patients. Post-transplantation management of hyperlipidemia typically includes lifestyle modifications, adjustments to immunosuppressive regimens, and the use of lipid-lowering agents. However, initiating lipid-lowering medications requires careful monitoring for potential drug interactions, especially with immunosuppressive drugs. Additionally, caution is necessary when combining different lipid-lowering agents because such combinations can lead to severe adverse effects, including myopathies and rhabdomyolysis [26]. Our study found a negative influence stemming from the hyperlipidemia induced by immunosuppressive medications such as Tacrolimus.

Our study observed minimal improvement in Hgb levels after post-therapeutic exercises compared to pre-therapeutic exercises in the control group. However, the difference was not statistically significant, likely owing to chronic anemia associated with decompensated liver cirrhosis. Previous research indicates that anemia is prevalent in approximately 75% of individuals with chronic liver disease. Additionally, the use of immunosuppressive medications can contribute to abnormal hematologic indices after LT. Anemia is a common issue among adults after LT [27].

Additionally, our study revealed an improvement in the low platelet count associated with portal hypertension resulting from cirrhosis after LT [28]. Compared to the pre-transplant cirrhotic state, characterized by low albumin levels, high bilirubin levels, and coagulopathy, similar improvements in liver function indicators were observed after LT. The study results demonstrate that LT is effective in restoring

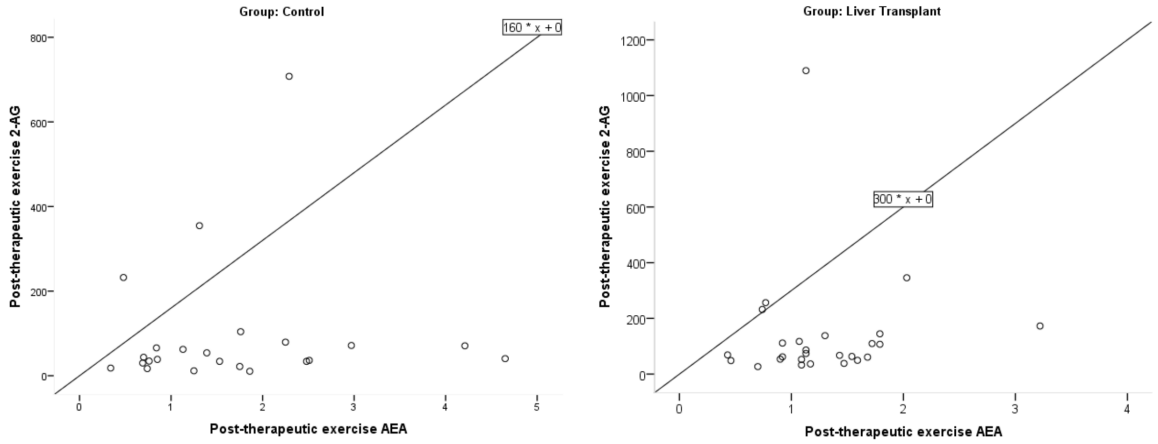

Figure 4. Scatter plot illustrating the relationship between 2-AG and AEA after therapeutic exercises in both the control and liver transplant groups. 2AG: 2-arachidonoylglycerol, AEA: anandamide.

Table 3. Comparison of hematological and biochemical markers before and after therapeutic exercises in the control group

Hematological and biochemical markers	Normal range	Pre-Exs (n = 23)	Post-Exs (n = 23)	Sig.
Hemoglobin	12.5-16.5 (g/dL)	10.97 ± 2.08	11.32 ± 2.20	0.400
Platelet count	150-400 (× 10^9/L)	106.17 ± 48.60	116.78 ± 74.69	0.225
Total bilirubin	3.4-20.5 (μmol/L)	81.97 ± 106.61	54.26 ± 7.13	0.143
Albumin	35-52 (g/L)	27.70 ± 5.16	34.96 ± 29.99	0.261
Total protein	64-83 (g/L)	65.48 ± 9.61	68.83 ± 8.68	0.129
A/G (ratio)	1.1-2.2	0.78 ± 0.25	0.75 ± 0.25	0.342
INR (ratio)	0.8-1.2	1.59 ± 0.53	1.52 ± 0.67	0.436
Glucose fasting	4.6-6.4 (mmol/L)	7.34 ± 4.88	6.92 ± 5.02	0.583
HbA1c	< 5.7 (%)	5.76 ± 2.16	5.77 ± 1.97	0.955
Calcium	2.1-2.5 (mmol/L)	2.16 ± 0.21	2.12 ± 0.16	0.224
Phosphorus	0.74-1.52 (mmol/L)	1.01 ± 0.22	1.03 ± 0.18	0.727
Alkaline phosphatase	40-150 (unit/L)	166.43 ± 92.04	201.26 ± 99.42	0.065
Magnesium	0.66-1.07 (mmol/L)	0.72 ± 0.10	0.73 ± 0.11	0.962
Sodium	136-145 (mmol/L)	137.17 ± 4.50	135.70 ± 3.95	0.178
Potassium	3.5-5.1 (mmol/L)	3.97 ± 0.59	4.17 ± 0.45	0.096
BUN	3.1-8.5 (mmol/L)	5.19 ± 2.93	4.75 ± 2.38	0.349
Creatinine	57-105 (μmol/L)	76.04 ± 23.53	76.26 ± 25.61	0.946

Non-significant difference in the mean between pre-exercise and post-exercise in the control group at the 5% level of significance. Exs: Therapeutic exercises, A/G: Albumin/globulin ratio, INR: International normalized ratio, HbA1C: Hemoglobin A1C, BUN: Blood urea nitrogen.

liver function, as evidenced by significant improvements in metrics that are either directly or indirectly related to liver health [29]. These indicators include the A/G ratio, INR ratio, albumin concentration, platelet count, and bilirubin level.

Renal function tests were normal in both groups before and after therapeutic exercises. However, advanced liver cirrhosis can sometimes impact renal function. Recent studies have reported that patients with decompensated liver cirrhosis may develop hepatorenal syndrome (HRS), a type of functional renal failure associated with advanced liver disease. Cirrhotic patients with acute kidney injury have a 20%-40% risk of developing HRS. For those with chronic liver disease, HRS often indicates a poor prognosis. LT is the definitive treatment for HRS. After LT, renal function tends to

Table 4. Comparison of hematological and biochemical markers before and after therapeutic exercises in the liver transplant group

Hematological and biochemical markers	Normal range	Pre-Exs (n = 26)	Post-Exs (n = 26)	Sig.
Hemoglobin (Hgb)	12.5-16.5 (g/dL)	11.30 ± 2.23	11.30 ± 1.64	0.987
Platelet count	150-400 (× 10^9/L)	127.73 ± 95.19	220.54 ± 91.47*	0.001
Total bilirubin	3.4-20.5 (μmol/L)	112.25 ± 136.75	17.61 ± 34.13*	0.001
Albumin	35-52 (g/L)	27.27 ± 5.79	38.31 ± 4.12*	< 0.001
Total protein	64-83 (g/L)	66.50 ± 9.61	65.00 ± 5.60	0.453
A/G (ratio)	1.1-2.2	0.76 ± 0.30	1.47 ± 0.28*	< 0.001
INR (ratio)	0.8-1.2	1.71 ± 0.96	1.11 ± 0.33*	0.006
Glucose fasting	4.6-6.4 (mmol/L)	7.55 ± 5.24	6.66 ± 2.55	0.422
HbA1c	< 5.7 (%)	5.38 ± 1.41	5.55 ± 0.92	0.548
Calcium	2.1-2.5 (mmol/L)	2.11 ± 0.15	2.19 ± 0.11	0.067
Phosphorus	0.74-1.52 (mmol/L)	1.01 ± 0.17	1.10 ± 0.21	0.092
Alkaline phosphatase	40-150 (unit/L)	187.42 ± 142.67	220.62 ± 213.61	0.537
Magnesium	0.66-1.07 (mmol/L)	0.75 ± 0.08	0.69 ± 0.07*	0.016
Sodium	136-145 (mmol/L)	135.38 ± 5.43	139.27 ± 2.88*	0.001
Potassium	3.5-5.1 (mmol/L)	3.99 ± 0.64	4.46 ± 0.38*	0.001
BUN	3.1-8.5 (mmol/L)	5.20 ± 2.98	7.18 ± 3.92*	0.033
Creatinine	57-105 (μmol/L)	79.04 ± 21.73	100.31 ± 45.06*	0.014

*Indicates a significant difference in the mean between pre-exercise and post-exercise in the liver transplant group at the 5% level of significance. Exs: Therapeutic exercises, A/G: Albumin/globulin ratio, INR: International normalized ratio, HbA1C: Hemoglobin A1C, BUN: Blood urea nitrogen.

improve, liver function is restored, and portal hypertension is alleviated owing to reduced kidney vasoconstriction [30]. Our research indicates that alkaline phosphatase levels increased after LT, likely owing to the side effects of immunosuppressive medications, which increased blood enzyme levels. Similarly, Al-Nattah et al. (2022) reported an increase in alkaline phosphatase levels after the initiation of immunotherapy with pembrolizumab [31].

Fasting blood glucose levels increased in both groups before and after therapeutic exercises. Hyperglycemia and diabetes mellitus are associated with cirrhosis and advanced liver disease. Liver cirrhosis exacerbates glucose intolerance and diabetes through several mechanisms, including insulin resistance and reduced insulin production [32]. Additionally, post-liver transplantation diabetes mellitus (PLTDM) can develop owing to the use of immunosuppressive medications, particularly calcineurin inhibitors such as tacrolimus and cyclosporine, which are major risk factors. PLTDM is one of the most frequent complications after LT [33].

The findings highlight the benefits of exercise before and after LT, including improvements in blood and biochemical markers associated with liver health. Additionally, increasing protein intake and implementing nutritional therapy after LT may further enhance nutritional status and liver function outcomes [34]. A previous study found that a large proportion of patients (61%) awaiting LT experienced notable anxiety and depression, and these levels remained unchanged even when they participated in an HBEP [35].

Strengths and limitations

This study is the first to evaluate the impact of exercise on the ECS in LTx with a well-defined treatment protocol. However, it has several limitations. The primary limitation is the relatively small sample size, with only 26 patients undergoing LT and 23 patients in the control group, which restricts the ability to draw broad conclusions. Additionally, ethical considerations prevented the design of a control group without exercise interventions. Furthermore, some patients in the control group who passed away did not meet the study's requirements for measuring the eCB response before and after therapeutic exercises. The duration of therapeutic exercises was also limited, potentially insufficient to fully capture the benefits of exercise for

Table 5. Comparison of hematological and biochemical markers after therapeutic exercises between the control and liver transplant groups

Hematological and biochemical markers	Normal range	Control (n = 23)	Liver transplant (n = 26)	Sig.
Hemoglobin	12.5-16.5 (g/dL)	11.32 ± 2.20	11.30 ± 1.64	0.896
Platelet count	150-400 (× 10^9/L)	116.78 ± 74.69	220.54 ± 91.47*	< 0.001
Total bilirubin	3.4-20.5 (μmol/L)	54.26 ± 7.13	17.61 ± 34.13*	< 0.001
Albumin	35-52 (g/L)	34.96 ± 29.99	38.31 ± 4.12*	< 0.001
Total protein	64-83 (g/L)	68.83 ± 8.68	65.00 ± 5.60*	0.049
A/G (ratio)	1.1-2.2	0.75 ± 0.25	1.47 ± 0.28*	< 0.001
INR (ratio)	0.8-1.2	1.52 ± 0.67	1.11 ± 0.33*	< 0.001
Glucose fasting	4.6-6.4 (mmol/L)	6.92 ± 5.02	6.66 ± 2.55	0.245
HbA1c	< 5.7 (%)	5.77 ± 1.97	5.55 ± 0.92	0.645
Calcium	2.1-2.5 (mmol/L)	2.12 ± 0.16	2.19 ± 0.11*	0.025
Phosphorus	0.74-1.52 (mmol/L)	1.03 ± 0.18	1.10 ± 0.21	0.218
Alkaline phosphatase	40-150 (unit/L)	201.26 ± 99.42	220.62 ± 213.61	0.652
Magnesium	0.66-1.07 (mmol/L)	0.73 ± 0.11	0.69 ± 0.07	0.065
Sodium	136-145 (mmol/L)	135.70 ± 3.95	139.27 ± 2.88*	< 0.001
Potassium	3.5-5.1 (mmol/L)	4.17 ± 0.45	4.46 ± 0.38*	0.013
BUN	3.1-8.5 (mmol/L)	4.75 ± 2.38	7.18 ± 3.92*	0.003
Creatinine	57-105 (μmol/L)	76.26 ± 25.61	100.31 ± 45.06*	0.004

*Indicates a significant difference in the mean between the control and liver transplant groups at the 5% level of significance. A/G: Albumin/globulin ratio, INR: International normalized ratio, HbA1C: Hemoglobin A1C, BUN: Blood urea nitrogen.

Table 6. Comparison of the effects of pre- and post-therapeutic exercises on mean lipid levels between the control and liver transplant groups

Lipid profile	Control (n = 23)		Sig.	Liver transplant (n = 26)		Sig.	Post-Exs. Control vs. LT
	Pre-Exs	Post-Exs		Pre-Exs	Post-Exs		Sig.
Total cholesterol	3.49 ± 1.41	3.61 ± 1.13	0.605	3.40 ± 1.07	4.17 ± 1.0*	0.014	0.072
HDL	0.82 ± 0.64	0.77 ± 0.41	0.601	0.79 ± 0.47	1.0 ± 0.36	0.054	0.041*
LDL	2.18 ± 0.98	2.25 ± 0.88	0.328	2.06 ± 0.75	2.46 ± 0.84	0.063	0.411
Triglycerides	1.08 ± 0.44	0.99 ± 0.50	0.723	1.06 ± 0.33	1.37 ± 1.37	0.121	0.052

*Indicates a significant difference in the mean between the control group and the liver transplant group at the 5% level of significance. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, Exs: Therapeutic exercises.

LTx patients. Another limitation is the inability to perform Western blotting tests for ECS receptors owing to the high costs of sample preparation and blotting, which were prohibitive for student-funded research. Western blotting is essential for understanding the distribution, function, and regulation of G protein-coupled receptors, and it provides crucial quantitative data on eCB expression levels from the ECS [36].

Conclusions

The study findings revealed an increase in 2-AG concentration after therapeutic exercises in LTx but not in the control group. In contrast, AEA levels were elevated post-therapeutic exercises in both groups. The effect of post-therapeutic exercises on hematological and biochemical markers showed significant differences between the control and LTx groups, specifically regarding platelet count, total bilirubin, total protein, A/G ratio, INR ratio, and calcium.

A large-scale study investigating the impact of exercise on eCBs and related signaling pathways would be highly valuable. It is recommended to collaborate with a multidisciplinary team to integrate psychological and nutritional interventions alongside HBEP for LT recipients. Given the variability in patient responses and influencing factors, outcomes may differ. Fur-

ther research is needed to understand the long-term effects of exercise on post-LT recovery and patient well-being. Exploring these factors and potentially refining research methods could help address discrepancies and enhance our understanding of the study results.

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Disclosure of conflict of interest

None.

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