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Effect of laser acupuncture combined with electrical stimulation on recovery from exercise fatigue in mice

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In sports events, the rapid recovery after high-intensity training or sport competition performance is very important for athletes' performance and health. The aim of this study is to evaluate the effect of laser acupuncture and electrical stimulation on the recovery from exercise fatigue, using mice with swimming fatigue as experimental model and the electromyography (EMG) and the Raman spectroscopy of blood as evaluation indicators. Root mean square (RMS) and mean power frequency (MPF) of EMG were analyzed after laser acupuncture and electrical stimulation. The amplitude frequency combined analysis (JASA) showed that the proportion of muscles in the fatigue recovery area of the control group, the laser acupuncture group, the multi-channel laser acupuncture group and the laser combined with electrical stimulation group were 34.78%, 39.13%, 39.13% and 43.48%, respectively. Raman spectroscopy of the mice blood during fatigue

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recovery showed there is a significant difference between the multi-channel laser acupuncture group and the laser combined with electric stimulation group compared with the recovery period and fatigue period ($P < 0.05$) at the peak of $997\,\text{cm}^{-1}$ and the laser combined electrical stimulation group had a statistical difference in the recovery period compared with the fatigue period ($P < 0.05$) at the peak of $1561\,\text{cm}^{-1}$. The results showed that laser acupuncture combined with electrical stimulation was beneficial to fatigue recovery in mice, and had the potential value in sports fatigue recovery.

Keywords: Laser acupuncture; electrical stimulation; electromyography; Raman spectroscopy.

1. Introduction

Timely medical intervention can accelerate the fatigue recovery, but the available fatigue therapies methods in modern medicine are very limited, therefore the substitutes and mechanisms of traditional medicine are worth studying.[1] Compared with the conventional treatment methods such as acupuncture, cupping and massage,[2] laser acupuncture is relatively safe with almost no adverse events.[3] Laser acupuncture has been used to enhance muscle endurance before exercise and promote the recovery after exercise.[4,5] With good penetration and strong irritation in biological tissues, electrical stimulation is widely used in muscle rehabilitation, fatigue recovery and pain treatment.[6]

At present, bioelectrical signals and biochemical indicators are used as the criteria for evaluating fatigue and recovery.[7] The electromyography (EMG) analysis is taken as the gold standard for muscle fatigue detection,[8,9] in which the action potential of a single muscle is identified by the amplitude and envelope of intramuscular EMG, and the change of discharge rate of muscle motor unit before and after exercise is explored.[10] In the analysis of EMG during fatigue recovery, the integral electromyography (IEMG) value, root mean square (RMS) value of time domain index of EMG, mean power frequency (MPF) and median frequency (MF) of frequency domain index are usually used to judge whether the muscle reaches fatigue or not.[11,12] Based on the analysis of EMG signal in time domain and frequency domain, amplitude frequency joint analysis is also used to quantify the muscle fatigue and recovery period.[13]

Raman spectroscopy can detect the composition of substances at the molecular level. Raman spectroscopy can reflect a variety of substance components in samples, which has the advantages of rapid detection, unlabeling and unstaining,[14] and it has been widely used in botany, pharmacy, forensic medicine and other fields. Raman spectroscopy can detect blood glucose level.[15] Previous studies of our research group showed that Raman spectroscopy can analyze the changes of blood components after laser acupuncture at Zusanli point in mice.[16] This technique has also been used to quantify urea and creatinine in urine to analyze renal function.[17] During exercise, plasma urea level will increase due to the protein degradation, and this process could take place after a short period of intense physical activity. Additionally, these levels decrease after prolonged physical activity.[18]

In this paper, the effect of laser acupuncture and electrical stimulation on fatigue recovery were studied by analyzing the electrophysiological signal and the blood Raman spectroscopy, and it showed a potential value for applications in sports fatigue recovery and clinic.

2. Materials and Methods

2.1. *Laser acupuncture combined with electric stimulation device*

Laser acupuncture combined with electrical stimulation treatment device was developed with microcontroller unit STM32 as the main control

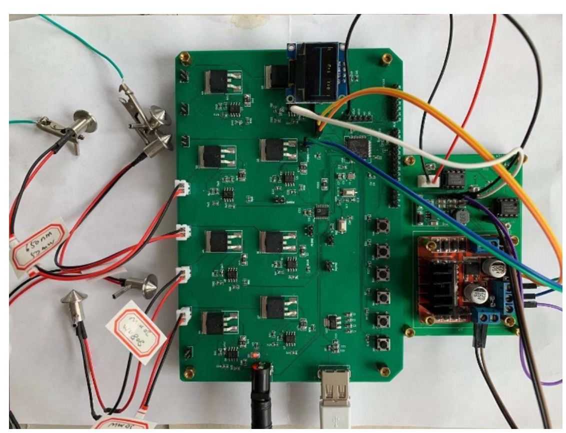


Fig. 1. Laser acupuncture combined with electric stimulation device.

chip (Fig. 1). In the laser acupuncture module, the multi-channel digital-to-analog conversion chip AD5676R was used to achieve the multi-channel output function of laser acupuncture, and manufacture the laser driving circuit module. In the electrical stimulation module, the bidirectional pulse output function was implemented by using H-bridge circuit and Howland current pump circuit. Laser acupuncture and electrical stimulation could be used separately or combination in this device. In addition, the laser output frequency, output time and output channel and laser acupuncture modes could be selected for laser acupuncture.

2.2. *Animal preparation*

Male BALB/c mice (8 weeks old, weight 26.5 ± 2.5 g) were fed at 22 ± 2°C, 12/12 h of time/dark cycle, free eating and drinking. The animal experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai University. Twenty healthy mice were randomly divided into four groups with five mice in each group: Control group (normal), laser acupuncture group (laser), multi laser acupuncture group (multi laser) and laser electric stimulation group (laser electric). The mouse Acupoint Zusanli (ST36) is located 4 mm below the knee joint of the hind leg[19] and Sanyinjiao (SP-6) is located at the posterior edge of the tibia, 0.5 mm above the medial malleolus,[20] which is selected as the stimulation point of laser acupuncture.

2.3. *Fatigue modeling*

After a week of feeding, the mice were trained for swimming. They were trained for 30 min without weight load for three days. At the beginning of swimming, the wooden stick was used to assist the mice to swim. When the mice float on the water without moving, the wooden stick was used to gently stir the water surface to keep them in a swimming state. The transparent water tank is selected for swimming test. The water level is 30 cm and the water temperature is about 25°C. After swimming training, the mice hair was dried by a heater. During the experiment, the tail of mice was tied with a 5% lead wire for weight-bearing swimming. When sank into water for 7 s and did not float on the water surface, the mice were regarded to be fatigue.[21]

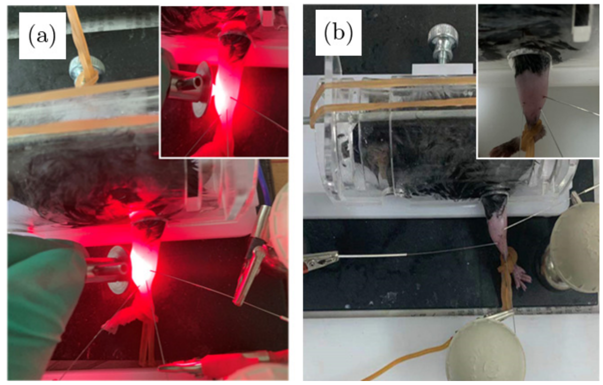


Fig. 2. (a) Schematic diagram of mouse ST36 laser acupuncture operation process (b) and EMG signal acquisition.

2.4. *Laser acupuncture and electrical stimulation parameter setting*

The mice in the control group were fixed in the fixator without any acupuncture stimulation. The laser acupuncture group (wavelength 650 nm, output power 10 mW, total energy 6J, continuous wave) stimulated ST36 acupoints, the multi-channel laser acupuncture group (wavelength 650 nm, 808 nm, output power 50 mW, continuous wave) stimulated ST36 and SP-6 acupoints, and the laser combined with electrical stimulation group (wavelength 650 nm, output power 50 mW, continuous wave, current 2 mA, ST36 acupoints were stimulated at a frequency of 100 Hz (Fig. 2(a)). All the laser acupuncture procedures used triangular wave simulation (Frequency: 10 Hz) to simulate the operation of lifting and inserting manipulation.

2.5. *EMG acquisition and processing*

The skin around the acupoints was shaved and disinfected with 75% alcohol before EMG collection. Electrophysiological signal acquisition needle electrodes (Shanghai Huatuo, 0.25 mm × 40 mm) were used to collect electromyographic signals during fatigue and recovery from biopac4.1 (USA) 8-channel physiological recorder. Three needle electrodes were inserted according to vertical muscle fibers, in which the positive and negative electrodes of the recording electrode were on gastrocnemius muscle,[22] the distance between the two electrodes was 3 mm and the reference electrode was inserted into muscle irrelevant to acquisition close to the positive electrode of the recording electrode (Fig. 2(b)). The sampling rate of EMG signal was

1000 Hz. BIOPAC was selected to collect EMG signal (5–1000 Hz) in mp36 mode, and the amplifier gain was set to 1000x. After the acquisition, MATLAB 2016 was used for data processing, by which a Butterworth bandpass filter was designed with passband frequency (50–150 Hz), stopband frequency (10–500 Hz) and notch filter (50 Hz) to minimize noise interference. EMG signal of 2 min is collected immediately when with the fatigue state; Two minutes of EMG signals collected after 5 min treatment as the recovery period. According to the sample size of each group (number of mice: 5), use case study to analyze the average value of each group of data.

2.6. *Raman spectrum acquisition and processing*

After the EMG collection of mice was completed, the mice rested for one week, and then the biochemical analysis of swimming fatigue was carried out after complete recovery. The blood components in fatigue period and recovery period were analyzed by Raman spectrometer. The treatment time of each group was 10 min. After fatigue modeling and treatment, the blood samples of each mouse were collected from caudal vein for Raman measurement. Raman spectra were collected using a Raman microscope (LabRAM HR evolution, Horiba, JP) equipped with a 633 nm He–Ne laser source. The initial parameters were configured as follows: The resolution was 1 cm^{-1}, the acquisition time was 10 s, and the scanning range was 400–1800 cm^{-1}. Before each measurement, the system was calibrated with a silicon reference sample through the 520 cm^{-1} band. The laser is focused on the surface of the blood sample through the 50x microscope objective (NA 0.5) (Fig. 3). Each sample was measured 8 times at different positions to reduce the measurement error as much as possible. The student's t test was used to calculate the significance of differences between groups of data. Otherwise, the nonparametric test and rank sum test were also used to test the significance of differences between the data groups. All raw Raman spectra data were obtained using LabSpec software (Horiba, Japan). The baseline correction of the raw data was proceeded using Hystudio SUBASE v2.10 software (self-developed) to remove the fluorescence background. For each group of data, all of the corrected spectral signal data of all samples were averaged as the mean

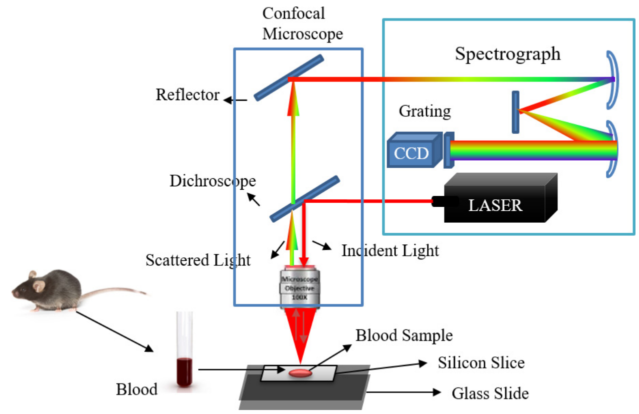


Fig. 3. Schematic diagram of confocal Raman microscope and blood sample spectrum measurement process.

Raman spectra, and the effects of different stimuli on the changes of blood components during fatigue recovery were evaluated by analyzing the changes of peak intensities.

3. Results and Discussion

3.1. *Analysis of EMG signal*

Figures 4 and 5, respectively, show the original EMG signal and filtered EMG signal of the four groups of data after treatment. The original EMG signal shows that there are differences in EMG signals of different groups. Among them, the amplitude of EMG signals of laser acupuncture, multichannel laser acupuncture and laser combined electrical stimulation changes significantly than that of the control group. EMG signal is an electrical pulse signal generated by motor neurons under the control of central nerve. It is characterized by muscle tension (action potential) when the electrical pulse causes muscle contraction. The short duration of action potential makes EMG to have intermittent positive and negative potential waveform. The original signal shows the characteristic peak of EMG signal after filtering. The characteristic peak of EMG signal after filtering is different in different groups, and its frequency and amplitude are different.

EMG signals are usually analyzed in time domain and frequency domain. In time domain analysis, the relationship between EMG signal amplitude and muscle relaxation during exercise is mainly explored, which is measured by integrated EMG value IEMG and RMS value. Frequency domain analysis can study the characteristics of EMG signals at

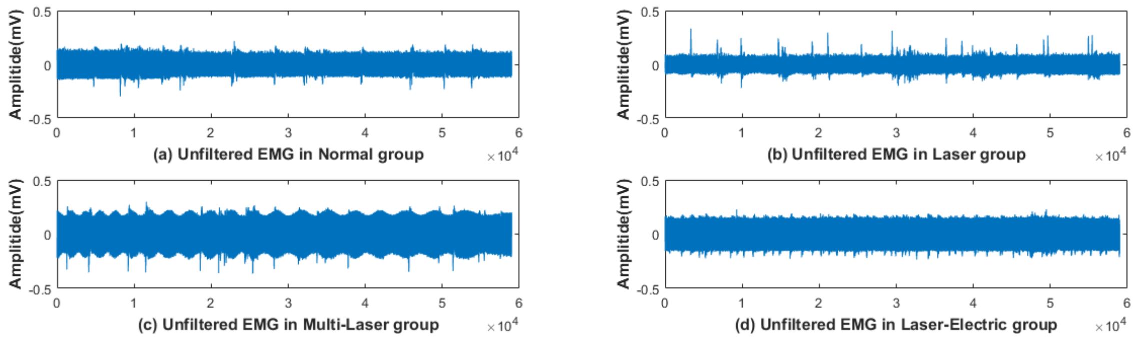


Fig. 4. Unfiltered EMG waveforms of different groups.

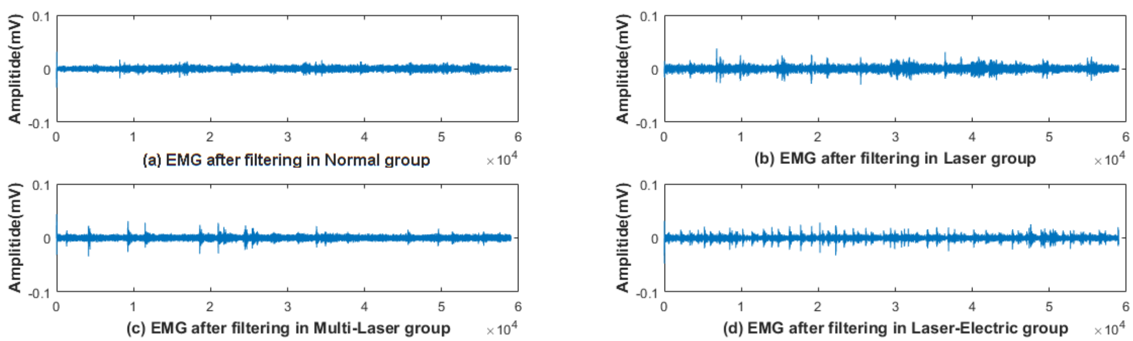


Fig. 5. Filtered EMG waveforms of different groups.

different frequencies and analyze the transformation law. The measurement indicators are usually mean power frequency (MPF) and MF. Table 1 shows the time domain and frequency domain indexes of fatigue period and recovery period of all data, and calculates mean ± SD.

Table 1 shows the time-frequency indexes of four groups of experimental data. The average value of each group of data shows that compared with the fatigue, the mean values of IEMG and RMS in recovery period are reduced to the following degrees: Normal group (IEMG = 0.34, RMS = 0.41), laser group (IEMG = 0.50, RMS = 0.64), multi-laser group (IEMG = 0.46, RMS = 0.75) and laser electric group (IEMG = 1.05, RMS = 1.46). The frequency domain index showed that the values of MPF and MF increased in recovery period compared with fatigue period. The increased values of each group were normal group (MPF = 9.11, MF = 4.66), laser group (MPF = 11.93, MF = 4.48), multi-laser group (MPF = 13.32, MF = 4.89) and laser electric group (MPF = 16.97, MF = 8.98). In addition, Fig. 6 shows the distribution of all data in time domain and frequency domain during fatigue period and recovery period. The amplitude of time domain indicators IEMG and RMS decreases during recovery period, the frequency of frequency domain indicators MPF and MF increases during recovery period, the overall amplitude of IEMG is lower than the overall amplitude level of RMS, and the overall frequency of MPF is higher than the overall frequency of MF, it is consistent with the fact that the index changing degree of RMS and MPF shown in Table 3 is greater than that of IEMG and MF.

Intra group analysis can only get the relationship of the same group of variables, which could not reflect the differences of different groups. Figures 7 and 8 show the effects of time domain and frequency domain indexes of different groups under different stimulation conditions on fatigue recovery of mice, and compare the difference between time-frequency indexes of each group during fatigue period and recovery period.

There are significant differences for the difference between fatigue period and recovery period among groups. For IEMG, the largest difference between recovery period and fatigue period is in the laser electric group, and the smallest difference is in the normal group. Under the condition of laser stimulation, the difference in multi-laser group is lower than that in the laser group. For RMS index, the four groups of data showed an increasing trend, and

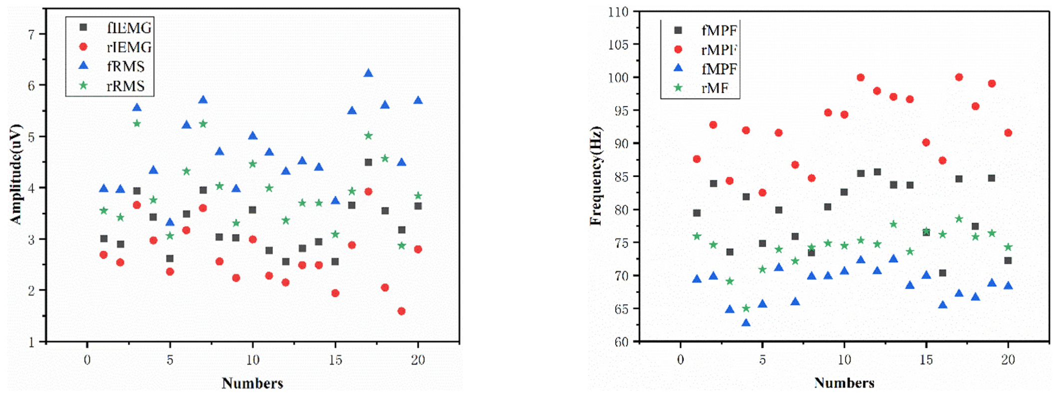
Table 1. Time frequency data of fatigue and recovery period under different groups.

Group	F/R	Index	1	2	3	4	5	Mean ± SD
Normal	F	IEMG	3.01	2.90	3.93	3.43	2.62	3.18 ± 0.51
		RMS	3.97	3.96	5.55	4.33	3.31	4.22 ± 0.83
		MPF	79.44	83.85	73.55	81.89	74.81	78.71 ± 4.44
		MF	69.35	69.8	64.76	62.69	65.58	66.44 ± 3.06
	R	IEMG	2.69	2.54	3.66	2.97	2.36	2.84 ± 0.51
		RMS	3.55	3.42	5.25	3.76	3.06	3.81 ± 0.85
		MPF	87.58	92.77	84.30	91.96	82.50	87.82 ± 4.54
		MF	75.90	74.61	69.09	65.01	70.88	71.10 ± 4.37
Laser	F	IEMG	3.48	3.95	3.04	3.02	3.57	3.41 ± 0.39
		RMS	5.21	5.70	4.69	3.97	5.00	4.91 ± 0.64
		MPF	79.90	75.92	73.42	80.40	82.62	78.45 ± 3.71
		MF	71.09	65.91	69.82	69.87	70.57	69.45 ± 2.05
	R	IEMG	3.17	3.60	2.56	2.24	2.99	2.91 ± 0.53
		RMS	4.32	5.24	4.03	3.31	4.46	4.27 ± 0.70
		MPF	91.56	86.71	84.71	94.6	94.32	90.38 ± 4.48
		MF	73.92	72.17	74.23	74.85	74.49	73.93 ± 1.04
Multi-Laser	F	IEMG	2.78	2.56	2.81	2.94	2.56	2.73 ± 0.17
		RMS	4.68	4.31	4.51	4.39	3.73	4.32 ± 0.36
		MPF	85.42	85.62	83.71	83.66	76.52	82.99 ± 3.73
		MF	72.23	70.6	72.44	68.4	69.95	70.72 ± 1.68
	R	IEMG	2.28	2.15	2.49	2.49	1.94	2.27 ± 0.23
		RMS	3.99	3.36	3.70	3.70	3.09	3.57 ± 0.35
		MPF	99.93	97.88	97.02	96.63	90.11	96.31 ± 3.70
		MF	75.29	74.71	77.75	73.61	76.67	75.61 ± 1.63
Laser-Electric	F	IEMG	3.66	4.49	3.55	3.18	3.64	3.70 ± 0.48
		RMS	5.49	6.22	5.60	4.48	5.69	5.50 ± 0.63
		MPF	70.37	84.54	77.38	84.7	72.21	77.84 ± 6.70
		MF	65.42	67.17	66.62	68.78	68.32	67.26 ± 1.35
	R	IEMG	2.88	3.92	2.05	1.59	2.80	2.65 ± 0.89
		RMS	3.93	5.01	4.57	2.87	3.84	4.04 ± 0.81
		MPF	87.39	100.00	95.56	99.03	91.55	94.71 ± 5.27
		MF	76.18	78.55	75.84	76.38	74.26	76.24 ± 1.54

Notes: F is fatigue period; R is recovery period; mean ± SD is mean ± standard deviation; In the table, the units of IEMG and RMS are μV, and the unit of MPF and MF is Hz; The numbers 1–5 represent different samples.

the difference in laser electric group is the largest. The change in multi-laser group and laser group was not obvious, and the difference in normal group was the smallest. The time domain index of EMG signal reflects the change of EMG signal amplitude. IEMG reflects the amplitude change trend in different time periods through the integration of time, and RMS reflects the amplitude change of overall EMG by finding the RMS of data. During the collection of mouse EMG signal, the fatigue recovery time of different mice is uncertain, and the changes of EMG signal in different time periods are different. From the results of data analysis in this paper, it is more suitable to use RMS index which can reflect the amplitude change of overall EMG signal to explore the change of EMG time-domain signal in mice during recovery, and the treatment effect of laser electric group is better.

Figure 8 shows that the difference of MPF between each group is significantly higher than that of MF, and the difference of MPF gradually increases between each group. The frequency domain index reflects the degree of muscle recovery by analyzing the frequency of EMG signal. MF is the median frequency, which reflects the median of EMG frequency in whole time period. In this paper, the change between each group is not obvious; and the change of MPF is more obvious in the four groups;



(a) Scatter of IEMG-RMS during fatigue and recovery (b) Scatter of MPF-MF during fatigue and recovery

Note: F in time-frequency index represents data in fatigue period and R represents data in recovery period.

Fig. 6. Scatter diagram of time-frequency information of fatigue and recovery period of all groups.

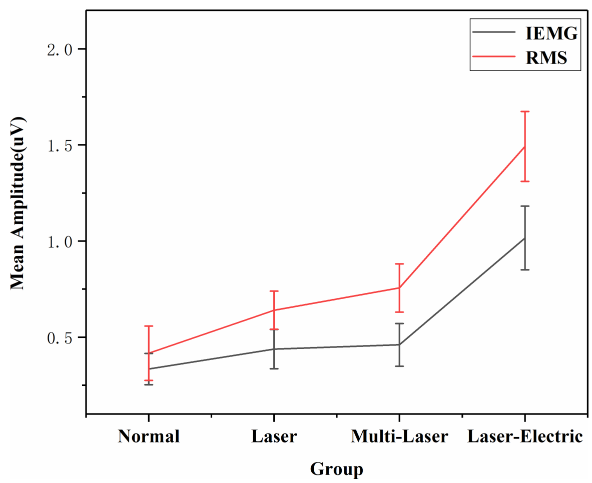


Fig. 7. IEMG and RMS during fatigue and recovery between difference groups.

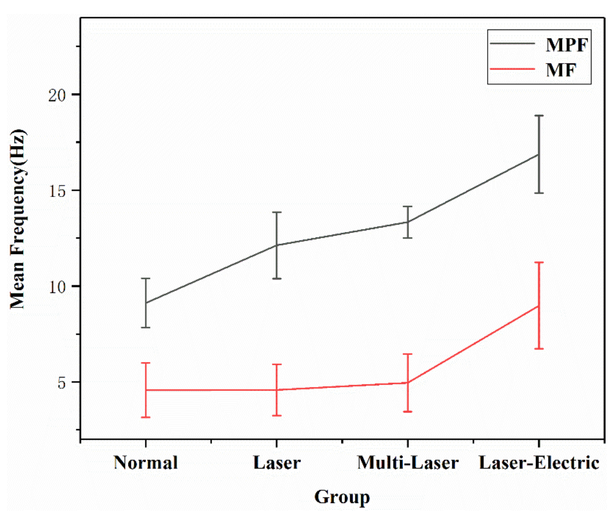
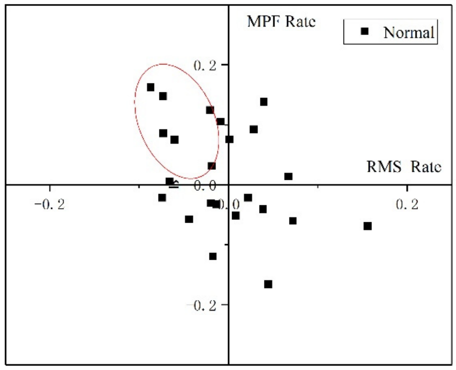


Fig. 8. MPF and MF during fatigue and recovery between difference groups.

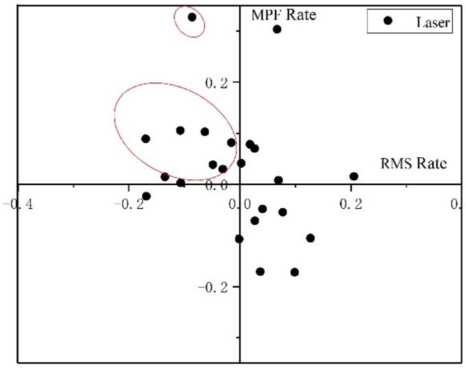
and laser electric is better than other groups. The results show that MPF is more sensitive to muscle activity and function than MF.

According to the time-domain and frequency-domain characteristics of EMG signal, the joint analysis of EMG spectrum and amplitude (JASA) is selected, which is proposed by Alvin Luttmann.[13] This method considers both amplitude and spectrum, taking the time-domain index as the abscissa and the frequency-domain index as the ordinate, and giving the fatigue state of four quadrants at different stages. The first and third quadrants are the increase and decrease of muscle strength separately. The second and fourth quadrants are muscle recovery and fatigue. According to the analysis of this paper, the changes of time-domain signal RMS and frequency-domain signal MPF reflect the state of muscle recovery obviously. Therefore, the horizontal axis of JASA is the change rate of RMS, while the vertical axis is the change rate of MPF. Taking each group of EMG signal (2 min) as a segment of 5 s, a total of 24 segments, we could calculate the change rate of RMS and MPF in each segment and draw the amplitude frequency joint analysis under different groups (Fig. 9). The change rates of RMS and MPF in different quadrants of each segment are counted (Table 2).

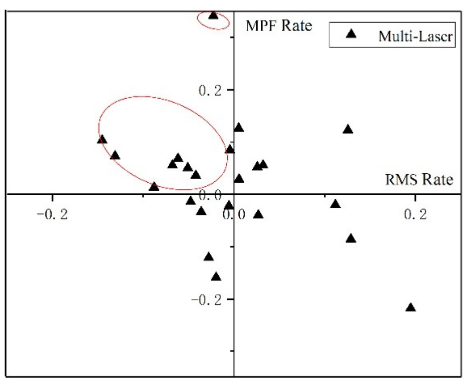
Figure 9 shows that although the data are from the fatigue recovery stage, the results of amplitude frequency analysis show that during the muscle recovery stage, the muscle also shows fatigue stage and muscle strength reduction. On the whole, the change rates of RMS and MPF in different time periods are concentrated in the range of −0.2–0.2.



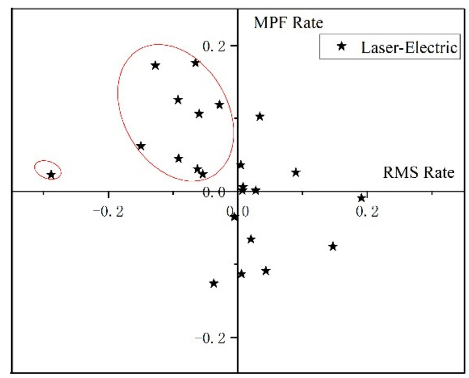
(a) Normal group JASA



(b) Laser group JASA



(c) Multi-laser group JASA



(d) Laser-electric group JASA

Note: The area circled in red is the data segment in recovery period.

Fig. 9. Schematic diagram of amplitude frequency joint analysis of different groups.

Table 2. Distribution proportion of scattered points in JASA of different groups (%).

Group	First quadrant muscle strength	Second quadrant recovery	Third quadrant decreased muscle	Fourth quadrant fatigue
Normal	17.39	34.78	21.74	26.09
Laser	26.09	39.13	8.70	26.09
Multi-laser	21.74	39.13	21.73	17.39
Laser-Electric	26.09	43.48	8.70	21.74

Table 2 records the data distribution of each section. The data in the recovery period account for the highest proportion in each group: 34.78% in the control group, 39.13% in the laser acupuncture group, 39.13% in the multi-channel laser acupuncture group and 43.48% in the laser combined with electrical stimulation group. The results show that laser combined with electrical stimulation is more effective for muscle recovery in fatigue recovery, which increases the muscle strength recovery by 8.7% compared with the control. But some muscles still have varying degrees of fatigue in the recovery process. For example, the proportion of muscle fatigue is still 21.74%. Laser acupuncture has a certain effect. But it only increased by 5.65% compared with the control group, while 26.09% of the muscle strength is still in the fatigue period of the fourth quadrant. Compared with the control group, the fatigue recovery of the multi-channel acupuncture group increased 5.56%, which had no difference with the laser acupuncture group, while the data of fatigue period accounted for 17.39%. In terms of muscle strength increase, the muscle strength of each group increased significantly. The gain effect of

Table 3. Tentative attribution of Raman spectral peaks related to this study.

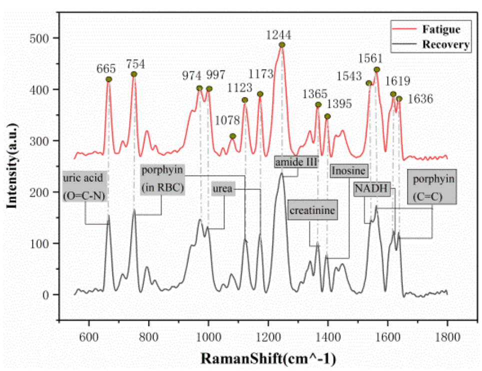
Shift/cm^{-1}	Major assignments
665	δ (O = C–N), Uric acid[23]
754	v_{15} (porphyrin deformation) in human RBC[24]
792	C_5' O–P–OC_3' phosphodiester bands in DNA[25]
974	C–O angel-bending glucose[26]
997	C–O ribose, Urea[27,28]
1078	C–C or C–O stretching mode of phospholipids[29]
1123	C–C stretching mode of lipids, C–N stretch[30]
1173	Tyrosine, Urea[31,32]
1244	Amide III (b sheet structure)[32]
1365	Creatinine[31]
1396	Nucleic acid, Inosine[31]
1449	C–H vibration ν (lipids)[25]
1543	Bound and free NADH[33,34]
1561	v_2 (C=C), porphyrin[35]
1619	Bound and free NADH[33]
1636	v_2 (C=C), porphyrin[35]

laser combined with electrical stimulation was the same as that of laser acupuncture group. In terms of muscle strength reduction, the laser combined with electrical stimulation group decreased the least.

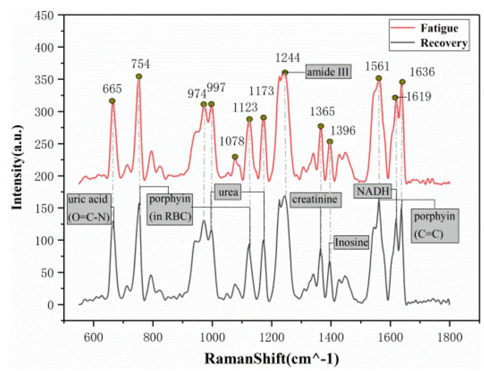
3.2. *Blood Raman spectrum analysis*

According to the tentative peaks of some biological components provided by previous studies (Table 3), the corresponding blood components can be inferred. The Raman spectra of the control group, laser acupuncture group, multi-channel laser acupuncture group and laser combined with electrical stimulation group were removed and baseline corrected. In order to reduce the random error of Raman data measurement, each group of data is averaged. Figure 10 shows the Raman spectra of mouse blood in the fatigue period and recovery period of four groups of data marks most of the main peak Raman shift values and related molecular structures. The size of curve peak in the Raman spectrum reflects the changes of components in mouse blood.

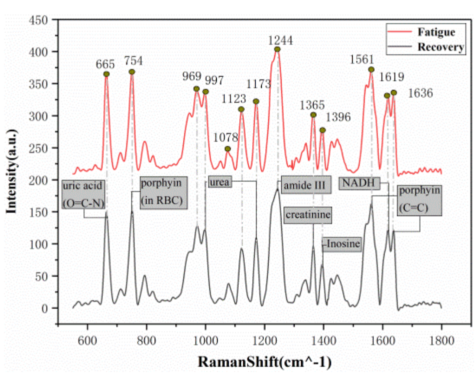
Raman spectrum can reflect the structure of corresponding molecules, while the intensity of the characteristic peak can reflect the relative content of related molecules in blood samples. Porphyrin, urea, uric acid, creatinine, free or bound reduced nicotinamide acypurine dinucleotide (NADH), lipid



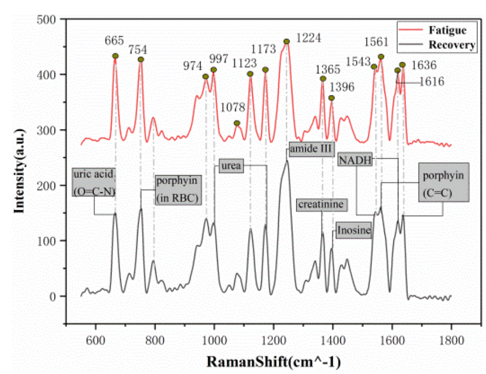
(a) Normal group blood Raman spectrum



(b) Laser group blood Raman spectrum



(c) Multi-laser group blood Raman spectrum

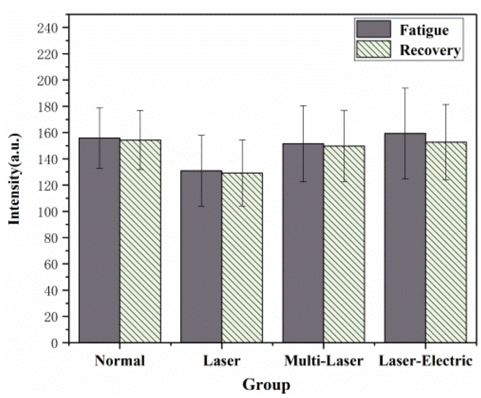


(d) Laser-electric group blood Raman spectrum

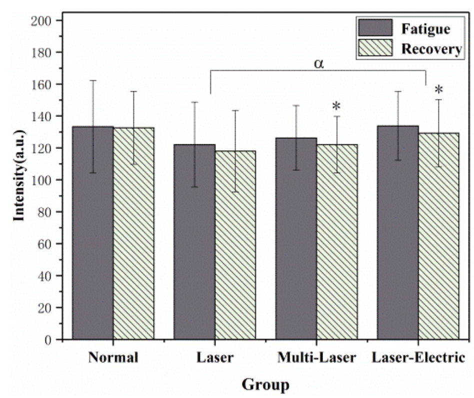
Fig. 10. Raman spectra of blood in fatigue and recovery mice in different groups.

and some others are different in fatigue and fatigue recovery in four groups of Raman spectra (Fig. 10). Laser acupuncture stimulation has certain physiological effects preliminarily. Raman peaks of 665, 997, 1173 and 1365 cm^{-1} are related to fatigue metabolites in blood (Table 3). The Raman characteristic peaks of 665, 997, 1173 and 1365 cm $^{-1}$ are reduced in the recovery period compared with the fatigue period. The peak intensity changes are different among different groups. The specific performance is as follows: Laser combined with electrical stimulation group > multi-channel laser acupuncture group > laser acupuncture group > control group. The intra group statistical analysis is carried out for each group (Fig. 11). According to the possible attribution of blood Raman peaks, Table 3 shows that 665 cm^{-1} indicates uric acid, 997 and 1173 cm^{-1} indicates urea and 1365 cm^{-1} indicates creatinine. These fatigue metabolites get accumulated in the body tissue and cause fatigue.

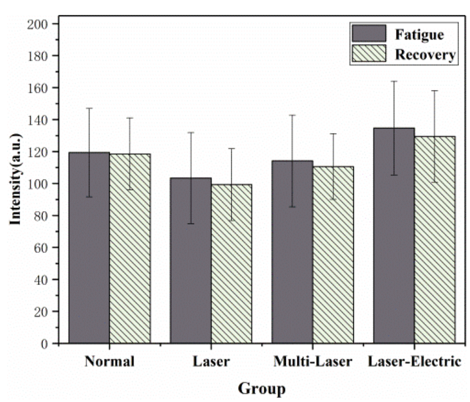
During high-intensity exercise, creatine phosphate will be consumed as the energy supply of muscle, resulting in a large amount of decomposition of adenine nucleoside triphosphate (ATP), increasing purine production, and the purine metabolism will eventually produce uric acid. The statistical results in Fig. 11(a) show that the peak intensity recovery group at 665 cm^{-1} is lower than that in the fatigue group ($P > 0.05$), indicating that both of the multi-channel laser acupuncture group and the laser combined with electrical stimulation group can reduce the level of uric acid during recovery and help to alleviate the symptoms of fatigue. The preliminary results are consistent with the conclusions of the published literature.[36] The results showed that the effect of laser combined with electric stimulation group was better than that of laser acupuncture group and laser multi-channel acupuncture group ($P > 0.05$), and there was no significant difference between laser acupuncture



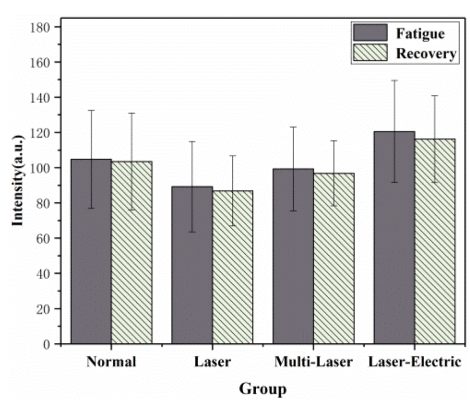
(a) Fatigue and recovery peak at 665 cm^{-1}



(b) Fatigue and recovery peak at 997 cm^{-1}



(c) Fatigue and recovery peak at 1173 cm^{-1}



(d) Fatigue and recovery peak at 1365 cm^{-1}

Note: $^{*}P < 0.05$, recovery in the group compared with fatigue; α indicates $P < 0.05$, difference between groups (pairwise comparison).

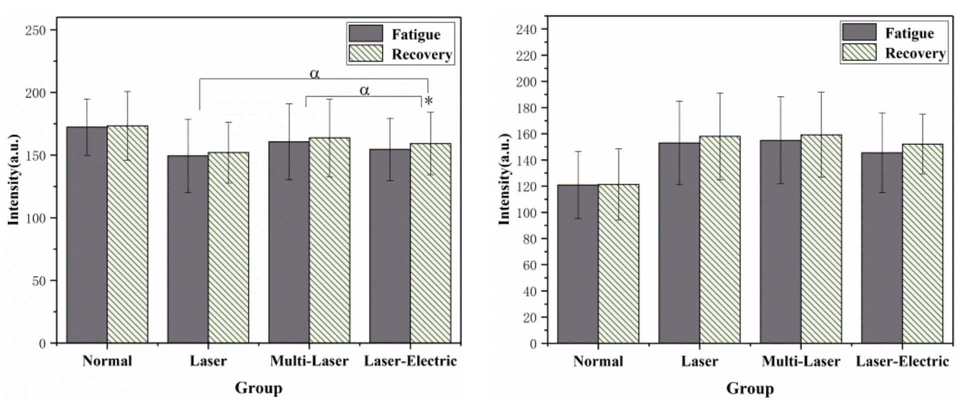
Fig. 11. Characteristic peak intensity of blood metabolites during fatigue and recovery in mice.

group and multi-channel laser acupuncture group ($P > 0.05$).

The change of urea can reflect the amount of exercise, the metabolism and energy supply of protein and amino acids. Blood urea nitrogen is also commonly expressed in blood. When a long time exercising takes place, the massive consumption of muscle glycogen leads to demand of energy supplement, the metabolic capacity of protein and amino acids is enhanced, the gluconeogenesis of amino acids is improved and the urea is accumulated rapidly. The Raman peaks of 997 and 1173 cm^{-1} were initially attributed to urea. The peak intensity of each group decreased in the recovery period compared with the fatigue period (Figs. 11(b) and 11(c)), and in the Raman intensity at 997 cm^{-1}, there is a significant difference between the multi-channel laser acupuncture group and the laser combined with electric stimulation group compared with the recovery period and fatigue period ($P < 0.05$), suggesting that the laser acupuncture group The multi-channel laser acupuncture group and the laser combined with electrical stimulation group may reduce the urea accumulation level after fatigue by reducing the protein oxidation function, which is consistent with the conclusions of the existing literature.[37] The results showed that the therapeutic effect of laser electric stimulation group is better than that of laser acupuncture group ($P < 0.05$), and there is no significant difference among other groups ($P > 0.05$).

Creatinine is the product of creatine metabolism during long-term exercise, and creatine and creatine phosphate can efficiently produce ATP for body energy supply. During fatigue, a large amount of creatine and creatine phosphate are consumed and accumulated. Much more creatinine are produced and increased the burden on the kidney. Through the value of 1365 cm^{-1} Raman spectral peak of mouse blood, creatinine could be reduced during fatigue recovery ($P > 0.05$), in the laser acupuncture group, multi-channel laser acupuncture group and laser combined with electrical stimulation group. This suggests that laser stimulation can reduce creatinine, a fatigue metabolite. The content of creatinine is related to exercise fatigue and recovery process.[38,39] The effect of laser combined with electric stimulation group is better than that of laser acupuncture group and multi-channel laser acupuncture group ($P > 0.05$), and the effect of laser acupuncture group and multi-channel laser acupuncture group is not obvious ($P > 0.05$).

The peaks of 754, 1561 and 1636 cm^{-1} (see Fig. 10) could be assigned to porphyrin in red blood cells (see Table 3 for details). Porphyrin, as a key component of heme in hemoglobin, is related to blood cells and plays an important role in the transport of oxygen in blood in organisms, acupuncture physiotherapy could increase the content of hemoglobin in fatigue recovery.[40] In this paper, the peak strength of each group before and after fatigue is compared. The peak values of 1561 and 1636 cm^{-1} are obviously changed, and these two peaks are selected for statistical analysis. Compared with the control group, laser acupuncture group, multi-channel laser acupuncture group and laser combined electrical stimulation group, the hematoporphyrin in 1561 and 1636 cm^{-1} Raman peaks increased (see Fig. 12).



(a) Fatigue and recovery peak at 1561 cm^{-1} (b) Fatigue and recovery peak at 1636 cm^{-1}

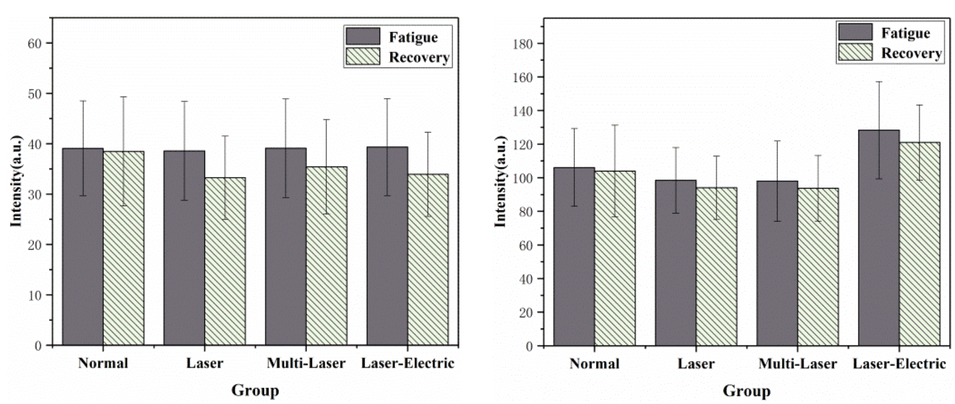
Note: $^{*}P < 0.05$, recovery in the group compared with fatigue; α indicates $P < 0.05$, difference between groups (pairwise comparison).

Fig. 12. Characteristic peak intensity of respiratory metabolites during fatigue and recovery in mice.

Among them, the laser combined electrical stimulation group had a statistical difference in the recovery period compared with the fatigue period ($P < 0.05$), and the recovery period of other groups increased compared with the fatigue period ($P > 0.05$). The results show that laser stimulation is conducive to increase the content of hemoglobin, which may be related to the exchange rate of O_2/CO_2 during recovery. The peak intensities of 1561 and $1636\,cm^{-1}$ show that the effect of laser combined with electrical stimulation group is better than that of laser acupuncture and multi-laser group. At the peak of $1561\,cm^{-1}$, there is a significant difference between laser combined with electrical stimulation group and laser acupuncture and multi-laser group ($P < 0.05$). There is no significant difference between laser acupuncture group and multi-channel laser acupuncture group ($P > 0.05$).

Excessive fatigue could lead to the increase of blood lipid concentration,[41] and laser acupuncture has been used to therapy the obesity and hyperlipidemia.[42] Blood lipid is explored to evaluate the laser acupuncture on fatigue recovery. The characteristic peaks related to lipid are 1078 and $1123\,cm^{-1}$ (see Table 3 for details). In Fig. 10, Raman spectral peaks of fatigue mice blood have lower intensity at $1078\,cm^{-1}$ and higher intensity at $1123\,cm^{-1}$ than that of the recovery mice blood. The Raman spectral peak intensities at 1078 and $1123\,cm^{-1}$ are analyzed statistically (Fig. 13). The results show that the laser acupuncture group, multi-channel laser acupuncture group and laser combined with electrical stimulation group decreased the lipid related substances in the blood of mice during the recovery period ($P > 0.05$). This indicated that the laser acupuncture stimulation has an effect on lipid substances in blood during the fatigue recovery. In addition, the peak intensities of 1078 and $1123\,cm^{-1}$ showed that the effect of laser combined with electrical stimulation is better than that of laser acupuncture group and multi-channel laser acupuncture group, but there is no significant difference between laser acupuncture group and multi-channel laser acupuncture group, indicating that the therapeutic effect of laser combined with electrical stimulation is better than that of laser acupuncture group and multi-channel laser acupuncture group.

Bioelectrical signals and biochemical indicators are commonly used criteria for evaluating fatigue at present.[7] In this paper, the time domain and frequency domain indicators of EMG signals are used to study the recovery from exercise fatigue with mice model. Some studies show that the time domain indicators in muscle recovery period are less than those in fatigue period,[43] and the frequency domain indicators are more than those in fatigue period, which is consistent with the analysis results of this study. During muscle fatigue, muscle fibers need to collect more energy to increase the amplitude of EMG signal. In the fatigue recovery stage, there is no need to provide a large number of recruitment units, resulting in the decrease of EMG amplitude, so as to reduce the values of IEMG and RMS. In this paper, the decrease of RMS is greater than IEMG. In addition, with the deepening of muscle fatigue, muscle fibers are not enough to support muscle movement, resulting in the reduction of the frequency of EMG signals, which reduces the values of MPF and MF. In the fatigue recovery



(a) Fatigue and recovery peak at $1078\,cm^{-1}$ (b) Fatigue and recovery peak at $1123\,cm^{-1}$

Fig. 13. Characteristic peak intensity of lipid related substances in mouse blood Raman spectrum.

stage, the biological self-regulation ability makes the muscle fibers recover gradually, and the values of MPF and MF increase. In this paper, the increase of MPF is greater than that of MF, which may be related to the fact that MPF index is more sensitive to muscle activity and function than MF index in practical application.[44] According to the data analysis results, it is more suitable to use RMS and MPF which can reflect the amplitude changes of overall EMG signal to explore the time-domain signal changes of EMG in mice during recovery, and the treatment effect of laser electric group is better.

4. Conclusions

In this study, laser acupuncture combined with electrical stimulation was used to fatigue recovery after mice exercise, and the effect was analyzed by EMG and Raman spectroscopy. The results showed laser acupuncture combined with electrical stimulation induced obvious changes in RMS and MPF of EMG signal. Different stimulation methods could regulate the body metabolites in the blood during fatigue recovery, in which the contents of uric acid, urea, creatinine and lipid in the blood were reduced, and the contents of respiratory related porphyrins were increased. The results showed that laser acupuncture combined with electrical stimulation could have a beneficial effect on fatigue recovery in mice, and it was better than that of single laser acupuncture and multi-laser acupuncture.

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Conflict of Interest

The authors have no conflicts of interest relevant to this article.

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