Establishment of a murine, lipopolysaccharide-induced sepsis model for testing anaerobic exercise thresholds and early mobilization

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Research

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Abstract

Background: Several methods have been proposed to prevent post intensive care syndrome (PICS) after sepsis, including early mobilization, but controversy remains in mechanisms and outcomes. Translational studies are required to fully reveal the optimal exercise intensity during acute phase sepsis. The aim of this study is to establish a septic mouse model and and classify exercise intensi ties during the acute phase of sepsis.

Methods: Adult, male C57/B6 mice received lipopolysaccharide (LPS) injections (20mg/kg). We recorded survival rates and metabolic changes, such as resting energy expenditure (REE) compared with controls up to 72h after LPS administration. We also observed vital signs (rectal temperature and weight change) and inflammation (TNF- α and IL-6). We detected exercise intensity via metabolic monitoring treadmill, analyzed the anaerobic threshold (AT) by V-Slope Method and classified the intensity levels according to American College of Sports Medicine (ACSM) guidelines.

Result: Around 30% of the mice survived 72h after LPS induction with REE and rectal temperatures lowest at around 22-24h later (REE at 22h: 2.26 ± 0.34 kcal/day $p < 0.05$ vs. baseline; Rectal temperature at 24h: $29.17 \pm 0.48^\circ\text{C}$, $p < 0.05$ vs. baseline). Gradual recovery was seen out to 72h. Blood concentrations of TNF- α were highest at the 12h timepoint (41.23 ± 24.39 pg/ml, $p < 0.05$ vs. baseline) and IL-6 was highest at 24h after LPS induction (1476.5 ± 274.7 pg/ml, $p < 0.05$ vs. baseline). Using the V-Slope method, we observed the AT at each timepoint (6h:14m/min,12h: 9m/min, 24h:4m/min,36h:10m/min, 48h:16m/min,60h:20m/min,72h:22m/min) and classified these into very low intensity exercise training (VLIT), low intensity exercise training (LIT), or moderate intensity exercise training (MIT) at each timepoint.

Conclusion: We classified exercise intensities out to 72h after sepsis recovery using a mouse model. These data may serve as a bridge to clinical studies to fill gaps in best practice for sepsis rehabilitation.

Background

Sepsis is a critical life-threatening condition with over 19 million people affected worldwide and a mortality reported at 26%(1). Even after discharge, one-third of sufferers die and, for those who survive longer than 6 months suffer(2) multiple complications, including cognitive dysfunction(3) and muscle weakness (ICU-acquired weakness [ICU-AW])(4). Recently, these complications have been well studied and named post intensive care syndrome (PICS) at the 2012 SCCM Conference(5).

Current sepsis best practice attempts to control mortality and quality of life and thus the relationship between sepsis and PICS has been targeted by the intensive care field as evidenced by several reported methods for preventing PICS. Early mobilization, or exercise after recovery, is a well-known prevention method to prevent sepsis complications (6) and the American College of Critical Care Medicine (ACCM) emphasizes that early mobilization plays a key role for preventing delirium(7). Early mobilization is defined as applied physical activity within the first 2 to 5 days of critical illness or injury(8), and a recent

randomized, clinical study revealed that early mobilization during septic shock prevents muscle atrophy(9). Implicated pathways for muscle atrophy during sepsis include the mammalian target of rapamycin (mTOR)(10), interleukin-6 (IL-6)(11), and tumor necrosis factor-alpha (TNF-α)(12). However, conflicting studies report that early mobilization is beneficial for preventing muscle weakness(9) but may increase mortality(13). Additionally, potential prognostic markers have recently been investigated in septic shock patients(6). Therefore, to reveal critically needed hard outcomes, including mortality and sepsis progression mechanisms, translational studies in humans and animal models are required. Thus, the aim of this study is to find the appropriate exercise intensity for early mobilization in a severe septic mouse model.

Materials And Methods

Mouse Model

Male C57/BL6 mice (24–26 g, 11 weeks old) were purchased from CLEA, Tokyo, Japan for use in this study. Sepsis was induced by the intraperitoneal (IP) administration of bacterial LPS from *Escherichia coli* strain 055: B5 (20 mg/kg) dissolved in sterile saline. This LPS-induced sepsis model has been used previously used to generate animal models of sepsis(14–16). Mice were kept on a 12 hr:12 hr light:dark schedule at an ambient temperature of $23 \pm 1^\circ\text{C}$ under specific pathogen-free conditions.

Time course of metabolism and survival experiment

From our previously published experience with this LPS-induced sepsis model(14–16), 72h metabolism and 72h survival analyses were performed using a metabolic cage that automatically calculates VO₂ and VCO₂ (Muromachi, Tokyo, Japan). The measurement interval was set at 3 minutes and cage air flow was set at 0.5L/min. We used the hourly average of VO₂ and VCO₂ in our calculations. The cage was not opened during the observational period and standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and tap water were provided *ad libitum*. We calculated the resting energy expenditure (REE) using Lusk's formula(17) as follows: $\text{REE} = 3.816 \times \text{VO}_2 + 1.231 \times \text{VCO}_2$.

Time course of vital signs and inflammation

To analyze the status of inflammation after LPS administration over 72 hours, mice were divided into 5 groups: controls (n = 10), 12h (n = 10), 24h (n = 10), 48h (n = 10) and 72h (n = 10) post-LPS administration. We measured rectal temperatures and weight loss rate plus we harvested blood to analyze representative inflammation proteins TNF-α and IL-6 by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN).

Anaerobic Threshold test and defining intensity of exercise in sepsis model

The anaerobic threshold (AT) is the transition point from predominantly aerobic to anaerobic metabolism, (18) which occurs at around 60% of the maximum oxygen uptake capacity (max VO_2)(19). With regard to intensity, we adopted recommendations of the American College of Sports Medicine (ACSM), which defines exercise over the AT (60% of the max VO_2) as vigorous, intensity of under one-third as very low intensity exercise training (VLIT), between one-third to two-thirds as low intensity training (VIT), and between two-thirds to the AT as moderate intensity training (MIT).(20) All mice underwent 30 minutes of treadmill exercise (25m/min) three times per week for one week to become accustomed to the exercise protocol and handling. We performed exercise experiments three times on the first day after LPS administration (6h, 12h, 24h after induced LPS), two times on the second day after LPS administration (36h, 48h after induced LPS) and on the third day after LPS administration (60h, 72h after induced LPS). A metabolic monitoring, motor-driven, two-lane treadmill chamber was used for AT testing. The exercise protocol started at 1m/min and the exercise intensity was then increased by 1 m/min (1–10 m/min) every three minutes. From the 10m/min point, the exercise intensity was increased by 2 m/min every three minutes up to the all-out value. We defined this all-out value as the point where mice could not keep running even with encouragement. Anaerobic Threshold (AT) was evaluated by Ventilation Threshold (VT), a representative method for estimating AT, and derived by the V-Slope Method, which evaluates the point at which the increase in carbon dioxide production (VCO_2) is greater than the increase in oxygen uptake (VO_2) (21, 22). Briefly, we used each speed point as a cut off and calculated aerobic exercise slope and anaerobic exercise. Then, total residuals were calculated (residuals of aerobic exercise slope and anaerobic exercise slope) and we defined the minimum total residuals cut point which indicated the best fit for the two variable lines as the AT point. (Figure 1) The R-squared fit value for each regression line is also shown in Figure 1.

Experiment flow

The total number of mice used in these experiments was eighty. Twenty mice were used to analyze metabolic differences between sepsis ($n = 10$) and controls ($n = 10$) within 72h after LPS administration. Ten mice were used for the exercise tolerance experiment within 72h after LPS administration. A total of fifty mice were used to analyze inflammation and vital signs in control ($n = 10$), 12h ($n = 10$), 24h ($n = 10$), 48h ($n = 10$) and 72h ($n = 10$) after LPS administration groups. The Institutional Review Board (IRB) of the University of Tsukuba Affiliated Hospital approved all aspects of the study (Approval # 18–132) and we obeyed The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guideline(23) for all the procedures.

Statistical Analysis

Results are expressed as mean $\pm$ SE and, for parametric distribution, two-sided analysis of variance (ANOVA) testing was used. For non-parametric distribution among two groups, Kruskal-Wallis testing, followed by Steel-Dwass testing for multiple comparisons, was used. Survival data was analyzed by log-rank testing and $p < 0.05$ was considered statistically significant for all parameters. All statistics were performed in R software (<http://www.r-project.org>).

Results

Time course of metabolism and survival experiment

We evaluated survival after LPS administration over multiple timepoints until 72h. Within the LPS group, 70% of the mice died around 24h after LPS administration and 30% survived while 100% of the control mice survived. ($p < 0.01$) As shown in Figure 2, REE in the control group was synchronized to circadian rhythms in the control group. However, REE in the LPS group dramatically decreased at roughly 12h after LPS induction of inflammation (5.76 ± 0.49), was lowest at the 22h post-LPS timepoint (2.26 ± 0.34) and gradually recovered, although not to baseline, at 24h (2.26 ± 0.34), 48h (3.3 ± 0.4) and at 72h after LPS administration (3.58 ± 0.41).

Time course of vital signs and inflammation

Data of rectal temperatures and weight change rates until 72h after LPS induction are shown in Figure 3. The average rectal temperature before LPS (baseline) was $37.31 \pm 0.17^\circ\text{C}$ but dramatically decreased 12h after LPS induction ($31.15 \pm 0.67^\circ\text{C}$, $p < 0.05$ vs. baseline), was lowest 24h after LPS induction ($29.17 \pm 0.48^\circ\text{C}$, $p < 0.05$ vs. baseline) and gradually recovered at 48h ($32.31 \pm 0.46^\circ\text{C}$, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 24h after LPS induction) and 72h timepoints ($34.04 \pm 0.28^\circ\text{C}$, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 24h after LPS induction) although it did not return to baseline. Weight change rates were significantly decreased from the baseline at 12h ($92\% \pm 0.5\%$, $p < 0.05$ vs. baseline), 24h ($90\% \pm 0.5\%$, $p < 0.05$ vs. baseline), 48h ($83\% \pm 0.6\%$, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 12h after LPS induction, $p < 0.05$ vs. 24h after LPS induction), and 72h post-LPS timepoints ($76\% \pm 0.5\%$, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 12h after LPS induction, $p < 0.05$ vs. 24h after LPS induction, $p < 0.05$ vs. 48h after LPS induction).

Serum TNF- α and IL-6, representative inflammatory cytokines, experienced dramatic LPS-induced changes as shown in Figure 4. TNF- α levels were highest 12h after LPS induction (41.23 ± 24.39 pg/ml, $p < 0.05$ vs. baseline) and decreased by the 24h timepoint (14.37 ± 7.85 pg/ml, $p < 0.05$ vs. baseline). Levels then normalized 48h after LPS induction. IL-6, on the other hand, significantly increased 12h after LPS induction (1133.8 ± 182.4 pg/ml, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 72h after LPS induction), was highest at 24h after LPS induction (1476.5 ± 274.7 pg/ml, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 48h after LPS induction, $p < 0.05$ vs. 72h after LPS induction) but significantly decreased at the 48h timepoint (277.7 ± 160.8 pg/ml, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 24h after LPS induction) and did not fully normalize until 72h after LPS administration (56.6 ± 24.2 pg/ml, $p < 0.05$ vs. baseline, $p < 0.05$ vs. 24h after LPS induction).

Anaerobic Threshold (AT) test and defining intensity of exercise in sepsis model

AT test results are shown in Figures 5,6, and 7. Overall, the AT threshold decreased 24 h after LPS induction and gradually recovered out to the 72 h timepoint. At 6h after LPS induction, AT, as defined by the minimum total residuals cutoff point, was found to be 14 m/min and this dropped to 9m/min at the 12 h timepoint and 4 m/min at the 24 h timepoint. However, it recovered to 10 m/min at the 36 h timepoint, 16 m/min at the 48 h timepoint, 20 m/min at the 60 h timepoint and 22 m/min at the 72 h timepoint.

From these results, we classified activity into either VLIT (less than one-third the intensity of AT), LIT (from one-third to two-thirds the intensity of AT) or MIT (from two-thirds the intensity of AT to max AT) as follows: VLIT: 0 ~ 4 m/min, LIT: 5 ~ 9 m/min, MIT: 10 ~14 m/min at 6 h after LPS induction; VLIT: 0 ~ 3 m/min, LIT: 4 ~ 6 m/min, MIT: 7 ~ 9 m/min at 12 h after LPS induction; VLIT: 0 ~ 1 m/min, LIT: 2 m/min, MIT: 3 ~ 4 m/min at 24 h after LPS induction; VLIT: 0 ~ 3 m/min, LIT: 4 ~ 6 m/min, MIT: 7 ~10 m/min at 36 h after LPS induction; VLIT: 0 ~ 5 m/min, LIT: 6 ~ 10 m/min, MIT: 11 ~16 m/min at 48 h after LPS induction; VLIT: 0 ~ 6 m/min, LIT: 7 ~ 13 m/min, MIT: 14 ~ 20 m/min at 60 h after LPS induction; and VLIT: 0 ~ 8 m/min, LIT: 9 ~ 16 m/min, MIT: 17 ~22 m/min at 72 h after LPS induction. (Figure 8)

Discussion

Discussion

This study is the first to reveal the effect on exercise capacity in the acute phase of a murine, LPS-induced sepsis model. Several animal studies reported on exercise before the onset of sepsis(24, 25), but did not fully reveal effects of sepsis on exercise capacity that would be useful in exploring benefits of early mobilization.

Our LPS-induced sepsis model showed the lowest metabolic condition at the 22h timepoint after a reactive TNF- α and IL-6 cytokine surge 12h after induction that peaked at the 24h timepoint(26). Rectal temperature changes supported this observation and the protective immune reaction(27), as seen in previous reports, resulted in a lower metabolic condition/REE(28). The 70% mortality seen 24h after LPS administration is also in line with literature reports. For survivors, the 24h to 72h post-LPS timepoint sees improvements in inflammation status and AT but weight loss persists. This time course after sepsis is well described in humans,(29) indicating that our model is suitable for simulating the septic cascade.

In line with the inflammation results, the observed AT threshold diminished up to 24h after LPS induction but gradually recovered from then until the 72h timepoint. As a new diagnostic criterion for sepsis includes increases in lactate levels attributed to anaerobic metabolism, this seems to be in line with the 3-day recovery needed for AT.(30) Excess lactate caused by inflammation within the muscle tissue could adversely affect VO2 max and decrease fitness as seen in our results. Additionally, IL-6 is thought to be a

suitable marker for septic response and, in our model, IL-6 and AT changes occurred in parallel which indicates that appropriate exercise intensities progress in a condition-dependent fashion. Surprisingly, IL-6 is recognized as a myokine (a cytokine released by myocytes in muscle tissue) and plays roles in autocrine regulation of metabolism in muscle and other endocrine organs.(31) Skeletal muscle has a unique function as an acute sensor of external stress signals via IL-6 expression(32). In particular, during sepsis induced by LPS, the liver contains the major portion of tissue-bound LPS (~40%) but muscle contains the second highest tissue-bound LPS (~5%)(33). This relationship between muscle condition and IL-6 also supports the change in AT we observed over 72h after LPS induction.

Randomized, controlled trials in for early phase exercise in severely ill patients have utilized electrical muscle stimulation (EMS)(13), ambulation(13), walking(34), and therapeutic exercises up to MIT to improve survival rate and prevent subsequent complications. However, paradoxically, even though the stepwise progression of rehabilitation within these studies progresses from VLIT to MIT, there are scarce reports on which levels were most effective. Meanwhile, the effect of VLIT and LIT to improve health outcomes was epidemiologically examined by the National Health and Nutrition Examination Survey (NHANES) in the elderly(35). Cellular-level experiments showed that LIT improved the regenerative capacity of skeletal muscle in humans(36) and short-distance, voluntary exercise activated brain-derived neurotrophic factor (BDNF) signaling in the hippocampus, enhancing spatial memory in rats(37). A functional MRI study in humans found that only 10 minutes of VLIT was needed to improve hippocampal function(38). The clear benefits of exercise in the acute phase of sepsis still need to be revealed both clinically and fundamentally, but VLIT and LIT (e.g., sitting at the edge of a bed, standing/transfer, ADL Training) may be as valuable as MIT for improving the vital physical and cognitive functions of sepsis survivors.

Clinically, sepsis may adversely affect physical, cognitive, psychological function in survivors, a condition known as post-intensive care syndrome (PICS)(5). Various studies have sought the effect of early rehabilitation on mortality and PICS(34, 39, 40) while several systematic and Cochrane reviews(41–43) have been conducted over the past three years but none have fully revealed associations between early rehabilitation and outcomes. These studies are weakened by a reliance on observational studies (and subsequent inability to remove demographic bias) since randomized, controlled trials are difficult to conduct in sepsis cases. (44) On the other hand, animal studies allow for precise control of variables that remove bias in results(45, 46). There has been a recent push for animal studies that reveal not only physical and cognitive changes in sepsis, but also psychological function, represented as post-traumatic stress disorder, using mice models(47–51). Our establishment of a mouse model that allowed for exploration into the appropriateness of early mobilization and could easily be adapted for translational studies in sepsis that explore clinically relevant biomarkers (such as lactate) or exercise-related parameters.

Conclusion

We established an LPS-induced, severe sepsis mouse model appropriate for evaluation of early mobilization in sepsis and classified exercise intensities (VLIT, LIT, MIT) for 72h after LPS administration.

Declarations

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Ethical Approval and Consent to participate

The Institutional Review Board (IRB) of the University of Tsukuba Affiliated Hospital approved all aspects of the study (Approval # 18–132).

Authors' contributions

YM designed the study and carried out sample collection, data analysis, and wrote the manuscript. NS, HH, KM and YE participated in sample collection. SO, JS and SM support study management, and SK, SI and YI participated in designing study. BM support writing manuscript. All authors read and approved the final manuscript.

Competing interests

None.

Availability of supporting data

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable

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Figures

Example of the figure of V-slope and exercise intensity

Figure 1

Example V-slope and exercise intensity

Figure 2

Survival rate and transition of metabolism Hourly average number of VO2 and VCO2 was used to calculate the resting energy expenditure (REE) using Lusk's formula.

Figure 3

Rectal temperature and weight change rate

A. Blood TNF-α

	Number of subjects at each measurement time point									
	0h		12h		24h		48h		72h	
	Average	SE	Average	SE	Average	SE	Average	SE	Average	SE
Blood TNF-α level	0	0	41.23	24.39	14.37	7.85	0	0	0	0

*p < 0.05 vs. control, † p < 0.05 vs. LPS induced 12h. # p < 0.05 vs. LPS induced 24h.
§ p < 0.05 vs. LPS induced 48h. ‡ p < 0.05 vs. LPS induced 72h.

B. Blood IL-6

	Number of subjects at each measurement time point									
	0h		12h		24h		48h		72h	
	Average	SE	Average	SE	Average	SE	Average	SE	Average	SE
Blood IL-6 level	0	0	1133.8	182.4	1476.5	274.7	277.7	160.8	56.6	24.2

*p < 0.05 vs. control, † p < 0.05 vs. LPS induced 12h. # p < 0.05 vs. LPS induced 24h.
§ p < 0.05 vs. LPS induced 48h. ‡ p < 0.05 vs. LPS induced 72h.

Figure 4

Inflammation as represented by blood cytokines

A. Anaerobic Threshold after 6h induced LPS

Number of subjects at each measurement time point

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	23.7	17.6	-	-	-
2m/min	36.7	27.4	0	71.2	71.2
3m/min	34.7	24.8	0.7	248.5	249.2
4m/min	36.4	25.4	1.7	228.9	230.6
5m/min	36.5	26	1.8	211.3	213.1
6m/min	36.6	26.8	2	197.5	199.5
7m/min	36.8	28.1	4.2	189.6	193.8
8m/min	39	28.1	4.3	163.8	168.1
9m/min	44.6	35	9.2	152.2	161.4
10m/min	47.8	37.5	10.8	133.3	144.1
12m/min	48.1	37.5	11.1	101.2	112.3
14m/min	48.5	37.7	11.2	43.4	54.6*
16m/min	53.8	46.8	-	-	-
18m/min	60.9	62.9	-	-	-

B. Anaerobic Threshold after 12h induced LPS

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	17.2	14	-	-	-
2m/min	28	21.8	0	11816.7	11816.7
3m/min	31	24.6	0.1	193.2	193.3
4m/min	31.3	25	0.2	186.1	186.3
5m/min	36.8	27.3	1	157.9	158.9
6m/min	39.1	31.4	2.1	138.9	141
7m/min	41.7	32.3	2.1	93.9	96
8m/min	41.7	32.8	2.2	33.8	36
9m/min	45.6	37.9	24.7	9.4	34.2*
10m/min	47.8	47.2	-	-	-
12m/min	50	55	-	-	-

C. Anaerobic Threshold after 24h induced LPS

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	15.3	13.4	-	-	-
2m/min	27.3	22.6	0	8403.5	8403.5
3m/min	31.5	26.7	0.2	33.5	33.7
4m/min	35.7	29.1	0.4	21.4	21.8*
5m/min	41.1	34.8	-	-	-
6m/min	42.5	42.7	-	-	-

* most fitted point : Anaerobic Threshold

Figure 5

First day of the AT as estimated by V-slope

A. Anaerobic Threshold after 36h induced LPS

Number of subjects at each measurement time point

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	28.7	28.2	-	-	-
2m/min	37	35.3	0	1212.6	1212.6
3m/min	41.6	37.5	0.8	112.2	113
4m/min	48.5	37.9	8.9	110.8	119.7
5m/min	51.3	40.7	8.9	110.	118.9
6m/min	54.5	42.9	9.1	108.3	117.4
7m/min	55.5	42.9	9.1	102.9	112
8m/min	56.2	43.2	9.1	93.4	102.6
9m/min	57.1	44.3	9.3	81.7	91
10m/min	61.6	46.4	9.3	40	49.3*
12m/min	65.4	52.9	-	-	-
14m/min	69.8	65.7	-	-	-

B. Anaerobic Threshold after 48h induced LPS

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	34.8	31.6	-	-	-
2m/min	50.4	38.5	0	74.7	74.7
3m/min	60.3	46	1.6	63.1	64.7
4m/min	67.1	43.2	16.2	52.8	69
5m/min	62.1	43.3	16.3	52.7	69
6m/min	63.8	43.5	16.7	52.2	68.9
7m/min	64.2	43.8	16.8	51.4	68.3
8m/min	65	44	16.9	50	66.9
9m/min	66.7	45.9	17.5	49.5	67
10m/min	67.3	46.2	18	48.7	66.7
12m/min	68.3	46.6	18.4	46.9	65.3
14m/min	72.7	48	18.4	33.5	51.9
16m/min	73.2	49.4	19.8	20.3	40.1*
18m/min	80.5	55.9	-	-	-
20m/min	83.3	64.2	-	-	-

* most fitted point : Anaerobic Threshold

Figure 6

Second day of the AT estimated by V-slope

A. Anaerobic Threshold after 60h induced LPS

Number of subjects at each measurement time point

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	40.7	33.8	-	-	-
2m/min	50.3	40.3	0	532.3	532.3
3m/min	55.2	40.8	2.2	1244.9	1247.1
4m/min	53.3	43.2	6.4	1239.1	1245.5
5m/min	53.6	40.2	8	1204.8	1212.8
6m/min	53.7	42.2	8.6	1186.7	1195.3
7m/min	53.8	43.1	10.3	1172.7	1183
8m/min	56.5	43.1	10.4	1131.3	1141.7
9m/min	60.1	43.7	12.4	1036.7	1049.1
10m/min	60.2	44	13.1	926.1	939.2
12m/min	61.7	48	17.9	846.8	864.8
14m/min	62	49	23.9	762.1	786
16m/min	66	50.2	24.7	581.8	606.5
18m/min	70.7	58.6	49.4	433.9	483.3
20m/min	76.8	66	77.8	212.1	290*
22m/min	82.3	79.2	-	-	-
24m/min	85.5	103.2	-	-	-

B. Anaerobic Threshold after 72h induced LPS

Speed	Vo2	Vco2	Slope 1 residuals	Slope 2 residuals	Total residuals
1m/min	42.37	35.5	-	-	-
2m/min	47.8	38.7	0	699.7	699.7
3m/min	48.1	39.8	0.4	668.7	669.1
4m/min	49.7	41.7	1.2	633.9	635.1
5m/min	51.3	41.3	1.8	619.1	620.9
6m/min	53.1	44.3	2.6	595.2	597.8
7m/min	54	46	4.2	557.3	561.5
8m/min	55.3	46.6	4.3	523.5	527.8
9m/min	56.7	47	4.5	496.6	501.1
10m/min	57.8	49.1	5	446.1	451.1
12m/min	59.3	49.3	5.2	395.7	400.9
14m/min	62.2	52.1	5.2	340.5	345.7
16m/min	64.8	53.7	5.6	279.7	285.3
18m/min	68.1	57.3	5.7	126	131.7
20m/min	77	67.2	9	68.1	77.1
22m/min	82	77	35.1	1.7	36.8*
24m/min	87.5	89	105.3	0	105.3
26m/min	88.2	95	-	-	-
28m/min	93	120	-	-	-

* most fitted point : Anaerobic Threshold

Figure 7

Third day of the AT estimated by V-slope

Number of subjects at each measurement time point

	6h	12h	24h	36h	48h	60h	72h
VLIT	0~4 m/min	0 ~ 3 m/min	0 ~ 1 m/min	0 ~ 3 m/min	0 ~ 5 m/min	0 ~ 6 m/min	0 ~ 8 m/min
LIT	5~9 m/min	4 ~ 6 m/min	2 m/min	4 ~ 6 m/min	6 ~ 10 m/min	7 ~ 13 m/min	9 ~ 16 m/min
MIT	10 ~ 14 m/min	7 ~ 9 m/min	3 ~ 4 m/min	7 ~ 10 m/min	11 ~ 16 m/min	14 ~ 20 m/min	17 ~ 22 m/min

Figure 8

Appropriate intensity of exercise for early mobilization as determined by the V-Slope Method AT was detected at each time point and classified as very low intensity exercise training (VLIT), low intensity exercise training (LIT), or moderate intensity exercise training (MIT).