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# Dynamic metabolic changes observed in an LPS-induced systemic inflammation rat model using continuous long-term indirect calorimetry experiments

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## Abstract

**Background**: Nutritional management is crucial for severely ill patients. Measuring metabolism is believed to be necessary for the acute sepsis phase to accurately estimate nutrition. Indirect calorimetry (IDC) is assumed to be useful for acute intensive care; however, there are few studies on long-term IDC measurement in patients with systemic inflammation.

**Methods**: Rats were categorized into the lipopolysaccharide (LPS) received or control groups; LPS rats were categorized into underfeeding (UF), adjusted feeding (AF), and overfeeding (OF) groups. IDC measurement was performed until 72 or 144 hours. Body composition was measured at -24 and 72 or 144 hours and tissue weight was measured at 72 or 144 hours.

**Results**: Low energy consumption and loss of diurnal variation of resting energy expenditure (REE) were observed in the LPS group compared with the control group until 72 hours, after which the LPS group recovered. The REE in the OF group was higher than that in the UF and AF groups. In the first phase, low energy consumption was observed in all groups. In the second and third phases, higher energy consumption occurred in the OF group than in the UF and AF groups. In the third phase, diurnal variation recovered in all groups. Muscle atrophy caused body weight loss but fat tissue loss did not occur.

**Conclusions**: We observed metabolic changes with IDC during the acute systemic inflammation phase owing to differences in calorie intake. This is the first report of long-term IDC measurement using the LPS-induced systemic inflammation rat model.


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**Keywords**: intensive care, nutrition management, respiratory gas analysis, rodent model, metabolic observation

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## Introduction

Sepsis is one of the most severe and complex conditions for patients, with a high mortality rate in the current medical environment. Early recovery requires radical treatments, such as drainage, debridement, and administration of antibacterial drugs. In addition, there is awareness of the importance of nutritional management for patients with sepsis. Nutrition can be considered the foundation for patient treatment, and nutrition therapy is mentioned in clinical settings as major guidelines for critical care.[1-3] Although various studies have been conducted on metabolism and nutrition in critically severe situations, nutritional therapy for critically ill patients, such as those with sepsis, is poorly understood.[4] According to guidelines, approximately 60%–70% of the normal calorie intake is sufficient for approximately 1 week after the invasion. However, a few studies have indicated that the prognosis of those underfeeding or overfeeding is poor.[5,6]

There is limited knowledge about which nutritional substrate should be used in the acute, subacute, and recovery phases of sepsis.[7-9] In the acute phase of sepsis, few studies have demonstrated the importance of optimal nutrition and avoiding under- or over-nutrition. Furthermore, the guidelines recommend using indirect calorimetry (IDC) to treat critically ill patients.[1,2] However, which index should be used to understand the patient's condition and calculate the optimal energy is still unclear. Although significant changes occur in the body

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during the hyperacute phase of sepsis, no reports include continuous or long-term measurement, although many studies have used IDC for a short time or in spots.[10]

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The loss of muscle mass in the acute phase of sepsis, termed Intensive Care Unit-acquired weakness (ICU-AW) or post-intensive care syndrome (PICS), adversely affects recovery[10-12]. Muscle loss and metabolic alterations occur during sepsis; however, the clinical relevance of the two is not well-documented. Various studies have been conducted to answer above questions; however, definite explanations have not been given to date in basic or clinical research.[13-15]

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We established an experiment with continuous IDC measurement during the acute phase of systemic inflammation. The aims of this study were to: 1. investigate continuous long-term IDC in rats; 2. measure the difference in calorie intake using IDC and a body composition analyzer in a rat systemic inflammation model; and 3. examine a systemic inflammation model in rats using IDC and a body composition analyzer and tissue weight measurement.

**Methods**

**Reagents**

Lipopolysaccharide (LPS; from *Escherichia coli* O111:B4) was obtained from Sigma–

Aldrich (St. Louis, MO, USA). Intravenous solutions, 70% glucose, and AMIPAREN injection were obtained from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Intravenous fluids used for each group were prepared as presented in Table 1. All amino acids contained in the infusion were prepared using AMIPAREN (Supplement Table 1, http://links.lww.com/SHK/B701).

**Rats**

Seven-week-old male SD rats (Japan SLC, Hamamatsu, Japan) were acclimatized in aluminate cages for 5–7 days under a 12-hour light-dark cycle (light phase, 7:00 to 19:00; dark phase: 19:00 to 7:00) and constant temperature (23°C ± 3°C) and humidity (65% ± 15%) before use in experiments. Rats had free access to water and food pellets (CRF-1 pellets, Oriental Yeast Co., Ltd., Tokyo, Japan). This study was conducted following the ethical guidelines of the Ehime University Animal Experimentation Committee and Otsuka Pharmaceutical Factory, Inc. and in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Systemic inflammatory rat model**

An intravenous LPS infusion model was used as a rat septic model. One day before starting measurement with IDC, an intravenous catheter was inserted through the external jugular vein



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into the superior vena cava under intraperitoneal anesthesia (5 mL/kg BW). Intraperitoneal anesthesia included midazolam (2.0 mg/mL), medetomidine hydrochloride (0.15 mg/mL), and butorphanol tartrate (2.5 mg/mL). The tubing was tunneled subcutaneously and excited through a stab wound in the skin of the mid-scapular region. Furthermore, the externalized tubing was routed through a Teflon button and stainless-steel spring attached to a flow through the swivel. After catheterization, the rats were randomly assigned to each group and placed in the IDC cages. After that, all rats were administered intravenous fluid at 210 mL/kg BW/day without LPS. On the following day of catheterization, rats were administered intravenous fluid at 210 mL/kg BW/day with or without LPS 45 μg/kg BW/h.

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**Dietary regimens**

Rats received total parenteral nutrition. LPS and control group rats received 172.2 kcal/kg BW/day energy. Furthermore, we determined the caloric dose based on a previous experiment.[16] The adjusted feeding (AF) group was a model of optimal calorie-receiving patients in the acute phase of sepsis, and AF rats received 137.8 kcal/kg BW/day as the basal calorie intake. The underfeeding (UF) group was an undernutrition sepsis model and was established as a group that resembled the low nutritional intake in the acute phase of patients with sepsis. UF rats received 76.4 kcal/kg BW/day (55% of AF group intake). The overfeeding (OF) group was an overnutrition sepsis model and was established as a group that resembled

the high nutritional intake in the acute phase of patients with sepsis. OF rats received 206.6 kcal/kg BW/day (150% of AF group intake). Intake of water and other nutrients, including nitrogen, was equal for all groups.

**IDC through respiratory gas analysis**

IDC through respiratory gas analysis was performed from 0 to 72 or 144 hours using a mass spectrometer for respiratory analysis and a bioprocess monitoring system (ARCO-2000, Arco System, Chiba, Japan). The measurement interval was 10 minutes, and the data obtained when the chamber was opened for each treatment were eliminated because these values could skew the results. The mean of the data collected at every 6-hour point was calculated and used for analysis. We used Lusk's formula and Frayn's formula to calculate resting energy expenditure (REE), carbohydrate oxidation (CHO), fatty acid oxidation (FAO), and respiratory quotient (RQ) rates as follows:

Lusk's formula: REE (kcal/min) = 3.816 * VO2 + 1.231 * VCO2

CHO (mg/min) = 4.55 * VCO2 − 3.21 * VO2

FAO (mg/min) = 1.67 * VO2 − 1.67 * VCO2

RQ = VCO2/VO2



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**Measurement of body composition**

Rats were weighed, and their lean and fat masses were analyzed using a body composition analyzer (EchoMRI-700; Hitachi, Tokyo, Japan) at -24 and 72 or 144 hours.

**Measurement of tissue weight**

Rats were euthanized 72 or 144 hours after the start of LPS administration under isoflurane anesthesia. From the right limb, the soleus muscle, gastrocnemius muscle (GAS), tibialis anterior muscle, and extensor digitorum longus (EDL) muscle were harvested, and epididymal fat was collected from both sides.

**Statistical analysis**

Data are expressed as mean ± standard deviation. If a significant difference existed by paired two-way ANOVA, Student's t-test and Tukey test were used for analysis at each time point of measurement. Log-rank tests were performed to analyze the difference in survival among the groups. Statistical significance was set at $p<0.05$. All statistical analyses were performed using EXSUS Ver.10.0 (EPS Corporation Ltd. Japan).

**Results**

We categorized the acute phase of the LPS reaction into three phases based on the

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experimental results. The first phase (grey zone) was from the start time of LPS injection (0 hours) to 24 hours, the second phase (dotted zone) was from 24 to 72 hours, and the third phase (white zone) was from 72 to 144 hours.

**Effect of LPS infusion on IDC**

REE: Diurnal variation of REE was observed in the control group but not in the LPS group (Figure 1A). Based on the IDC results, we divided the 144 hours from the start of the invasion into three phases. In the first phase, energy consumption in the LPS group was lower than that in the control group. In the second phase, energy consumption in the LPS group was higher than that in the control group. In the third phase, the diurnal variation recovered in the LPS group. However, no significant difference was observed between the groups throughout the phases.

CHO: Diurnal variation of CHO was observed in the control group but not in the LPS group (Figure 1B). In the first phase, the CHO in the LPS group was lower than that in the control group. In the second phase, the CHO in the LPS group was higher than that in the control group. In the third phase, the diurnal variation recovered in the LPS group. A significant difference was observed between the groups.

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FAO: Diurnal variation of FAO was observed in the control group but not in the LPS group (Figure 1C). In the first phase, the FAO in the LPS group was higher than that in the control group. In the second phase, the FAO in the LPS group was lower than that in the control group. In the third phase, the diurnal variation recovered in the LPS group. However, no significant difference was observed between the groups throughout the phases.

RQ: Diurnal variation of RQ was observed in the control group but not in the LPS group (Figure 1D). In the first phase, the RQ in the LPS group was lower than that in the control group. In the second phase, the RQ in the LPS group was higher than that in the control group. In the third phase, the diurnal variation recovered in the LPS group. However, no significant difference was observed between the groups throughout the phases.

The survival proportions in the LPS and control groups were 66.7% and 100%, respectively (Supplemental Figure 1, http://links.lww.com/SHK/B702). No significant difference was observed between the LPS and control groups (p=0.0652).

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**Effects of different calorie intake on IDC**

REE: The REE in the UF and AF groups was comparable; however, the REE in the OF group was higher than that in the other two groups (Figure 2A). All three groups showed almost the

same energy consumption in the first phase. In the second and third phases, higher energy consumption than that in the first phase occurred in the OF group but not in the UF and the AF groups. In the third phase, REE recovered diurnal variation in all three groups.

CHO: The kinetics of CHO in each group are shown in Figure 2B. Throughout the experiment, the higher the intake energy, the higher CHO (Figure 2B). In the first phase, CHO increased once and decreased subsequently. In the second phase, CHO increased and became stable. In the third phase, CHO recovered diurnal variation in all three groups gradually.

FAO: The kinetics of FAO in each group are shown in Figure 2C. Throughout the experiment, the higher the energy intake, the lower FAO (Figure 2C). In the first phase, the FAO was lower than that at the start in the AF and OF groups. In the first and second phases, the FAO in the UF group showed flat graphs. In the second phase, the FAO was almost unchanged from the first phase. In the third phase, FAO recovered diurnal variation in all three groups gradually.

RQ: The kinetics of RQ in each group are shown in Figure 2D. Throughout the experiment, the higher the intake energy, the higher RQ (Figure 2D). In the first phase, RQ was higher than that at the start. In the second phase, RQ was almost unchanged from the first phase. In the third phase, RQ recovered diurnal variation in all three groups gradually.



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The survival proportions in the UF, AF, and OF groups were 66.7%, 88.9%, and 44.4%, respectively (Supplemental Figure 2, http://links.lww.com/SHK/B703). However, no significant difference was observed among the three groups.

**Change of rat body composition with or without LPS infusion**

The body weight did not decrease in the control group; however, that in the LPS group decreased at 72 and 144 hours (Figure 3A). Similarly, the lean body weight did not decrease in the control group; however, that in the LPS group decreased (Figure 3B). Conversely, the fat weight decreased at 72 and 144 hours in the control group; however, that in the LPS group increased gradually (Figure 3C). At 72 and 144 hours, the weight of lower limb muscles was lower in the LPS group than in the control group (Figure 3D), whereas the weight of the epididymal fat was not (Figure 3E).

**Changes of body composition between different calorie intakes in septic rats**

No effect of intake calorie difference on the body weight of systemic inflammation rats was observed at 72 hours; however, the body weight in the UF and AF groups was lower than that in the OF group at 144 hours (Figure 4A). At 72 hours, the lean body weight in all groups decreased from each baseline, and the lean body weight in the OF group recovered at 144 hours (Figure 4B). The UF group fat weight decreased daily, whereas the AF group fat weight was



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almost unchanged, and the OF group fat weight gradually increased (Figure 4C). No difference was observed in muscle weight three groups at 72 hours; however, the UF group EDL and GAS weights were lower than those in the OF group at 144 hours (Figure 4D). Additionally, the UF group EDL muscle weights were lower than those in the AF group at 144 hours. Fat tissue weight did not differ at 72 hours; however, the OF group fat tissue weight was higher than that in other groups at 144 hours (Figure 4E).

## Discussion

We succeeded in clarifying three purposes through our experiments. First, we aimed to investigate with continuous measurement of respiratory gas monitoring of rats using IDC. From our experiments, we observed dramatic metabolic changes in the LPS group compared with the control group. Second, we aimed to evaluate the metabolic effects of different intake calorie doses during the acute phase of systemic inflammation with IDC and a body composition analyzer. For this purpose, we observed in our 144-hour experiments that the metabolism changes dynamically depending on the calorie administered. Third, we aimed to examine these rat body composition analyses and issue measurements. We observed close agreement between body composition analysis and tissue weight measurements.



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Sepsis is an acute complex syndrome with inflammatory and metabolic complications. Severe bacterial infections cause pathogen-associated molecular pattern molecules, including LPS, to spread throughout the body, followed by a cytokine storm that causes whole-body inflammatory systemic damage.[17-19] Therefore, hyperinflammation and uncontrolled infection lead to hypoperfusion, hypotension, multiple organ failure, and death. Although optimal energy supports patient care, inadequate energy supply exacerbates severe patients caused by hyperglycemia, hypoglycemia, and excessive or insufficient energy expenditure. Several beneficial effects of nutritional management for patients with sepsis have been reported for the clinical application of IDC measurement in humans.[20-23] However, previous studies have analyzed metabolic alterations with sepsis using IDC only in the short term.[13,24-26] Guidelines, such as the European Society of Clinical Nutrition and Metabolism, state that the acute phase of sepsis is categorized into the following three phases: the phase when the invasion suddenly occurs, the phase when the body reacts and withstands the invasion, and the phase when the body adapts to the invasion.[2] In this report, we refer to these three phases as the first, second, and third phases. The second phase is almost the same as the ebb phase, and the third phase is similar to the flow phase. For this purpose, our study uniquely calculated the utilization rates of the carbohydrates and lipids by IDC of rat experiments for 144 consecutive hours of the acute systemic inflammation phase, including these phases.

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Our report analyzed the acute phase by categorizing it into three phases with the LPS rat model. In the first phase (from LPS injection to 24 hours after injection), the LPS group FAO was higher and CHO was lower than those in the control group, and the LPS group REE, CHO, and RQ were lower than those in the control group. The body overreacts to the outrageous insult, the overall metabolism drops, and the diurnal variation disappears. Additionally, the LPS group activity was decreased, fur condition worsened, and eye discharge accumulated (data not shown). This is probably because septic shock impairs blood flow to systemic tissues and reduces systemic activity. In the second phase (from 24 to 72 hours after LPS injection), the LPS group REE, CHO, and RQ were higher than those in the control group, and the LPS group FAO was lower than that in the control group. Furthermore, the LPS group CHO and FAO diurnal variation was still lost. This might be caused by the energy demand increasing during a cytokine storm.[27] In the third phase (from 72 to 144 hours after LPS injection), the LPS group REE, CHO, FAO, and RQ recovered almost the same as those in the control group. Because of the recovery or acquisition of tolerance, the LPS group became more active, had better fur, and lost eye discharge (data not shown). Metabolic diurnal variation gradual recovery reflects the fact that the LPS rat model became accustomed to LPS invasion. These reactions are called LPS tolerance, and there are many reports *in vivo* and *in vitro*.[28-32] However, there have been no reports with IDC to date. This is the first study to measure LPS tolerance metabolism change.



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Another purpose of our research was to determine whether IDC can be a useful tool to approach adjusted feeding.[20,33] The OF group has significantly lower FAO and higher REE, CHO, and RQ than the UF and AF group rats. Although the UF group has no difference in REE compared with the AF group, it has higher FAO, lower CHO, and RQ than the AF group. Specifically, REE alone can distinguish whether nutritional management is appropriate or inappropriate. However, it cannot differentiate between the OF and UF groups; therefore, it was found that CHO, FAO, and RQ should also be evaluated. These results showed for the first time the possibility of using IDC to grasp the status of each patient's underfeeding, adjusted feeding, and overfeeding in real-time. Recently, the miniaturization of IDCs has progressed, making it easier to measure in clinical scenes. Additionally, continuous IDC measurements may have more usefulness for proper nutritional management in sepsis care than intermittent or short-time measurements.

Regarding the nutritional dose for critically ill patients, there is currently no clear answer; therefore, it is customary to administer less energy than usual.[3] The optimal nutritional administration, such as nutrition start timing, patient severity, given dosage, and nutrition components, is still unknown. Several beneficial effects of nutritional management for patients with sepsis have been reported for the clinical application of IDC measurement in humans.[20] For example, one of the results of IDC measurements, the change in RQ reflects the metabolic

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change from FAO to CHO, which may be responsible for the change in the phase of inflammation. Therefore, to study these, our model appears to be the best, ethically, technically, and scientifically.

A body composition analyzer measures body composition, such as body weight, fat mass, and lean body mass, without killing individuals. Our results showed that the body composition analyzer reflects the actual tissue situations. A body composition analyzer and direct tissue measurement revealed that the LPS group body weight loss occurs by muscle atrophy rather than fat loss. Notably, the four removed muscles lost 20% in weight in the LPS group compared with the control group. This situation is very similar to ICU-AW or PICS, which are critically ill patients losing muscle while in intensive care.[34,35] From our results, this experiment using rats can be considered to reproduce the pathology of patients with sepsis in intensive care. These results explained why the LPS group IDC results differed from those in the control group. These results suggest that, in the acute phase of systemic inflammation, the administered energy is used to some extent for metabolism, and muscle catabolism is also involved as another energy source. Furthermore, it was consistent with the results of both IDC and tissue measurements that the energy shortfall in muscle tissue metabolism was secured by lipid metabolism in the UF group. Only the OF group was found to increase muscle mass in this study. The reason for this is whether muscle catabolism does not occur because the nutrient



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dose is high or whether muscle catabolism does occur; however, some accumulation occurs. Increased muscle mass is generally considered a good factor; however, it is unclear whether it is a meaningful increase in muscle mass. All that can be stated from these results is that at least the UF and the OF groups tend to have a higher mortality rate than the AF group, and the reason for this was not investigated in this study. The difference was larger when measured using a body composition analyzer than tissue measurement; however, this is considered to be owing to the comprehensive evaluation of the whole body. In clinical practice, body composition analyzers might be useful in the current status of critically ill patients without invasion, and the results might be reflected in treatment promptly. Regarding tissues, we investigated the effects of metabolic changes only on tissue weight, not examining microscope, DNA, RNA, or protein. Moreover, the results of our study alone do not link the effects of muscle weight loss on the whole body effects and prognosis of patients. Therefore, in the future, it is necessary to promote research that links IDC measurement results with clinical significance.

In the future, our research method might be useful for studying the appropriate nutritional treatment, such as when to restrict calories, when to increase calories, how many calories to intake, and what nutrients to intake, among others, in treating patients with sepsis. Therefore, we plan to create evidence of nutritional therapy during intensive care for critically ill patients by investigating this change in metabolism and nutritional administration using our rat

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systemic inflammation model with IDC measurement experiments. It is also possible that continuous respiratory IDC measurements will become widespread in clinical practice to ascertain the timing of these events.

In conclusion, this is the first study of IDC measured long period using the LPS-induced systemic inflammation rat model for 144 consecutive hours. Based on our LPS rat IDC measurements, body composition analyzer, and the actual weight of muscle and fat, energy demand increases, energy metabolism ratio change from lipid to glucose, and protein catabolism increases. Moreover, we examined changes in metabolism owing to differences in calorie intake; further research on the potential benefits of nutritional interventions in treating severe sepsis would be beneficial.

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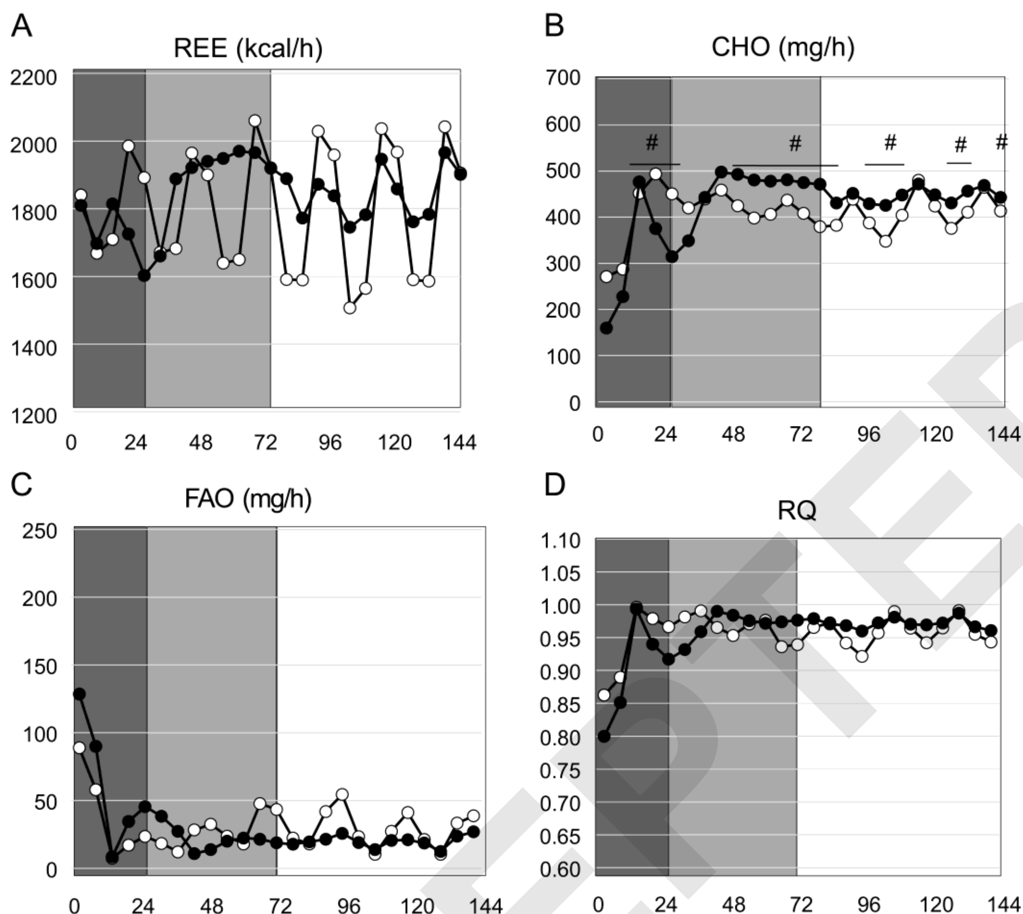
**Figure 1**




These figures show the results of IDC for the LPS and the control group performed for 144 hours consecutively (n=9). The first, second, and third phase graph areas are shown in grey, dotted, and white, respectively. The closed and open circle shows the LPS and control groups, respectively. The differences between groups were analyzed using repeated measures ANOVA, followed by Student's t-test at each point. Statistical significance was set at $p < 0.05$. Significant differences are indicated with the symbol #. A) shows two groups REE, B) shows CHO, C) shows FAO, and D) shows RO.

IDC, indirect calorimetry; LPS, lipopolysaccharide; REE, resting energy expenditure; CHO, carbohydrate oxidation; FAO, fatty acid oxidation; RQ, respiratory quotient

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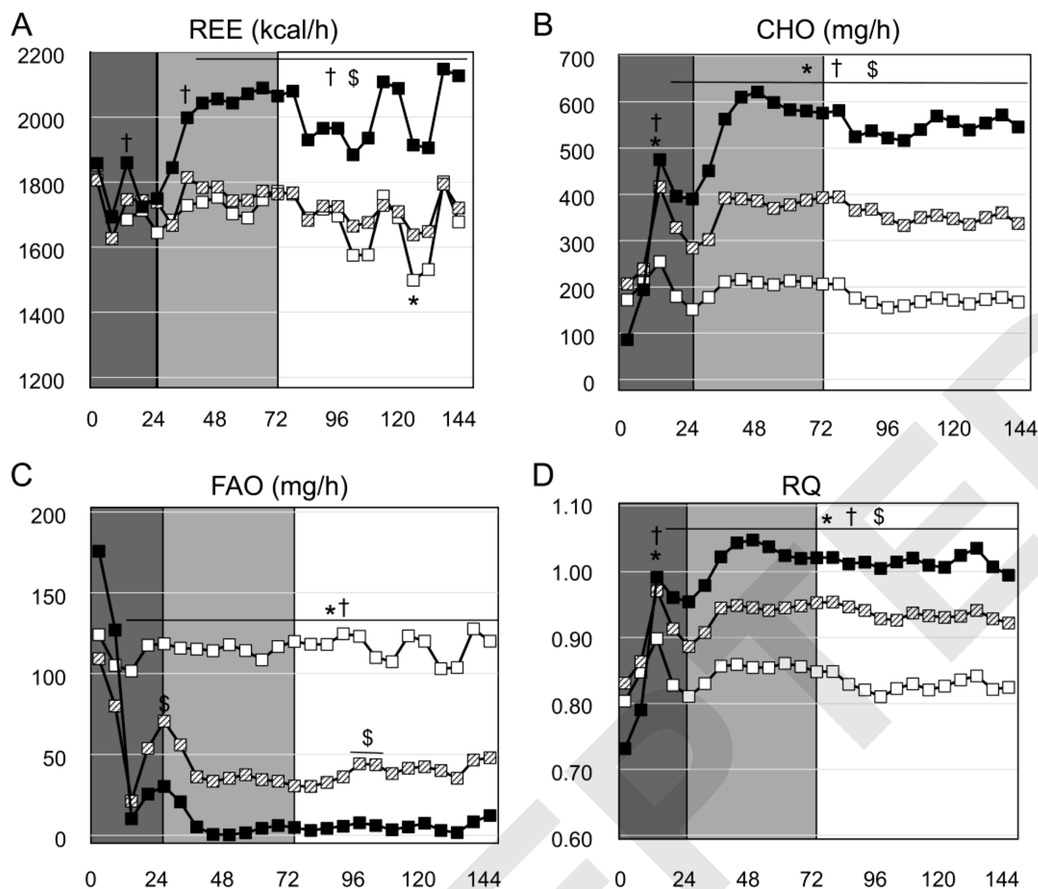
**Figure 2**




These figures show the results of IDC for the UF, AF, and OF groups performed for 144 hours consecutively (n=9). The first, second, and third phase graph areas are shown in grey, dotted, and white, respectively. The open, grey, and closed square shows the UF, AF, and OF groups, respectively. The differences between groups were analyzed using repeated measures ANOVA, followed by Tukey type test at each point. Statistical significance was set at $p < 0.05$. Significant differences are indicated with the symbol, *: UF versus AF. †: UF versus OF. $: AF versus OF. A) shows three groups REE, B) shows CHO, C) shows FAO, and D) shows RO.

IDC, indirect calorimetry; LPS, lipopolysaccharide; REE, resting energy expenditure; CHO, carbohydrate oxidation; FAO, fatty acid oxidation; RQ, respiratory quotient; UF, underfeeding; AF, adjusted feeding; OF, overfeeding

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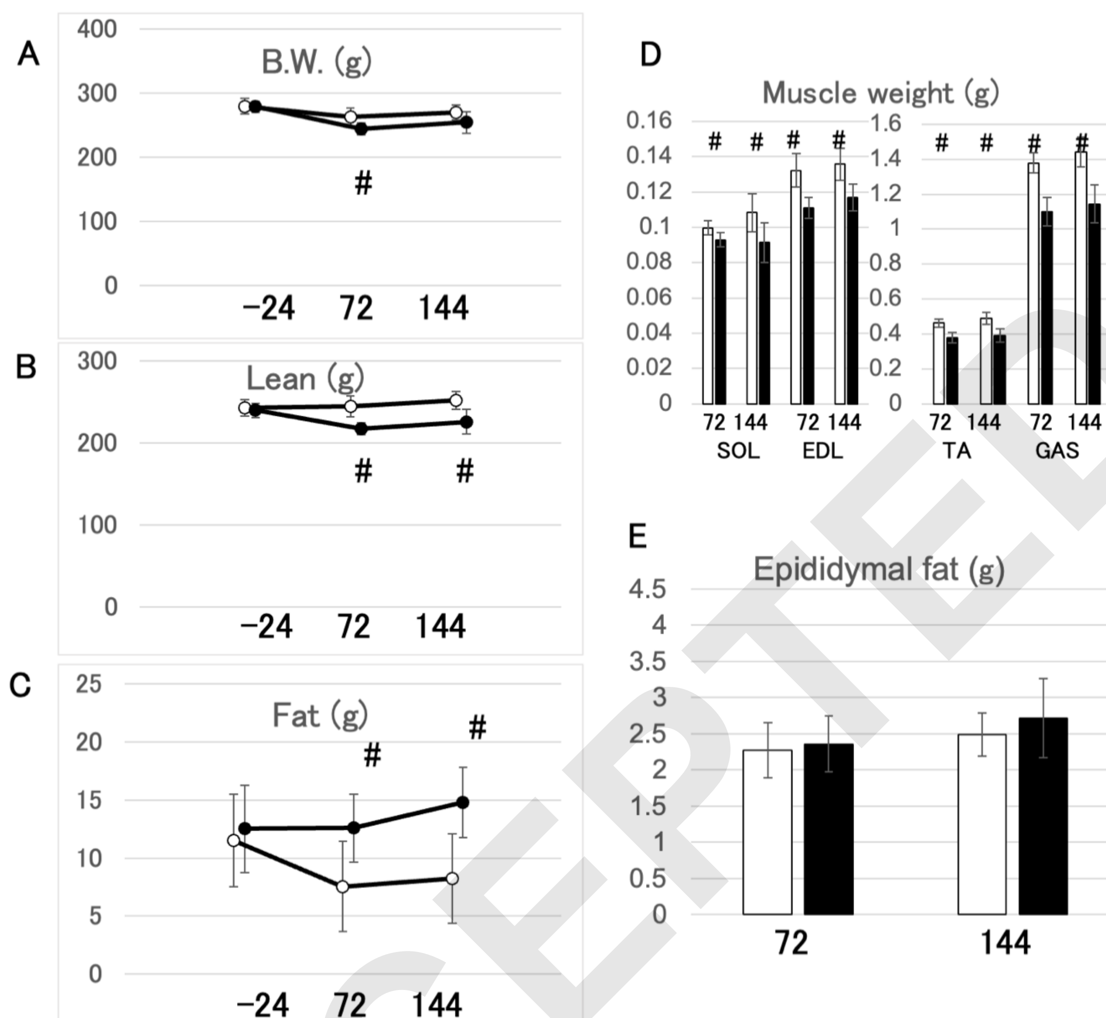
**Figure 3**




These figures show the body balance in the LPS and control groups (n=9): A) shows the LPS and control group body weight; B) shows lean mass; C) shows fat mass analyzed using the body composition analyzer EchoMRI-700. The closed and open circles indicate the LPS and control groups, respectively; D) shows direct muscle weight; and E) shows direct adipose tissue weight. The closed and open bars show the LPS and control groups, respectively. The differences between the groups were analyzed using the Student's t-test at each point. Statistical significance was set at $p < 0.05$. Significant differences are indicated with the symbol #.

LPS, lipopolysaccharide; SOL, soleus; EDL, extensor digitorum longus; TA, tibialis anterior; GAS, gastrocnemius

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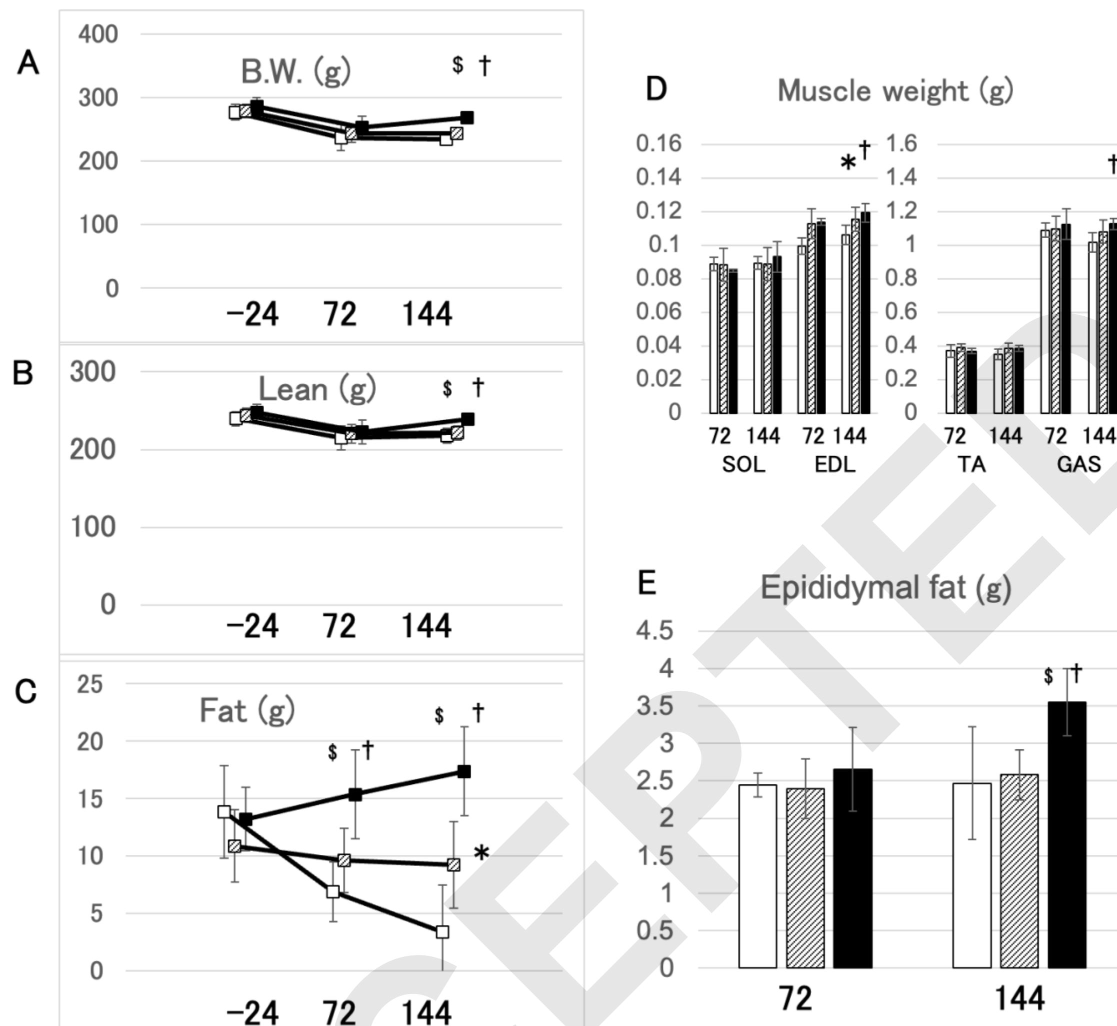
**Figure 4**




These figures show the intake calorie effect of LPS rats' body balance (n=9): A) shows the UF, AF, and OF groups' body weight; B) shows lean mass; C) shows fat mass analyzed using the body composition analyzer EchoMRI-700. The open, grey, and closed squares show the UF, AF, and OF groups, respectively; D) shows direct muscle weight; and E) shows direct adipose tissue weight. The open, grey, and closed bars indicate the UF, AF, and OF groups, respectively. The differences between the groups were analyzed using the Tukey test at each point. Statistical significance was set at $p < 0.05$. Significant differences are indicated with the symbol, *: UF versus AF. †: UF versus OF. $: AF versus OF.

UF, underfeeding; AF, adjusted feeding; OF, overfeeding; SOL, soleus; EDL, extensor digitorum longus; TA, tibialis anterior; GAS, gastrocnemius

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**Table 1**

| | | Control | LPS | UF | AF | OF |
|---|---|---|---|---|---|---|
| Glucose | g/L | 175 | 175 | 60.9 | 133.7 | 216.3 |
| Amino acid | g/L | 30 | 30 | 30 | 30 | 30 |
| Na+ | mEq/L | 35 | 35 | 35 | 35 | 35 |
| K+ | mEq/L | 20 | 20 | 20 | 20 | 20 |
| Cl- | mEq/L | 35 | 35 | 35 | 35 | 35 |
| Lactate- | mEq/L | 20 | 20 | 20 | 20 | 20 |
| LPS | mg/L | ー | 5.14 | 5.14 | 5.14 | 5.14 |
| Calorie | kcal/L | 820 | 820 | 364 | 656 | 984 |

Intravenous fluids were used for each group. LPS, lipopolysaccharide

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**Supplemental Figure 1**

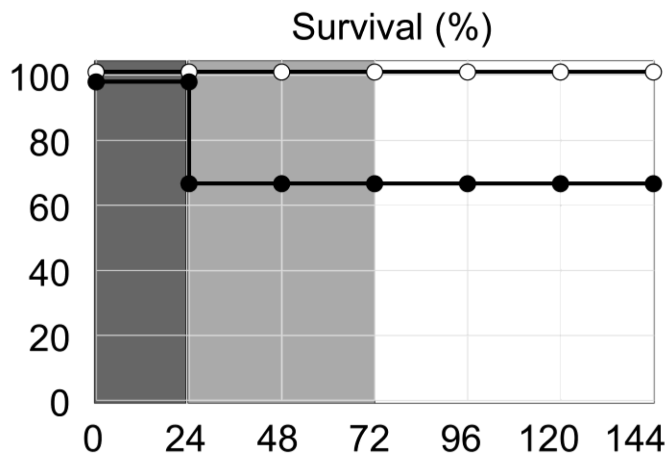


Figure S1 shows the results in the LPS and control groups for 144 hours (n=9). The closed and open circles show the LPS and control groups, respectively. The differences between the groups were analyzed with a log-rank test. The difference between the LPS and control groups was insignificant ($p = 0.065$).

LPS, lipopolysaccharide

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**Supplemental Figure S2**

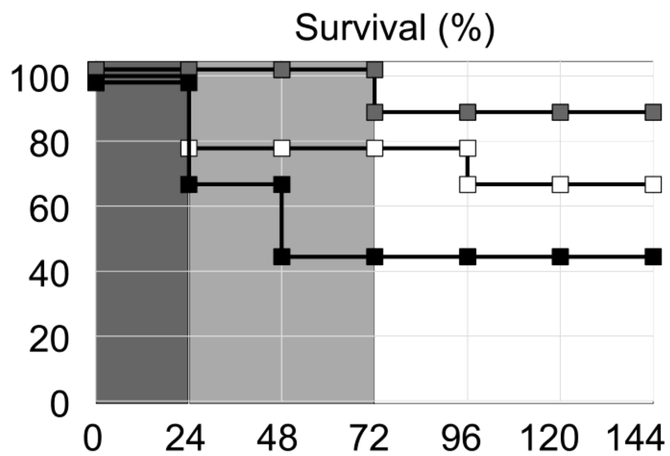


Figure S2 is the Kaplan–Meier plot showing the intake calorie effect of sepsis rats for 144 hours (n=9). The open, grey, and closed squares show the UF, AF, and OF groups, respectively. The differences between the groups were analyzed using the log-rank test. The difference between each group was insignificant (UF versus AF: $p = 0.5945$, UF versus OF: $p = 0.0974$, AF versus OF: $p = 0.5326$).

UF, underfeeding; AF, adjusted feeding; OF, overfeeding

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**Supplemental Table 1**

| AMIPAREN | g/L |
|---|---|
| L-Leucin | 14 |
| L-Isoleucin | 8 |
| L-Valine | 8 |
| L-Lysine acetate | 14.8 |
| L-Threonin | 5.7 |
| L-Tryptophan | 2 |
| L-Methionin | 3.9 |
| L-Cysteine | 1 |
| L-Phenylalanine | 7 |
| L-Tyrosine | 0.5 |
| L-Arginine | 10.5 |
| L-Histidin | 5 |
| L-Alanin | 8 |
| L-Proline | 5 |
| L-Serin | 3 |
| Glycine | 5.9 |
| L-Asparatic acid | 1 |
| L-Glutamic acid | 1 |

Ingredients of AMIPAREN.

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