

NEUROMUSCULAR ELECTRICAL STIMULATION IMPROVES ENERGY SUBSTRATE METABOLISM AND SURVIVAL IN MICE WITH ACUTE ENDOTOXIC SHOCK

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ABSTRACT—This study investigated the therapeutic benefits of neuromuscular electrical stimulation (NMES). C57BL/6 mice were administered lipopolysaccharide (LPS; 20 mg/kg body weight) by intraperitoneal injection and divided into control (C) and NMES groups (n = 10–12 each). The latter received NMES to the bilateral gastrocnemius muscle for 1 h at low or high frequency (LF = 2 Hz and HF = 50 Hz, respectively) and low or high voltage (LV = 10 V and HV = 50 V, respectively). In LF–LV and LF–HV groups, NMES was performed twice and the results were compared with those for mice that received one round of NMES. Changes in energy metabolism were measured by indirect calorimetry up to 24 h; survival was evaluated up to 72 h after LPS administration; peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α expression in the liver and gastrocnemius muscle was evaluated by quantitative PCR; and plasma concentration of interleukin (IL)-6 was determined by enzyme-linked immunosorbent assay. Survival was improved only in the LF–LV group with one round of NMES ($P < 0.01$) and the LF–HV group with two rounds of NMES ($P < 0.05$). Fatty acid oxidation (FAO) was slightly increased in these two groups, whereas carbohydrate oxidation (CHO) was decreased or not changed. Significant upregulation of PGC-1 α in muscle as well as a decrease in plasma IL-6 level were also observed in these two groups ($P < 0.05$). Thus, NMES exerts therapeutic effects under conditions that induce a mild switch in energy metabolism from glucose to lipid predominant metabolism through PGC-1 α upregulation and suppression of inflammation, and may be an effective early intervention even in hemodynamically unstable patients.

KEYWORDS—Endotoxic shock, energy substrate metabolism, muscle stimulation, nutritional management

ABBREVIATIONS—BW—body weight; C—control; CHO—carbohydrate oxidation; FAO—fatty acid oxidation; HF—high frequency; HV—high voltage; ICU—intensive care unit; IL-6—interleukin 6; LF—low frequency; LPS—lipopolysaccharide; LV—low voltage; NMES—neuromuscular electrical stimulation; PGC-1 α —peroxisome proliferator-activated receptor gamma coactivator 1 α ; VCO₂—maximum rate of CO₂ consumption; VO₂—maximum rate of O₂ consumption

INTRODUCTION

There is growing awareness of the importance of nutritional management for critically ill patients affected by stress conditions such as trauma or sepsis; indeed, nutrition can be considered as the foundation for patient treatment. Several major guidelines for critical care nutrition have been published and are being used in clinical settings (1–4). However, the nutritional state of intensive care patients can worsen during prolonged or recurrent stress; moreover, malnutrition can result in a poor outcome. This catastrophic change in immunological and metabolic states is known as the two-hit theory (5) or persistent inflammation, immunosuppression, and catabolism syndrome (6). However, the optimal forms of therapy for critically ill patients are not known.

We previously investigated changes in energy substrate utilization during sepsis, and found that it changed from glucose to predominantly lipid utilization, with protein catabolism also increasing, as a function of sepsis severity (7). We also demonstrated that low-intensity exercise in the acute phase of endotoxic shock has therapeutic effects such as improving lipid metabolism and prolonging survival by stimulating peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α expression; additionally, exercise suppressed carbohydrate metabolism and plasma interleukin (IL)-6 levels (8). These results suggest that upregulation of PGC-1 α after exercise causes a metabolic switch from glucose to lipid utilization and suppresses inflammation, thereby improving the nutritional state and leading to a better outcome.

In a clinical setting, it may be unsafe for patients in the acute phase of sepsis to exercise. Neuromuscular electrical stimulation (NMES) has effects similar to exercise and was shown to be beneficial as an early rehabilitation strategy for patients in the intensive care unit (ICU) who have sepsis (9) or who have recently undergone cardiovascular surgery (10). NMES has also been reported to prevent muscle weakness (11) and mobilize endothelial progenitor cells (12) in sepsis patients and attenuate postprandial hyperglycemia in type 2 diabetes

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(13). NMES also upregulated PGC-1 α expression in cultured rat skeletal muscle (14). However, there have been no studies to date examining the effect of NMES on energy metabolism and survival of septic patients. In the present study, we tested the hypothesis that like exercise, NMES can improve lipid metabolism and survival using a mouse model of acute endotoxic shock.

MATERIALS AND METHODS

Acute endotoxic shock mouse model

Male C57BL/6 mice (11 weeks old; Japan SLC, Hamamatsu, Japan) were used for experiments. The mice were housed in individual cages and allowed to acclimate for 5 to 7 days before experiments under a 12:12-h light/dark cycle (lights on at 6:00) and constant temperature ($25 \pm 2^\circ\text{C}$) and humidity ($60 \pm 10\%$), with free access to standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and tap water. The study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and was in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experimental procedures were approved by the Kyoto University Animal Experimentation Committee. The hair of bilateral lower legs of each mouse was shaved under gas anesthesia (isoflurane/Forane; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) on the day before experiments.

On the day of the experiment, mice were given 20 mg/kg body weight (BW) lipopolysaccharide (LPS; Sigma-Aldrich, St. Louis, Mo, cat. no. L-2880) by intraperitoneal injection along with 10 mL/kg BW normal saline 4 h after the start of the light phase, then divided into control (C) and NMES groups ($n = 10\text{--}12/\text{group}$). The food was removed, but the mice had free access to water until sacrifice (immediately after NMES, see below) because PGC-1 α expression is affected by food intake (15).

NMES

Mice were fixed on a table and adhesive pads with electrodes were placed on bilateral lower legs. NMES to the bilateral gastrocnemius muscle for 1 h was performed at 4 h for one round or at 2 and 6 h for two rounds of NMES following LPS administration. Isometric and intermittent stimulation (5 s contraction and 10 s rest) was delivered using an electrical stimulation device (SEN-1101; Nihon Kohden, Tokyo, Japan) under gas anesthesia (2%–3% of isoflurane/Forane). Low- and high-frequency stimulation (LF = 2 Hz and HF = 50 Hz, respectively) was delivered at low and high voltages (LV = 10 V and HV = 50 V, respectively) (Fig. 1). Mice in the control groups were also treated in the same way as NMES groups. These settings were based on previous studies of muscle strength in mice (16, 17) or glucose metabolism in humans (13). In LF–LV and LF–HV groups, NMES was performed twice and the results were compared with those for mice that received one round of NMES. Timing of NMES once or twice was determined relatively early after LPS administration based on our previous study (7, 8).

Indirect calorimetry and survival analysis

Changes in energy utilization were measured by indirect calorimetry 4 h before and 24 h after NMES ($n = 10\text{--}12/\text{group}$ after one round, $n = 7\text{--}11/\text{group}$ after two rounds), using a mass spectrometer for respiratory gas analysis and a bioprocess monitoring system (ARCO-2000; Arco System, Chiba, Japan) at

constant temperature ($25 \pm 2^\circ\text{C}$) and humidity ($60 \pm 10\%$). During measurements, mice were deprived of food and had access only to water. Data were obtained every 10 min.

Carbohydrate oxidation (CHO) and fatty acid oxidation (FAO) were calculated using Frayn's formula (18) as follows.

$$\text{CHO} = 4.55 \times \text{VCO}_2 - 3.21 \times \text{VO}_2$$

$$\text{FAO} = 1.67 \times \text{VO}_2 - 1.67 \times \text{VCO}_2$$

After indirect calorimetry, mice were given free access to food and water. Survival was monitored for up to 72 h after LPS administration.

Measurement of PGC-1 α and IL-6 levels

Mice were sacrificed by decapitation immediately after NMES sessions, and bilateral gastrocnemius muscle, liver, and plasma samples were obtained ($n = 4/\text{group}$). Quantitative PCR was performed to evaluate PGC-1 α mRNA expression in gastrocnemius muscle and liver. Total RNA was extracted from muscle and liver tissues using TriPure Isolation Reagent (Roche Diagnostics, Basel, Switzerland) and reverse transcribed into cDNA. Real-time PCR was performed using the TaqMan probe method on a Light Cycler (Roche Diagnostics). Hypoxanthine guanine phosphoribosyltransferase (HPRT) was used as the internal standard. The forward and reverse primer sequences were as follows: *pgc1a*, TGTGGAAGCTCTCTGGAAGTGC and AGGGTTATCTTGGTTGGCTTTA; *hppt*, TCCTCCTCAGACCGCTTTT and CCTGGTTCATCATCGCTAATC. Plasma concentrations of IL-6 after NMES were measured using a Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions.

Statistical analysis

Values are presented as mean \pm SEM. Survival data were analyzed with the log-rank test. The D'Agostino & Pearson omnibus normality test was used to verify whether metabolic data (FAO and CHO) were normally distributed, in which case they were analyzed with the Student *t* test with Welch's correction. Non-normally distributed data along with PGC-1 α and IL-6 expression levels were analyzed with the Mann–Whitney *U* test. Statistical analyses were performed using Prism 6 software (GraphPad Inc., La Jolla, Calif), with $P < 0.05$ considered statistically significant.

RESULTS

Effect of a single round of NMES

Results of indirect calorimetry and survival analysis after a single round of NMES are shown in Figure 2. NMES altered energy substrate utilization and survival depending on the stimulation conditions. Compared with the control group, FAO was increased ($P < 0.05$) and CHO was decreased ($P < 0.05$) in the LF–LV group, whereas in the LF–HV group, FAO was also increased ($P < 0.01$) but CHO was unchanged. There were no changes in FAO and CHO in the HF–LV group. In the HF–HV group, FAO was increased ($P < 0.0001$), whereas no change was observed in CHO. Survival was prolonged only in the LF–LV group ($P < 0.01$).

NMES conditions	Low Voltage 10V	High Voltage 50V
Low Frequency - - - 2Hz	LF-LV group	LF-HV group
High Frequency ----- 50Hz	HF-LV group	HF-HV group

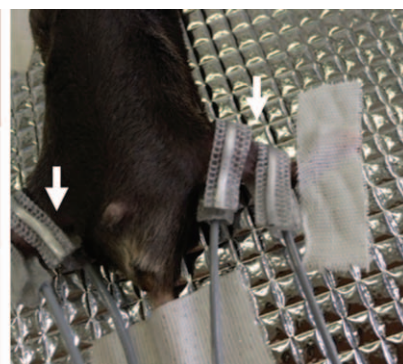


FIG. 1. Neuromuscular electrical stimulation (NMES) protocol. Mice were divided into four groups ($n = 10\text{--}12/\text{group}$) depending on frequency and voltage conditions. NMES of the bilateral gastrocnemius muscle was performed for 1 h.

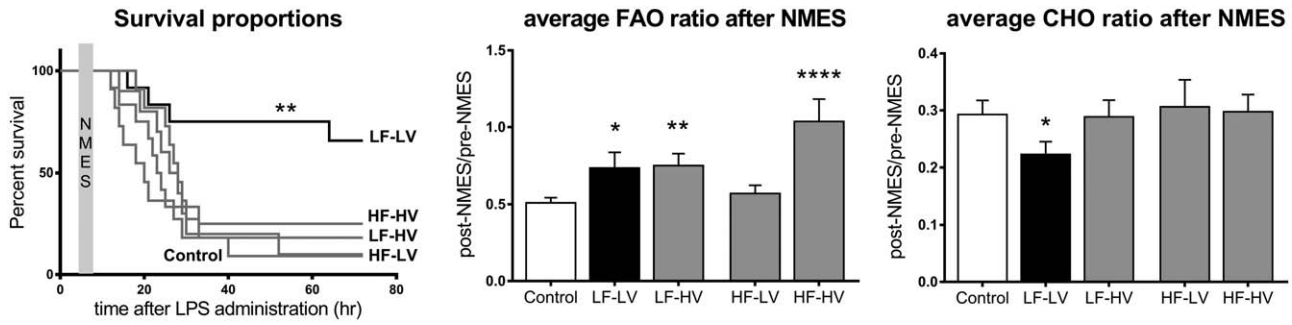


FIG. 2. Changes in energy substrate utilization and survival after one round of neuromuscular electrical stimulation (NMES). Values represent mean \pm SEM ($n = 10-12$ /group). * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$ vs. control.

Effect of two rounds of NMES

Mice in the LF-LV group that underwent two rounds of NMES (LF-LV $\times 2$) showed both FAO and CHO increased compared with control and LF-LV $\times 1$. Mice in the LF-HV group that underwent two rounds of NMES (LF-HV $\times 2$) showed that FAO increased, whereas CHO was unchanged compared with control and LF-HV $\times 1$. Survival was improved in the LF-HV $\times 2$ group ($P < 0.05$), but was reduced in the LF-LV $\times 2$ group ($P < 0.05$) as compared with mice that underwent a single round of NMES (Figs. 3 and 4).

Effect of NMES on PGC-1 α and IL-6 levels

PGC-1 α expression in the liver did not change in the LF-LV group after one round of NMES or in LF-HV group after two

rounds, but was upregulated ($P < 0.05$) in gastrocnemius muscle (Fig. 5). On the contrary, plasma concentrations of IL-6 decreased in the LF-LV group after one round of NMES ($P < 0.05$) (Fig. 6).

DISCUSSION

Energy substrate utilization typically switches to a catabolic pathway under stress conditions such as sepsis. Although carbohydrates are used initially, as energy demands increase, endogenous lipids and proteins are preferentially mobilized because liver glycogen is exhausted within 12 to 24 h; free fatty acids are released from adipose tissues; and gluconeogenesis occurs in muscles, providing the required energy (19).

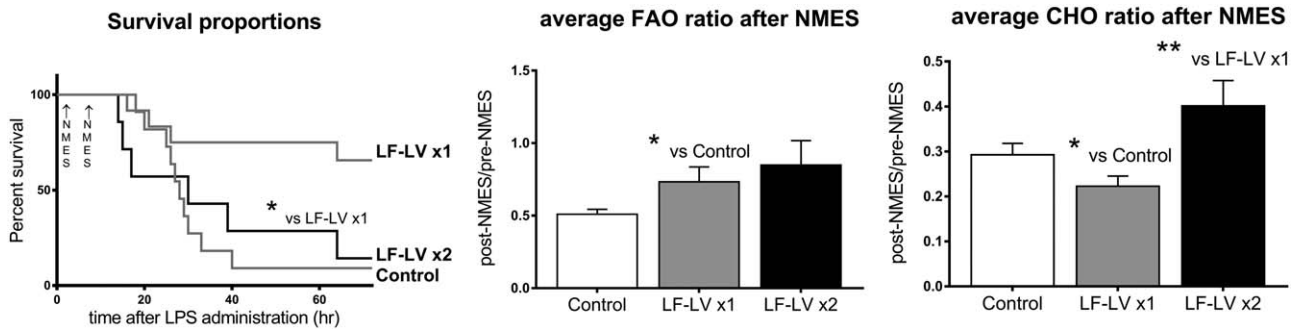


FIG. 3. Changes in energy substrate utilization and survival in low frequency-low voltage (LF-LV) group after two rounds of neuromuscular electrical stimulation (NMES). Values are mean \pm SEM ($n = 7-11$ /group). * $P < 0.05$; ** $P < 0.01$ vs. control or 1 \times LF-LV.

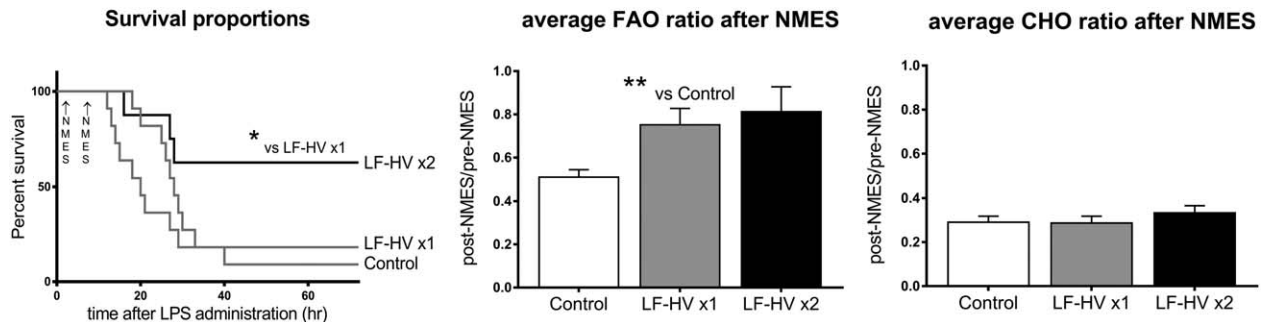


FIG. 4. Changes in energy substrate utilization and survival in low frequency-high voltage (LF-HV) group after two rounds of neuromuscular electrical stimulation (NMES). Values are mean \pm SEM ($n = 7-11$ /group). * $P < 0.05$; ** $P < 0.01$ vs. control or 1 \times LF-HV.

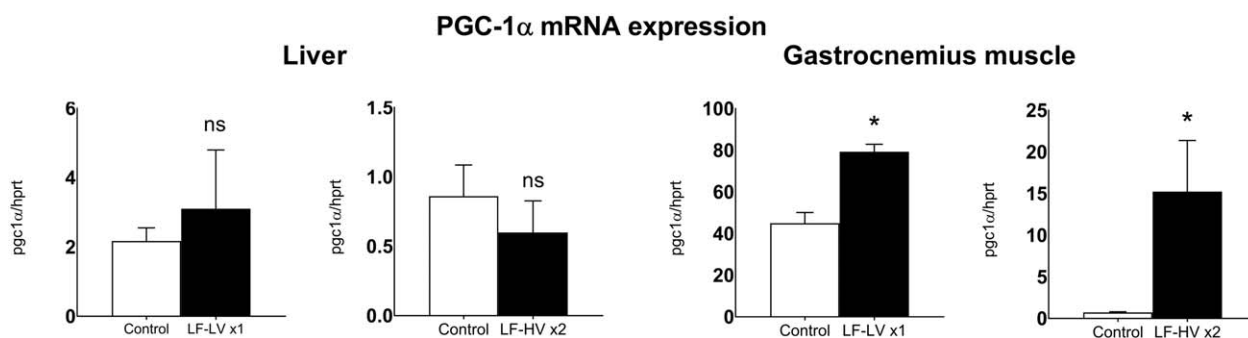


FIG. 5. **PGC-1 α expression in liver and gastrocnemius muscle after neuromuscular electrical stimulation (NMES).** Values represent mean \pm SEM (n=4/group). * $P < 0.05$ vs. control.

We previously reported alterations in energy substrate utilization during different degrees and durations of sepsis, which were determined based on indirect calorimetry, urinary nitrogen excretion, and tissue analysis (7). During sepsis, lipids are the predominant energy source and are transported to and stored in the liver. However, in this study we observed that FAO was paradoxically suppressed in the hyperacute phase of endotoxemic shock induced by high-dose LPS administration.

PGC-1 α is a key molecule related to fat metabolism whose expression in adipose tissue, muscle, and liver is increased by cold exposure, exercise, and fasting, respectively (15). Upregulation of PGC-1 α induced by exercise was shown to improve muscle physiology and suppress inflammation (20). However, PGC-1 α is suppressed by LPS (21–23). We speculated that the inhibition of FAO by high-dose LPS administration was due to a decrease in PGC-1 α expression. We have demonstrated that when mice performed a low-intensity exercise, PGC-1 α level was increased and caused a switch in energy utilization from glucose into lipid predominance while also suppressing inflammation, thereby improving the nutritional state and leading to a better outcome (8). Given the potential harm associated with exercise during the acute phase of sepsis, in this study we

investigated whether NMES is an equally effective alternative therapeutic intervention.

In summary of the present study, we found that NMES applied once at low frequency and low voltage (LF–LV \times 1) or applied twice at low frequency and high voltage (LF–HV \times 2) increased PGC-1 α expression, caused a switch in energy metabolism, and suppressed inflammation, confirming that it has similar beneficial effects to exercise.

Conditions such as postintensive care syndrome (24) or ICU-acquired weakness (25, 26) are important issues in critical care medicine. Malnutrition and muscle weakness are major problems after intensive care; the latter can be prevented by early rehabilitation. Recent studies have demonstrated the therapeutic potential of NMES for preventing muscle atrophy and preserving muscle mass (27, 28); for instance, NMES alleviated muscle wasting in comatose patients in the ICU (29). However, the effects of NMES vary according to the conditions of stimulation (30); patients receiving vasopressors or remaining in the ICU for a longer period of time are less likely to respond to NMES (31), and early in-bed cycling in combination with NMES did not have significant benefits in ICU patients (32).

NMES may alter cytokine levels in a manner similar to exercise in healthy humans (33), and was shown to influence

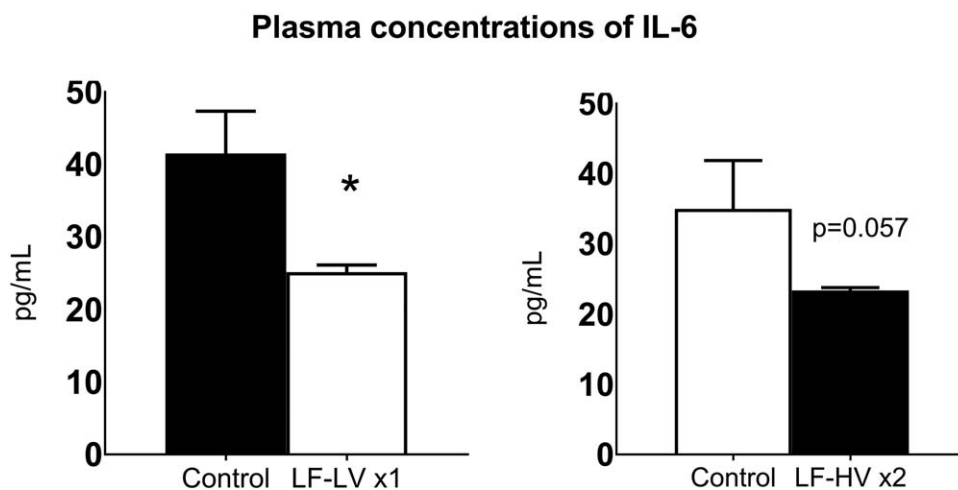


FIG. 6. **Plasma IL-6 levels after neuromuscular electrical stimulation (NMES).** Values represent mean \pm SEM (n=4/group). * $P < 0.05$ vs. control.

blood glucose concentration and body fat percentage in patients with type 2 diabetes mellitus (34). The effects of NMES in septic shock patients have been previously investigated (35); these earlier results and ours suggest that NMES exerts therapeutic effects under conditions that slightly alter energy metabolism from glucose utilization to predominantly lipid utilization. However, the appropriate conditions of NMES for critically ill patients remain to be determined.

There are some limitations of this study. Data using PGC-1 α knockout mice were not obtained. Besides, the protein levels of PGC-1 α , the enzyme levels for carbohydrate or lipid metabolism, and the indicators of inflammation other than IL-6 were not measured. Therefore, there remained some unclear points about the mechanism of the effect of NMES for improving metabolism and survival. However, our study at least suggests the potential possibility of NMES for improving outcome of septic patients.

Although there are various challenges for the clinical application of NMES, such as the difficulty in attaching the NMES device to trauma or burn patients and the potential influence of NMES on hemodynamic state or electrical monitoring devices, NMES has beneficial effects on metabolism and inflammation under the appropriate conditions. In the field of critical care nutrition, the importance of early enteral nutrition is emphasized by the saying “If the gut works, use it!” We offer the slogan “If the muscle works, use it!” to underscore the therapeutic benefits of early muscle stimulation through exercise or NMES in the acute phase of sepsis.

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