ORIGINAL RESEARCH

Supplement of Lipid Emulsion to Epinephrine Improves Resuscitation Outcomes of Asphyxia-Induced Cardiac Arrest in Aged Rats

Lijun Huang[1]
Qiusheng Ren[1]
Shenghui Yu[1]
Ya Shao[1]
Yijun Chen[2]
Xin Huang[1]

[1]Department of Anesthesiology, Ningbo Yinzhou People's Hospital, The Affiliated People's Hospital of Ningbo University, Ningbo, Zhejiang, People's Republic of China; [2]Department of Anesthesiology, Ningbo First Hospital, Ningbo Hospital of Zhejiang University, Ningbo, Zhejiang, People's Republic of China

Objective: The goal of the study was to investigate the efficacy of lipid supplement to epinephrine-based therapy in resuscitation of asphyxia-induced cardiac arrest in aged rats.

Methods: The study included two parts: in experiment A, rats underwent asphyxial cardiac arrest and cardiopulmonary resuscitation, randomized to receive epinephrine and normal saline (control group, n=22), epinephrine and intralipid 20% (long-chain triglycerides (LCT) group, n=22) or epinephrine and lipovenoes 20% (LCT/medium-chain triglcerides (MCT) group, n=22). Return of spontaneous circulation, recurrence of asystole after resuscitation, hemodynamic metrics, arterial blood gas values, neurological assessment score and indexes of pulmonary transudation were recorded. In experiment B, rats using the same model and resuscitation protocol were randomly divided into 21 groups: Control $_0$, Control $_{20}$, Control $_{40}$, Control $_{60}$, Control $_{80}$, Control $_{100}$, Control $_{120}$, LCT $_0$, LCT $_{20}$, LCT $_{40}$, LCT $_{60}$, LCT $_{80}$, LCT $_{100}$, LCT $_{120}$, LCT/MCT $_0$, LCT/MCT $_{20}$, LCT/MCT $_{40}$, LCT/MCT $_{60}$, LCT/MCT $_{80}$, LCT/MCT $_{100}$ and LCT $_{120}$ (n=10, the subscripts represent respective endpoint of observation in minutes). Myocardial bioenergetics were determined.

Results: In experiment A, the LCT and LCT/MCT groups had a shorter time to return of spontaneous circulation (ROSC) (P=0.001and P<0.001, respectively) and higher survival rate (P=0.033 and P=0.014, respectively) compared with the Control group. The LCT/MCT group had higher MAP (P<0.001 and P=0.001, respectively), HR (P<0.001 and P=0.004, respectively) and RPP (P<0.001 and P<0.001, respectively) compared with the Control and LCT groups, respectively. In experiment B, the LCT/MCT group had a higher energy charge compared with the control group at 20 (P<0.001) and 40 (P<0.001) minutes. The LCT group had higher energy charge compared with the Control group at 40 (P<0.001) and 60 (P<0.001) minutes.

Conclusion: The supplement of lipid emulsion to epinephrine improves resuscitation outcomes of asphyxia-induced cardiac arrest than epinephrine alone in our in vivo model of aged rat. LCT/MCT emulsion may be superior to LCT emulsion in epinephrine-based resuscitation.

Keywords: asphyxia, cardiac arrest, epinephrine, lipid emulsion, cardiopulmonary resuscitation

Correspondence: Lijun Huang
Department of Anesthesiology, Ningbo Yinzhou People's Hospital, The Affiliated People's Hospital of Ningbo University, No. 251, East Baizhang Road, Ningbo, Zhejiang Province 315040, People's Republic of China
Email huanglijunyz@163.com

Introduction

Cardiac arrest (CA) is a main cause of death and disability, which affects approximately 300,000 people per year in the United States.[1] Despite advances in resuscitation therapies and techniques, the survival rate of CA remained unchanged at 8% over the past decades.[2] In addition, neurological disorders, cardiac insufficiency and

other organ dysfunction after return of spontaneous circulation (ROSC) are still intractable.[3,4] Several epidemiological studies have revealed that the risk of CA increases with age.[5] Decreased organ function and underlying diseases make aged populations more prone to low success rate of cardiopulmonary resuscitation (CPR), and substantial mortality and morbidity after ROSC.[6] So far, the majority of researches investigating CA have used juvenile animal models.[7,8] The main discouraging factor for conducting a study on aged animals is the low success rate of CPR and the poor post-CA survival. However, performing CA in aged animals may be more clinically relevant, for CA of aged populations is intractable and in large numbers.

Epinephrine, which is recommend by the American Heart Association guidelines, is the first-line medication in resuscitation of CA.[9] It exerts a vasoconstriction effect, augments coronary perfusion pressure (CCP), and facilitates restoration of cardiovascular circulation.[10] However, its role in CPR is controversial. The activation of adrenergic receptors increases myocardial oxygen consumption and peripheral vascular tone, which may lead to post-resuscitation myocardial dysfunction and deterioration of pulmonary oxygen exchange.[11,12] In addition, a large dose of epinephrine is not effective in improving neurological outcomes after resuscitation.[13] These unwarranted side effects of sole epinephrine application during CPR suggest that further treatment is required.

Lipid emulsions, including long-chain triglycerides (LCTs) and long- and medium-chain triglycerides (LCTs/MCTs), are widely used in clinical scenarios. Intravenous administration of lipid emulsion has been shown to successfully reverse local anesthetic-induced cardiovascular collapse in animal models.[14] Clinical case reports have also confirmed efficacy of lipid emulsion in rescuing local anesthetic overdose.[15] The application of lipid emulsion in rescuing drug overdose associated cardiac arrest is not confined to local anesthetics, but also to other lipophilic drugs.[16–18] These data may hint the "lipid sink" theory, in which lipophilic drug are extracted by lipid emulsion, reducing tissue concentration of the toxin.[19] An alternative theory is the "lipid flux," whereby lipid supplies the myocardial cell with sufficient fatty acid which can be directly utilized by mitochondria, thus countering the impeded energy supply induced by myocardial ischemia and anoxia.[20] Furthermore, there are differences between LCT and LCT/MCT in efficiency of oxidation and hydrolysis, ketone bodies production and metabolic pathways of

energy utilization.[21] Nevertheless, few studies focus on the rescuing effect of lipid on CA aside from lipophilic drug poisoning, nor addressing the choice of lipid emulsions.

No present data have reported the effects of lipid supplement to epinephrine during resuscitation of asphyxia-induced cardiac arrest in aged rat. We hypothesized that the combination of lipid emulsion and epinephrine provided better resuscitation outcomes than epinephrine alone after asphyxia-induced cardiac arrest in aged rat. Furthermore, LCT/MCT provides benefits over LCT lipid emulsion. Accordingly, we established an aged rat model of asphyxia-induced cardiac arrest with the primary aim of recording hemodynamic parameters and energy charge during the initial observation time of 120 minutes after onset of resuscitation, and determining resuscitation outcomes and hemodynamic metrics at the endpoint of 120 minutes. Secondarily, neurological outcomes, hemodynamic parameters, arterial blood gas values, lung wet-to-dry ratio and permeability index were determined 72 hours after onset of resuscitation to account for the possible difference between them.

Materials and Methods
Animal Preparation

All the experimental procedures were conducted according to the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH publication No 80–23), with the approval of the Animal Care and Use Committee of the Ningbo University. 20-month-old healthy male Sprague-Dawley rats (Sippe-Bk Lab Animal Co., Ltd., Shanghai, People's Republic of China) were used. They were housed in cages (4 rats per cage) at the animal center of Ningbo University in a standard 12-hour reverse day/night cycle at a humidity of 55 to 60% and an ambient temperature of 20–26°C. The rats had an *ad libitum* diet of stock laboratory diet and tap water. They had a recovery period of at least 2 weeks following transportation to Ningbo University before experimental manipulations.

A behavioral test for neurologic assessment was performed the day before CA/CPR. The rats were fasted for 12 hours with free access to water before surgery. Anesthesia was induced with 4% sevoflurane. After reaching a deep level of anesthesia, tracheal intubation was performed orally and rats were mechanically ventilated (tidal volume, 6–7.5 mL/kg; FiO2, 0.3; respiratory rate,

70–80 breaths/min; inspiratory/expiratory ratio, 2:3, maintaining the end-tidal carbon dioxide tension at 40 mmHg). Sevoflurane was adjusted to 3–4% to maintain the depth of anesthesia during surgery. Body temperature was maintained at 37.0–37.5°C by a thermal blanket underneath the rat's body and a heating lamp kept at a safe distance. Electrocardiogram was recorded using three subcutaneous needle electrodes. The skin of the invasive operating area was shaved, sterilized and locally anesthetized with 1% lidocaine before incision. The right femoral artery was cannulated for blood sample collection and continuous monitoring of arterial blood pressure. The right femoral vein was cannulated for intravenous drug and fluid administration. The left internal jugular vein was cannulated for central venous pressure monitoring. All the rats were allowed to stabilize for 20 minutes to recover from anesthesia induction and invasive procedures. Then an arterial blood gas analysis was performed for measuring baseline parameters.

Experiment A
Experimental Protocol

A total of 66 rats were divided by the random table method into three groups: Control, LCT, and LCT/MCT groups according to a random number table (n=22). After the post-surgical stabilization, the rats received 0.5 mg/kg pancuronium for prevention of respiratory movement. CA was induced by stopping mechanical ventilation. CA was defined as a decrease in mean arterial pressure (MAP) below 10 mmHg. Resuscitation was initiated 30 seconds after onset of CA (baseline time, designated T_0). Mechanical ventilation was restarted (tidal volume, 6–7.5 mL/kg; FiO2, 1.0; respiratory rate, 70–80 breaths/min; inspiratory/expiratory ratio, 2:3), external chest compression over the lower-middle sternum was manually performed (300 compressions per minute, a depth of 1/3 anterior-posterior chest diameter) and intravenous medications (preheated to 37°C before administration) were given: a bolus of 10 μg/kg epinephrine was administered at 1-minute intervals until ROSC (limiting maximum cumulative dose to 100 μg/kg), meanwhile normal saline (Control group), Intralipid 20% (LCT group) or Lipovenoes 20% (LCT/MCT group) were administered as a 3mL/kg bolus over 30 seconds followed by a 3 mL/kg infusion at a rate of 1.0 mL/kg/min for 3 minutes. The criterion of ROSC was defined as a spontaneous heart rate-blood pressure product (RPP) more than 20% of baseline value for at least 1 minute. Chest compression was ceased

once spontaneous circulation was achieved or an elapsed time of 40 minutes without return of ROSC. Hemodynamic parameters, central venous pressure were monitored for 120 minutes after onset of resuscitation (T_0).

After the observation period of 120 minutes, arterial blood was collected for blood gas analysis, all the intravascular catheters were removed, the vessels were ligated, and the incisions were sutured. Anesthesia was discontinued and the tube was removed after reaching adequate spontaneous respiration. All the rats were placed in an incubator tempered at 35°C for 3 days. A behavioral test for neurologic assessment was performed for the survival rats once more 72 hours after resuscitation. Then the rats were generally anesthetized, orally intubated and mechanically ventilated in the same protocol as before, the arterial blood pressure and the central venous pressure were measured as before except that the catheters were inserted at a more proximal point than the past ligation. Arterial blood was collected for blood gas analysis and further study, and the animals were sacrificed by overdose anesthesia with intraperitoneal injection of ketamine and xylazine.

Lung Wet-to-Dry Ratio and Permeability Index

After thoracotomy, the inferior lobe of the right lung was taken and weighed immediately (wet weight) and again after drying at 65°C for 72 hours (dry weight). The lung wet-to-dry ratio was calculated as wet weight/dry weight. The left lung was intubated and lavaged three times with 10 mL of normal saline. The bronchoalveolar lavage fluid (BALF) was centrifuged at 1000 revolutions per minute (rpm) for 10 minutes in a thermostatic centrifuge at 4°C, and the supernatant was removed. The blood sample collected 72 hours after onset of resuscitation was centrifuged at 3000 rpm for 10 minutes, the separated plasma was recentrifuged at 3000 rpm for 10 minutes, and the supernatant was removed. Protein concentrations of BALF in the supernatant and serum were measured by the bicinchoninic acid (BCA) protein assay, using the BCA Protein Assay Kit (Beyotime Biotechnology Inc, Shanghai, People's Republic of China) according to the manufacturer's instructions. Lung permeability index was calculated as the protein concentration in BALF/serum ratio.[22]

Neurological Assessment Score

A behavioral test for neurologic assessment was performed the day before CA/CPR and 72 hours after onset

of resuscitation. Testing was performed in a quiet and dim room by an investigator who was blinded as to the grouping of the rats. Consciousness, breathing pattern, food and water intake, vibrissae movement, motoric function, and interaction with the environment were scored (0 = no deficit; 100 = most severe deficit).[23–25]

Experiment B
Experimental Protocol
A second group of 210 rats were assigned to 21 groups (10 rats per group) by the random table method based on differing observation time. The groups were defined as Control $_0$, Control $_{20}$, Control $_{40}$, Control $_{60}$, Control $_{80}$, Control $_{100}$, Control $_{120}$, LCT $_0$, LCT $_{20}$, LCT $_{40}$, LCT $_{60}$, LCT $_{80}$, LCT $_{100}$, LCT $_{120}$, LCT/MCT $_0$, LCT/MCT $_{20}$, LCT/MCT $_{40}$, LCT/MCT $_{60}$, LCT/MCT $_{80}$, LCT/MCT $_{100}$ and LCT $_{120}$ (the subscripts represent respective endpoints of observation in minutes). The rats in Control $_{0–120}$, LCT $_{0–120}$ and LCT/MCT $_{0–120}$ received the same protocol of cardiac arrest and resuscitation as those in Control, LCT and LCT/MCT groups in experiment A. At the end of respective observation period in each group (0, 20, 40, 60, 80,100, 120 minutes), all the surviving rats were killed with overdose anesthesia, cardiac tissues were rapidly taken, rinsed to remove any residual blood and stored at - 80°C for further study.

Energy Charge
Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) of cardiac tissue were measured by high-performance liquid chromatography. Sample preparation was conducted by the Stehr[26] and Li[14] procedure. Separations were carried out at 30°C. The mobile phase was a mixture of eluent A and B (pH: 6.0, 99:1, v:v) at a flow rate of 1.0 mL/min. Eluent A was 12 mmol/L Na_2HPO_4 and 88 mmol/L NaH_2PO_4 (pH: 6.0) and eluent B was a mixture of 85% eluent A and 15% acetonitrile. An injection volume of 20 μL was used for both standard samples and tissue extract samples. The detection wavelength was 245 nm. Equally treated external standards of known concentrations were applied to check retention times and to permit sample quantification based on the analysis of peak area. Energy charge was calculated as: (ATP+0.5*ADP)/(ATP+ADP+AMP).

Statistical Analysis
The number of animals in each group was determined based on our preliminary study (n=10) in which the survival rates at 120 minutes were 40.0%, 80.0%, and 80.0%, respectively. Effect size of 0.40 was acquired by calculation according to the survival rate. A sample size of 21 per group was obtained by PASS 11.0, with α = 0.05 and β = 0.2. We enrolled 22 rats per group to account for attrition.

Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) for Windows. Normal distribution measurement data were presented as mean ± standard deviation (SD), non-normally distributed data were presented as medians and interquartile range, and frequencies were used in the case of categorical variables. Differences in baseline parameters, time to CA, time to first heartbeat, time to ROSC, arterial blood gas values, hemodynamic metrics at time point, RPP recovery ratio, neurological assessment score, wet-to-dry ratio, lung permeability index and energy charge in the three groups were examined by one-way ANOVA, and Bonferroni post-tests for data that had homogeneity of variance, or Dunnett T3 post-tests for data that exhibited heterogeneity of variance when significance was achieved. Dichotomous outcomes were analyzed with Fisher exact test. The cumulative epinephrine dose was analyzed using Wilcoxon and Mann–Whitney U-test. Differences in continuously recorded heart rate (HR), mean arterial blood pressure (MAP), heart rate-blood pressure product (RPP) and coronary perfusion pressure (CPP) during the 120-min ute observation period were analyzed by two-way repeated-measure ANOVA and Bonferroni post-tests when indicated by significance of difference. $P < 0.05$ was considered significant. Curve fitting and bar charts were performed by GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA).

Results
Baseline Values
No differences were observed in weight, baseline hemodynamic metrics or baseline arterial blood gas values among the Control, LCT and LCT/MCT groups in experiment A (Table 1), and the Control$_{0-120}$, LCT$_{0-120}$ and LCT/MCT$_{0-120}$ groups in experiment B (Tables 2 and 3).

Resuscitation Outcomes
Resuscitation outcomes for the three groups are shown in Table 4. Animals in each group achieved CA for approximately 100 seconds, and no significant difference was demonstrated among the three groups in time to CA.

Table 1 Baseline Values of Weight, Blood Gas Values and Hemodynamic Metrics for Control, LCT and LCT/MCT Groups in Experiment A

	Control (n=22)	LCT (n=22)	LCT/MCT (n=22)
Weight, g	574±20	574±17	579±15
pH	7.40±0.03	7.40±0.03	7.40±0.04
PO_2, mmHg	124±13	125±8	123±10
PCO_2, mmHg	40±3	40±3	40±3
HCO_3^-, mmol/L	23.4±1.3	24.7±2.0	24.6±1.7
BE, mmol/L	−0.3±1.5	−0.4±1.6	0.1±1.2
Lactate, mmol/L	1.2±0.6	1.1±0.6	1.3±0.6
HR, beat/min	355±21	346±18	345±16
MAP, mmHg	90±4	91±6	90±6
RPP, mmHg*beat/min	43595±3035	42928±3458	41939±2671

Notes: Baseline values showed no difference among the three groups; All values are mean ± SD.
Abbreviations: BE, base excess, HR, heart rate; MAP, mean arterial pressure; RPP, heart rate pressure product.

Table 2 Baseline Values of Weight, Hemodynamic Metrics for Control $_{0-120}$, LCT $_{0-120}$ and LCT/MCT $_{0-120}$ Groups in Experiment B

	Weight (g)	HR (Beat/Min)	MAP (mmHg)	RPP (mmHg*Beat/Min)
Control $_0$	568±16	356±18	91±5	43562±3034
Control $_{20}$	572±22	346±12	89±6	41452±3829
Control $_{40}$	565±17	352±20	93±7	42364±2980
Control $_{60}$	574±16	356±18	90±5	43212±2943
Control $_{80}$	578±22	348±14	91±6	41962±3003
Control $_{100}$	576±14	355±21	91±5	41879±2993
Control $_{120}$	568±17	345±23	90±6	42892±2894
LCT $_0$	573±13	350±17	91±5	41864±2653
LCT $_{20}$	573±13	351±19	89±6	40873±3634
LCT $_{40}$	569±12	354±16	94±5	41682±3213
LCT $_{60}$	570±21	358±24	91±9	42751±3034
LCT $_{80}$	570±18	348±14	93±5	41893±3123
LCT $_{100}$	575±20	349±16	91±7	42421±3064
LCT $_{120}$	567±14	348±17	90±8	41985±2519
LCT/MCT $_0$	571±17	345±15	91±5	43416±3328
LCT/MCT $_{20}$	572±16	348±16	92±5	41933±3274
LCT/MCT $_{40}$	573±18	349±17	91±6	39893±3452
LCT/MCT $_{60}$	578±19	348±21	90±8	39989±3246
LCT/MCT $_{80}$	570±13	352±17	88±7	40472±3122
LCT/MCT $_{100}$	571±12	351±19	89±6	40321±3004
LCT/MCT $_{120}$	569±14	357±13	93±8	42783±3123

Notes: Baseline values showed no difference among the 21 groups; All values are mean ± SD.
Abbreviations: HR, heart rate; MAP, mean arterial pressure; RPP, heart rate pressure product.

Compared with the Control group, the LCT and LCT/MCT groups had shorter time to ROSC (Control group vs LCT group, 113±36 vs 78±20 seconds, P=0.001; Control group vs LCT/MCT group, 113±36 vs 73±16 seconds, P<0.001), and higher survival rate (Control group vs LCT group, 36.4% vs 72.7%, P=0.033; Control group vs LCT/MCT group, 36.4% vs 77.3%, P=0.014). All the rats that survived at 120 minutes had no further deaths during the 72 hours after onset of resuscitation. The comparison of time to CA, time to first heartbeat, rate of ROSC, mortality after ROSC or epinephrine cumulative dose among the three groups demonstrated no statistical differences.

Hemodynamic Measures

Hemodynamic parameters are represented as MAP, HR, and RPP in the three groups, and are presented graphically in Figure 1. Within 120 minutes after the onset of resuscitation, the MAP of the LCT and LCT/MCT groups were higher than that of the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the MAP of the LCT/MCT groups was higher than that of LCT group (LCT group vs LCT/MCT group, P=0.001). Significant differences were demonstrated among the three groups in MAP at 2 (P=0.037; Control group vs LCT group, P=0.038), 4 (P=0.018; LCT group vs LCT/MCT group, P=0.018), 10 (P<0.001; Control group vs LCT group, P=0.005; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 20 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT

Table 3 Baseline Values of Blood Gas Values for Control $_{0-120}$, LCT $_{0-120}$ and LCT/MCT $_{0-120}$ Groups in Experiment B

	pH	PO$_2$ (mmHg)	PCO$_2$ (mmHg)	HCO$_3^-$ (mmol/L)	BE (mmol/L)	Lactate (mmol/L)
Control $_0$	7.40±0.05	120±9	40±4	23.5±1.9	0.1±1.5	0.9±0.7
Control $_{20}$	7.38±0.03	126±11	40±2	24.6±1.3	−0.3±1.2	1.6±0.6
Control $_{40}$	7.41±0.02	125±14	38±6	23.7±2.1	−0.2±1.6	0.8±0.5
Control $_{60}$	7.39±0.03	129±14	41±3	24.5±1.3	0.2±1.2	1.4±0.7
Control $_{80}$	7.42±0.04	118±11	40±3	23.3±1.6	0.4±1.1	1.2±0.4
Control $_{100}$	7.38±0.03	123±8	39±2	22.9±2.2	−0.1±0.9	1.0±0.6
Control $_{120}$	7.41±0.04	125±7	41±2	23.5±1.9	0.2±1.3	1.3±0.8
LCT $_0$	7.40±0.05	124±14	39±3	22.5±1.7	0.9±1.1	1.1±0.8
LCT $_{20}$	7.40±0.05	119±12	41±2	24.9±1.5	−0.2±1.8	1.6±0.7
LCT $_{40}$	7.38±0.03	121±9	39±3	24.2±1.4	−0.4±1.3	1.0±0.4
LCT $_{60}$	7.40±0.06	122±10	40±2	24.7±1.1	0.5±1.6	1.5±0.6
LCT $_{80}$	7.39±0.04	125±11	39±3	23.8±1.5	−0.8±1.2	1.2±0.8
LCT $_{100}$	7.38±0.03	127±7	41±3	23.7±1.2	0.4±1.5	0.9±0.6
LCT $_{120}$	7.41±0.04	124±15	40±2	24.1±1.5	0.3±1.2	1.2±0.9
LCT/MCT $_0$	7.39±0.03	123±9	39±3	24.5±1.4	0.4±1.3	1.2±0.7
LCT/MCT $_{20}$	7.41±0.06	128±11	39±2	24.5±1.3	−0.5±1.1	1.3±0.6
LCT/MCT $_{40}$	7.40±0.04	122±8	40±2	23.1±1.7	0.2±1.2	1.1±0.9
LCT/MCT $_{60}$	7.39±0.02	124±11	40±3	23.9±2.1	0.4±1.3	1.3±0.4
LCT/MCT $_{80}$	7.41±0.03	116±12	39±3	23.6±2.0	−0.1±1.6	1.0±0.7
LCT/MCT $_{100}$	7.37±0.06	122±6	40±3	23.9±1.6	−0.3±1.1	1.4±0.9
LCT/MCT $_{120}$	7.37±0.03	129±12	39±2	24.1±1.9	−0.2±1.4	0.9±0.6

Notes: Baseline values showed no difference among the 21 groups; All values are mean ± SD.
Abbreviations: PO$_2$, partial pressure of oxygen; PCO$_2$, partial pressure of carbon dioxide; HCO$_3^-$, bicarbonate radical; BE, base excess.

group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). The HRs of the LCT and LCT/MCT groups were higher than that of the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the HR of the LCT/MCT groups was higher than that of the LCT group (LCT group vs LCT/MCT group, P=0.004). Significant differences were demonstrated among the three groups in HR at 4 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001), 6 (P=0.024; LCT group vs LCT/MCT group, P=0.020), 20 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.027), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.013), 40 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group,

P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.030), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). The RPP of the LCT and LCT/MCT groups were higher than that of the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the RPP of the LCT/MCT groups was higher than that of the LCT group (LCT group vs LCT/MCT group, P<0.001). Significant differences were demonstrated among the three groups in RPP at 4 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P=0.019), 6 (P=0.024; Control group vs LCT/MCT group, P=0.016; LCT group vs LCT/MCT group, P=0.016), 10 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT

Table 4 Resuscitation Outcomes for Control, LCT and LCT/MCT Groups in Experiment A

	Control (n=22)	LCT (n=22)	LCT/MCT (n=22)
Time to CA, seconds	105±18	107±15	104±16
Time to first heartbeat, seconds	59±13	55±11	60±15
Time to ROSC, seconds	113±36	78±20**	73±16†††
Rate of ROSC, %	50.0% (11)	72.7% (17)	77.3% (17)
Mortality after ROSC, %	27.3% (3)	5.9% (1)	0 (0)
Survival rate, %	36.4% (8)	72.7% (16)*	77.3% (17)†
Epinephrine cumulative dose, μg/kg	20 (20,20)	20 (20,20)	20 (10,20)

Notes: The Control, LCT and LCT/MCT groups displayed differences in time to ROSC (P<0.001); Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in survival rate (P=0.012); Control group vs LCT group, P=0.033; Control group vs LCT/MCT group, P=0.014; LCT group vs LCT/MCT group, P=1.000. No significant differences were demonstrated among the three groups in time to CA, time to first heartbeat, rate of ROSC, mortality after ROSC or epinephrine cumulative dose. Normal data are mean ± SD, whereas non-normal data are median and interquartile values. *Control group vs LCT group, †Control group vs LCT/MCT group. *P<0.05, †P<0.05, **P<0.01, †††P<0.001.
Abbreviations: ROSC, return of spontaneous circulation; CA, cardiac arrest.

group, P<0.001; LCT group vs LCT/MCT group, P=0.001), 20 (P<0.001; Control group vs LCT group, P=0.023; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/

MCT group, P<0.001), 50 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.037), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.026), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). The LCT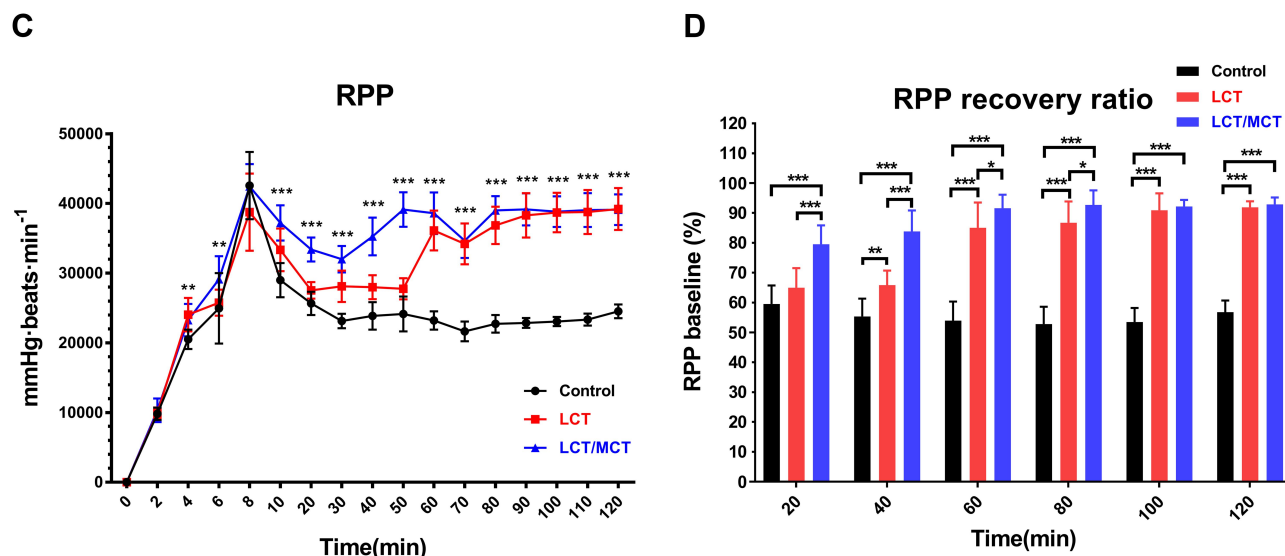/MCT group resulted in a more complete recovery of RPP at 20 (P<0.001), 40 (P<0.001), 60 (P<0.001), 80 (P<0.001), 100 (P<0.001) and 120 minutes (P<0.001) than did the Control group. The LCT group resulted in a more complete recovery of RPP at 40 (P=0.001), 60 (P<0.001), 80 (P<0.001), 100 (P<0.001) and 120 minutes (P<0.001) than the Control group. The LCT/MCT group resulted in a more complete recovery of RPP at 20 (P<0.001), 40 (P<0.001), 60 (P=0.023) and 80 minutes (P=0.021) than the LCT group.

Coronary Perfusion Pressure

CPP (coronary perfusion pressure, defined as simultaneously recorded diastolic arterial pressure minus central

A MAP

B HR

Figure 1 Continued.

C

D

Figure 1 Hemodynamic parameters for all rats that survived to 120 minutes in experiment A.

Notes: Data are presented as mean ± SD. (**A**) MAP vs time for rats during the 120-minute observation period after onset of resuscitation. Significant differences were demonstrated among the three groups in MAP during 120 minutes (P<0.001). The LCT and LCT/MCT groups produced superior MAP compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the LCT/MCT group produced superior MAP compared with the LCT group (LCT group vs LCT/MCT group, P=0.001). Significant differences were demonstrated among the three groups in MAP at 2 (P=0.037; Control group vs LCT group, P=0.038), 4 (P=0.018; LCT group vs LCT/MCT group, P=0.018), 10 (P<0.001; Control group vs LCT group, P=0.005; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 20 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). (**B**) HR vs time for rats during the 120-minute observation period after onset of resuscitation. Significant differences were demonstrated among the three groups in HR during 120 minutes (P<0.001). The LCT and LCT/MCT groups produced superior HR compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the LCT/MCT group produced superior HR compared with the LCT group (LCT group vs LCT/MCT group, P=0.004). Significant differences were demonstrated among the three groups in HR at 4 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001), 6 (P=0.024; LCT group vs LCT/MCT group, P=0.020), 20 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.027), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.013), 40 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.030), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). (**C**) RPP vs time for rats during the 120-minute observation period after onset of resuscitation. Significant differences were demonstrated among the three groups in RPP during 120 minutes (P<0.001). The LCT and LCT/MCT groups produced superior RPP compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), and the LCT/MCT group produced superior RPP compared with the LCT group (LCT group vs LCT/MCT group, P<0.001). Significant differences were demonstrated among the three groups in RPP at 4 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P=0.019), 6 (P=0.024; Control group vs LCT/MCT group, P=0.016; LCT group vs LCT/MCT group, P=0.016), 10 (P<0.001; Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.001), 20 (P<0.001; Control group vs LCT group, P=0.023; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.037), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.026), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). (**D**) RPP recovery ratio at 20, 40, 60, 80, 100 and 120 minutes. Significant differences were demonstrated among the three groups in RPP recovery ratio at 20 (P<0.001), 40 (P<0.001), 60 (P<0.001), 80 (P<0.001), 100 (P<0.001) and 120 minutes (P<0.001). The LCT/MCT group had higher RPP recovery ratios compared with the Control group at 20 (P<0.001), 40 (P<0.001), 60 (P<0.001), 80 (P<0.001), 100 (P<0.001) and 120 minutes (P<0.001). The LCT group had higher RPP recovery ratios compared with the Control group at 40 (P=0.001), 60 (P<0.001), 80 (P<0.001), 100 (P<0.001) and 120 minutes (P<0.001). The LCT/MCT group had higher RPP recovery ratios compared with the LCT group at 20 (P<0.001), 40 (P<0.001), 60 (P=0.023) and 80 minutes (P=0.021). *P<0.05, **P<0.01, ***P<0.001.

Abbreviations: MAP, mean arterial pressure; HR, heart rate; RPP, heart rate pressure product.

venous pressure[12]) in the three groups is graphically demonstrated in Figure 2. Within 120 minutes after the onset of resuscitation, the CPP of LCT and LCT/MCT groups wa higher than that of the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/ MCT group, P<0.001). There was no statistical signifi-cance when comparing CPP between the LCT and the LCT/MCT group during the 120-minute observation per-iod. Significant differences were demonstrated among the three groups in CPP at 2 (P=0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P=0.039), 4 (P=0.041; LCT group vs LCT/MCT group, P=0.037), 10 (P=0.011; Control group vs LCT group, P=0.033; Control group vs LCT/MCT group, P=0.011;
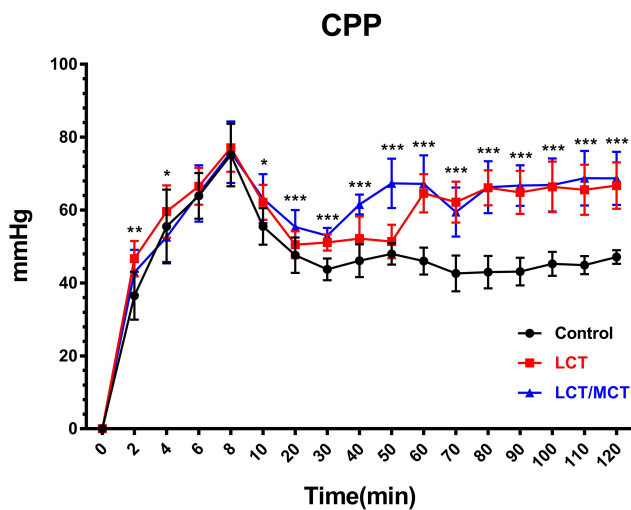
LCT group vs LCT/MCT group, P<0.001), 20 (P<0.001; Control group vs LCT/MCT group, P=0.001; LCT group vs LCT/MCT group, P=0.008), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/ MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P=0.014; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001).

Blood Gas Analysis and Hemodynamic Metrics 120 Minutes and 72 Hours After Onset of Resuscitation

Arterial blood gas values and hemodynamic metrics 120 min-utes and 72 hours after onset of resuscitation in Experiment A are shown in Table 5. In regard to blood gas values 120 minutes after onset of resuscitation, the LCT group had higher PO_2 (Control group vs LCT group, P<0.001), $HCO3^-$ (Control group vs LCT group, P=0.027) and BE (Control group vs LCT group, P=0.016), and lower lactate (Control group vs LCT group, P=0.043), when compared with the Control group; the LCT/MCT group had higher pH (Control group vs LCT/MCT group, P=0.001), PO_2 (Control group vs LCT/MCT group, P<0.001), $HCO3^-$ (Control group vs LCT/MCT group, P<0.001) and BE (Control group vs LCT/MCT group, P<0.001), and lower lactate (Control group vs LCT/MCT group, P=0.002), when compared with the Control group; the LCT/MCT group had higher $HCO3^-$ (LCT group vs LCT/ MCT group, P=0.020) and BE (LCT group vs LCT/MCT group, P=0.018) compared with the LCT group. In regard to blood gas values 72 hours after onset of resuscitation, the LCT and LCT/MCT groups had higher pH (Control group vs LCT group, P=0.017; Control group vs LCT/MCT group, P=0.032), PO_2 (Control group vs LCT group, P<0.001; Control group vs

Figure 2 CPP vs time for rats that survived to 120 minutes in experiment A.
Notes: Data are presented as mean ± SD. Significant differences were demon-strated among the three groups during 120 minutes (P<0.001). The LCT and LCT/ MCT groups produced superior CPP compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). No significant difference was demonstrated between the LCT and the LCT/MCT group in CPP. Significant differences were demonstrated among the three groups in CPP at 2 (P=0.001; Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P=0.039), 4 (P=0.041; LCT group vs LCT/MCT group, P=0.037), 10 (P=0.011; Control group vs LCT group, P=0.033; Control group vs LCT/MCT group, P=0.011; LCT group vs LCT/MCT group, P<0.001), 20 (P<0.001; Control group vs LCT/MCT group, P=0.001; LCT group vs LCT/MCT group, P=0.008), 30 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 40 (P<0.001; Control group vs LCT group, P=0.014; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 50 (P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P<0.001), 60 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 70 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 80 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 90 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 100 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), 110 (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001) and 120 minutes (P<0.001; Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001).
*P<0.05, **P<0.01, ***P<0.001.
Abbreviation: CPP, coronary perfusion pressure.

Table 5 Blood Gas Values and Hemodynamic Metrics for Control, LCT and LCT/MCT Groups 120 Minutes and 72 Hours After Onset of Resuscitation in Experiment A

	Control	LCT	LCT/MCT
At 120 Minutes			
pH	7.19±0.07	7.26±0.07	7.32±0.08[††]
PO$_2$, mmHg	153±28	217±24[***]	236±29[†††]
PCO$_2$, mmHg	38±3	40±3	40±2
HCO$_3^-$, mmol/L	17.3±1.7	19.1±1.2[*]	20.6±1.7[†††,‡]
BE, mmol/L	−10.3±1.3	−7.7±2.8[*]	−4.7±3.1[†††,‡]
Lactate, mmol/L	10.2±1.5	7.6±2.7[*]	6.5±2.4[††]
HR, beat/min	292±2	335±16[***]	334±15[†††]
MAP, mmHg	61±2	85±6[***]	87±6[†††]
RPP, mmHg*beat/min	24520±974	39191±3032[***]	39108±2207[†††]
After 72 Hours			
pH	7.28±0.08	7.38±0.03[*]	7.37±0.03[†]
PO$_2$, mmHg	91±12	116±15[***]	113±10[††]
PCO$_2$, mmHg	37±2	38±3	39±3
HCO$_3^-$, mmol/L	19.9±1.7	23.0±2.3[**]	22.9±1.5[††]
BE, mmol/L	−5.1±2.1	−3.3±1.4[*]	−2.8±1.3[††]
Lactate, mmol/L	5.5±2.1	3.2±1.6[*]	3.4±1.7[†]
HR, beat/min	307±12	340±13[***]	337±12[†††]
MAP, mmHg	71±7	85±5[***]	83±7[†††]
RPP, mmHg*beat/min	28617±2546	41918±2992[***]	41506±2501[†††]

Notes: The Control, LCT and LCT/MCT groups displayed differences in pH at 120 minutes (P=0.001); Control group vs LCT group, P=0.109; Control group vs LCT/MCT group, P=0.001; LCT group vs LCT/MCT group, P=0.063. The Control, LCT and LCT/MCT groups displayed differences in PO2 at 120 minutes (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.142. The Control, LCT and LCT/MCT groups displayed differences in HCO3$^-$ at 120 minutes (P<0.001); Control group vs LCT group, P=0.027; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.020. The Control, LCT and LCT/MCT groups displayed differences in BE at 120 minutes (P<0.001); Control group vs LCT group, P=0.016; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.018. The Control, LCT and LCT/MCT groups displayed differences in lactate at 120 minutes (P=0.003); Control group vs LCT group, P=0.043; Control group vs LCT/MCT group, P=0.002; LCT group vs LCT/MCT group, P=0.581. The Control, LCT and LCT/MCT groups displayed differences in HR at 120 minutes (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.999. The Control, LCT and LCT/MCT groups displayed differences in MAP at 120 minutes (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=0.924. The Control, LCT and LCT/MCT groups displayed differences in RPP at 120 minutes (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in pH after 72 hours (P<0.001); Control group vs LCT group, P=0.017; Control group vs LCT/MCT group, P=0.032; LCT group vs LCT/MCT group, P=0.609. The Control, LCT and LCT/MCT groups displayed differences in PO2 after 72 hours (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in HCO3$^-$ after 72 hours (P=0.001); Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P<0.003; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in BE after 72 hours (P=0.005); Control group vs LCT group, P=0.029; Control group vs LCT/MCT group, P=0.005; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in lactate after 72 hours (P=0.009); Control group vs LCT group, P=0.011; Control group vs LCT/MCT group, P=0.021; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in HR after 72 hours (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in MAP after 72 hours (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. The Control, LCT and LCT/MCT groups displayed differences in RPP after 72 hours (P<0.001); Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; LCT group vs LCT/MCT group, P=1.000. No significant differences were demonstrated among the three groups in PCO2 at 120 minutes or 72 hours after onset of resuscitation. All values are mean ± SD. *Control group vs LCT group, †Control group vs LCT/MCT group, ‡LCT group vs LCT/MCT group. *P<0.05, †P<0.05, ‡P<0.05, **P<0.01, ††P<0.01, ***P<0.001, †††P<0.001.

Abbreviations: PO$_2$, partial pressure of oxygen; PCO$_2$, partial pressure of carbon dioxide; HCO$_3^-$, bicarbonate radical; BE, base excess; HR, heart rate; MAP, mean arterial pressure; RPP, heart rate pressure product.

LCT/MCT group, P=0.001), HCO3$^-$ (Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P<0.003) and BE (Control group vs LCT group, P=0.029; Control group vs LCT/MCT group, P=0.005), and lower lactate (Control group vs LCT group, P=0.011; Control group vs LCT/MCT group, P=0.021), when compared with the Control group. In regard to hemodynamic metrics, the LCT and LCT/MCT groups had higher HR (for 120 minutes, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; for 72 hours, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001), MAP (for 120 minutes, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; for 72 hours, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.0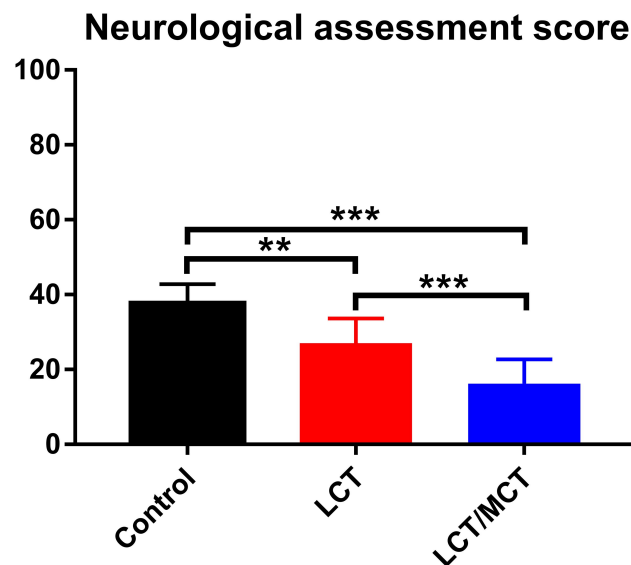01) and RPP (for 120 minutes, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001; for 72 hours, Control group vs LCT group, P<0.001; Control group vs LCT/MCT group,

Neurological assessment score

Figure 3 Neurological assessment score for rats that survived to 120 minutes in experiment A.
Notes: Significant differences were demonstrated among the three groups (P<0.001). The LCT and LCT/MCT groups had lower neurological assessment score compared with the Control group (Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001), and LCT/MCT groups had lower neurological assessment score compared with the LCT group (LCT group vs LCT/MCT group, P<0.001). **P<0.01, ***P<0.001.

P<0.001) compared with the Control group 120 minutes and 72 hours after onset of resuscitation. No significant differences were observed among the three groups in PCO$_2$ at 120 minutes or 72 hours after onset of resuscitation.

Neurological Assessment Score

Neurological assessment score in the three groups is graphically demonstrated in Figure 3. The LCT and LCT/MCT groups had lower neurological assessment score

(Control group vs LCT group, P=0.001; Control group vs LCT/MCT group, P<0.001) compared with the Control group, and the LCT/MCT group had lower neurological assessment score (LCT group vs LCT/MCT group, P<0.001) compared with the LCT group.

Lung Permeability Index and Wet-to-Dry Ratio

Lung permeability index and wet-to-dry ratio in the three groups are graphically demonstrated in Figure 4. The LCT and LCT/MCT groups had decreased lung permeability index compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). The LCT and LCT/MCT groups had decreased wet-to-dry ratio compared with the Control group (Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P=0.033).

Energy Charge

Energy charge in the three groups is graphically demonstrated in Figure 5. The LCT/MCT group resulted in superior energy charge at 20 (P<0.001) and 40 minutes (P<0.001) compared with the Control group. The LCT group resulted in superior energy charge at 40 (P<0.001) and 60 minutes (P<0.001) compared with the Control group. The LCT/MCT group resulted in superior energy charge at 20 (P<0.001) and 40 minutes (P<0.001), and inferior energy charge at 60 minutes (P<0.001) compared with the LCT group. The comparison of energy charge among the three
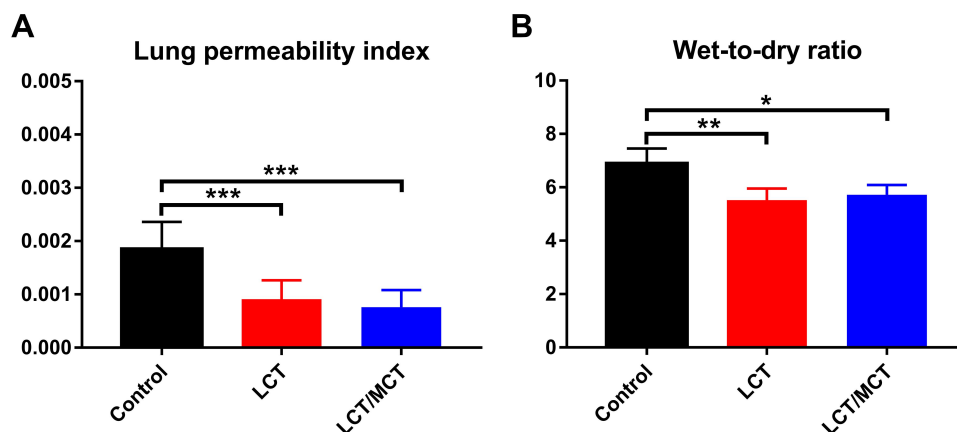

Figure 4 (**A**) Lung permeability index for rats that survived to 120 minutes in experiment A.
Notes: Significant differences were demonstrated among the three groups (P<0.001). The LCT and LCT/MCT groups had lower lung permeability index compared with the Control group (Control group vs LCT group, P<0.001; Control group vs LCT/MCT group, P<0.001). (**B**) Wet-to-dry ratio for rats that survived to 120 minutes in experiment A. Significant differences were demonstrated among the three groups (P=0.003). The LCT and LCT/MCT groups had lower wet-to-dry ratio compared with the Control group (Control group vs LCT group, P=0.002; Control group vs LCT/MCT group, P=0.033). *P<0.05, **P<0.01, ***P<0.001.
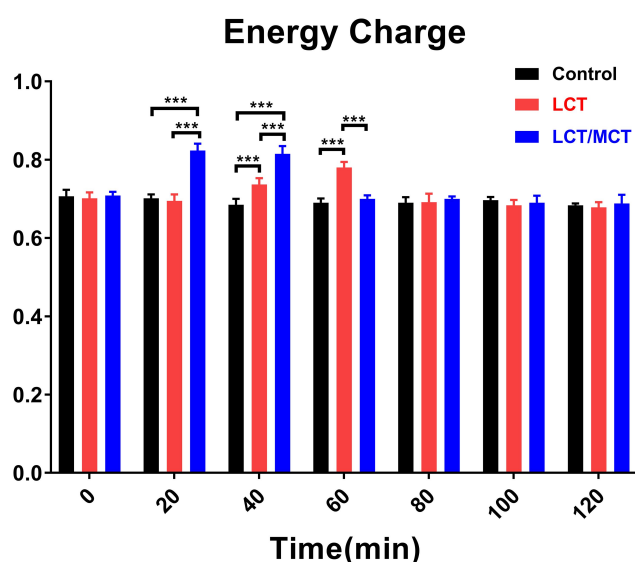
Energy Charge

Figure 5 Energy charge at 0, 20, 40, 60, 80, 100 and 120 minutes.
Notes: Significant differences were demonstrated among the three groups at 20 (P<0.001), 40 (P<0.001) and 60 minutes (P<0.001). The LCT/MCT group had higher energy charge compared with the Control group at 20 (P<0.001) and 40 minutes (P<0.001). The LCT group had higher energy charge compared with the Control group at 40 (P<0.001) and 60 minutes (P<0.001). The LCT/MCT group had higher energy charge at 20 (P<0.001) and 40 minutes (P<0.001), and lower energy charge at 60 minutes (<0.001) compared with the LCT group. No significant difference was demonstrated among the three groups in energy charge at 0, 80, 100 or 120 minutes ***P<0.001.

groups demonstrated no statistical significance at 0, 80, 100 or 120 minutes.

Discussion

This aged rodent model demonstrated that epinephrine supplemented with lipid emulsions resulted in significantly shorter time to ROSC and higher survival rate. The combination of epinephrine and lipid emulsions also exhibited significant improvement in hemodynamic status, pulmonary gas exchange, and neurological performance. In addition, LCT/MCT emulsion provides more benefits over LCT emulsion. These changes might be attributed to ATP generated by lipid emulsions and the different levels of energy supply between them.

Epinephrine is a first-line medication in resuscitation of CA because it can exert an α-adrenergic effect which increases diastolic blood pressure and CPP by peripheral vascular constriction, and a β-adrenergic effect which produces cardiac positive inotropic action.[10] However, a high dose of epinephrine is associated with augmented myocardial oxygen consumption, severe metabolic acidosis, pulmonary edema and even unsuccessful resuscitation.[11,12] Degenerated myocardium and impaired myocardial microcirculation make senior patients more vulnerable to the side

effects exerted by epinephrine.[27] Lipid emulsion plays a crucial role in multi-organ protection. Dong et al[28] reported that ω-3 fish oil lipid emulsion preconditioning mitigates myocardial damage from aldehyde stress.[28] Chen et al[29] reported that 15- hydroxyeicosatetraenoic acid promotes angiogenesis and improves neurological function in a mouse model of focal ischemia.[29] In a rat model of limb ischemia-reperfusion, LCT lipid emulsion mitigates impaired pulmonary function through attenuation of systemic inflammation.[30] At the background of epinephrine-based therapy, lipid emulsion was demonstrated to be effective in improving resuscitation outcomes of lipophilic toxin-induced cardiac arrest.[16,17] The main mechanisms behind it include the "lipid sink" hypothesis:[19] whereby lipid-soluble compartments in the blood provide a medium for lipophilic toxins to partition in, thus allowing the toxin to be transferred from heart to organs that can store them with high blood flow, and the "lipid flux" theory:[20] in which lipids supply the mitochondria with sufficient free fatty acids (FFA) to generate energy the heart needs to restore regular heartbeat under ischemia and hypoxia conditions. Different to local anesthetic-induced cardiac arrest, which is induced by directly interfering with myocardial iron channels and inhibition of myocardial contraction, asphyxia-induced cardiac arrest is associated with myocardial hypoxia and low supply of energy. The resuscitation effect of lipid emulsion on asphyxia-induced cardiac arrest in aged animal has not been reported so far.

In the current study, the combination of lipid emulsions (LCT or LCT/MCT) and epinephrine resulted in a significantly shorter time to ROSC and higher survival rate compared with epinephrine alone. What is more, the LCT and LCT/MCT groups showed much higher RPP and RPP recovery ratio than the Control group during the initial 120 minutes observation period. These results are consistent with the findings of Van de Velde et al[31] where infusion of LCT lipid emulsion improved recovery from myocardial ischemia. These authors reconfirm this conclusion in an isolated heart model of myocardial stunning, where contractile function was improved with intralipid (a kind of LCT lipid emulsion) perfusion.[32] In addition, Fettiplace et al[33] demonstrated that lipid emulsion could exert a direct positive inotropic effect in both intact animal and isolated heart models.[33] These data from animal studies concur with clinical evidence showing that lipid emulsion increases blood pressure when applied for total parental nutrition.[34,35] All the observed beneficial effects of lipid emulsions here might partially correlate

with increased energy supply from FFA oxidation. Fatty acids are the preferred fuel for cardiomyocyte under normal aerobic conditions and cycled from the intracellular triglyceride pool into mitochondria for oxidation and production of high-energy phosphates.[36,37] Lipid emulsions' administration could theoretically increase intracellular fatty acid content, and impede inhibition of ATP synthesis resulted from myocardial ischemia in the cardiomyocyte, thereby improving ATP production and cardiac function.[20,38] In this study, we found higher energy charge during the first 80 minutes of the observation period in the lipid-containing groups when compared with that in the Control group. This finding is consistent with Van de Velde et al's[31] discovery, in which they reversely verified the existence of exogenous energy supply from lipid emulsion by importing oxfenicine, a carnitine palmitoyltransferase-1 (CPT-1) inhibitor, which could block myocardial FFA metabolism effectively.[31] Independent of the cardiac effect, lipid emulsion was also confirmed to moderate blood pressure by increasing vascular resistance.[39] The vasoconstriction driven by elevated FFA is possibly due to modifying adrenergic sensitivity[40] and interfering with the nitric oxide signal pathway.[41] However, we were unable to test the vascular effect in such an intact animal model applied in the current study.

Not all studies testing the response of lipid in animal models of CA have reported positive results. Increased serum and myocardial triglyceride have been shown to suppress ischemic myocardial performance both in animal models[42,43] and human subjects.[44,45] Under conditions of hypoxia and ischemia, myocardial substrate preference shifts to Beta oxidation of FFA, which consumes approximately 11% larger phosphate-to-oxygen ratios compared to glucose.[46] In addition, the increased oxygen consumption of FFA oxidation resulted in a relatively low efficiency in ATP generation, and high FFA loads may activate cycling of FFA in and out of the triglyceride pool, which is futile and energy-consuming.[47,48] These authors believe that augmenting inefficient FFA metabolism makes the already low-perfused cardiomyocyte even worse. CPP, calculated as simultaneously recorded diastolic arterial pressure minus central venous pressure, is a crucial index reflecting myocardial perfusion, and is strongly associated with resuscitation outcomes.[49] Epinephrine is effective in increasing diastolic arterial pressure via peripheral vasoconstriction, thus elevating CPP. The combination of epinephrine and lipid has shown its superiority to lipid or epinephrine alone in

improving CPP and cardiac function, both in in vivo and ex vivo animal models.[50] In the current study, lipid emulsions were administered with epinephrine. We speculate that epinephrine rapidly and temporarily elevates the CPP, which increases oxygen supply for cardiomyocyte, thus affording the high oxygen consumption of FFA oxidation, and the sufficient oxidation of FFA provides higher ATP content for cardiac contraction, thereby forming a virtuous cycle. In addition, the bolus of 10 μg/kg epinephrine used in this study was shown to be an appropriate dosage which elevates CPP without increasing oxygen consumption. This may explain the higher CPP and hemodynamic variables in the lipid-ontaining groups, and the positive results of lipid therapy in the current study.

Carnitine is essential for long-chain fatty acids to be transported into the mitochondria.[51] Instead, medium-chain fatty acid can flux into the mitochondria via simple diffusion independent of the enzyme.[52] What is more, medium-chain fatty acid could be rapidly oxidized without deposition of fat stores and supply energy with higher ketone bodies.[21] Accordingly, we found that the LCT/MCT group showed earlier increase of energy charge and higher increase of CPP, thus leading to earlier and better recovery of hemodynamics compared with LCT group. Meanwhile we note that the increase of energy charge in the LCT group lasted for a longer time. The half-life may have accounted for this observation: 33 minutes for LCT and 17 minutes for MCT,[53] which indicates that eliminating of the plasma LCT/MCT is quicker than LCT.

We found that the results of blood gas analysis, lung permeability index and wet-to-dry ratio showed a parallel trend to the hemodynamics. We supposed that prolonged time of resuscitation and delayed recovery of effective circulation contribute to pulmonary transudation, deterioration of pulmonary gas exchange and insufficient perfusion of peripheral tissues, which manifested as low PaO_2 and metabolic acidosis. Recovery of neurological function is also a main concern after successful resuscitation of CA in aged subjects.[27] In this study, the LCT/MCT group showed a lower score compared with the other two groups. Neurocyte depends on glucose uptake as a major source of energy, and is sensitive to hypoxia and ischemia, where a few minutes of complete ischemia would lead to irreversible neuronal apoptosis.[54] The rats receiving LCT/MCT emulsion showing earliest recovery of RPP among the three groups, were considered to have the shortest period of hypoxia injury to neurons, thus having relatively minor damage to neurological function. Mechanisms underlying

neuroprotection and lung protection reported include interfering with vascular resistance, vasopermeability change, anti-oxidation, and anti-inflammation.[55,56] However, we consider that improvement of hemodynamics plays the major role in reservation of neurological and pulmonary function due to their consistency of trends.

Our study has several shortcomings. 1) The regimen of lipid emulsions and epinephrine may not be the optimal. However, the dosage was determined in preliminary experiments for the purpose of optimizing recovery. 2) Large doses of lipids may lead to eg, intravascular hemolysis, pulmonary arterial hypertension, hypersensitivity, deep venous thrombosis, and pancreatitis.,[57,58] however we were unable to estimate these negative effects in the current study. 3) General anesthesia and mechanical ventilation may have an effect on the pharmacokinetics of study drugs and the responses of the cardiovascular systems in the setting of asphyxia-induced cardiac arrest, although it is indispensable for both practical and ethical considerations. 4) The current study tentatively tested the effect of lipid emulsions in a rodent model, however massive efforts in both ethical and practical aspects should be made to translate the animal study into clinical human trials.

In conclusion, the supplement of lipid emulsion to epinephrine improves resuscitation outcomes of asphyxia-induced cardiac arrest as it provides a higher survival rate after resuscitation, more energy charge, superior hemodynamic recovery, and more reserved pulmonary and neurological function than did epinephrine alone in an in vivo model of aged rat. Moreover, LCT/MCT emulsion provides benefits over LCT emulsion in epinephrine-based resuscitation. Further studies are needed to investigate the optimal regimen of lipid emulsion and the possible application of lipid resuscitation besides existing treatment in the clinical scenario of asphyxia-induced cardiac arrest in senior populations.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest for this work.

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