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Kidney protection by hypothermic total liquid ventilation after cardiac arrest in rabbits

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Abstract

Background: Total liquid ventilation (TLV) with perfluorocarbons has been shown to induce rapid protective cooling in animal models of myocardial ischemia and cardiac arrest, with improved neurological and cardiovascular outcomes after resuscitation. Here, we hypothesized that hypothermic TLV can also limit kidney injury after cardiac arrest.

Methods: Anesthetized rabbits were submitted to 15-min of untreated ventricular fibrillation. After resuscitation, three groups of 8 rabbits each were studied: 1) life support plus hypothermia (32-33°C) induced by cold TLV (TLV group), 2) life support without hypothermia (Control group), 3) Sham group (no cardiac arrest). Life support was continued for 6 hours before euthanasia and kidney removal.

Results: Time to target esophageal temperature was less than 5-min in the TLV group. Hypothermia was accompanied by preserved renal function in the TLV group as compared to Control regarding numerous markers including creatinin blood levels (12±1 vs 16±2 mg/L, respectively; mean±SEM), urinary N-acetyl-β-(D)-glucosaminidase (1.70±0.11 vs 3.07±0.10 U/mol creat), γ-glutamyltransferase (8.36±0.29 vs 12.96±0.44 U/mol creat) or β2-microtubulin (0.44±0.011 vs 12±0.04 U/mol creat). Kidney lesions evaluated by electron microscopy and conventional histology were also attenuated in TLV vs Control. The renal-protective effect of TLV was not related to differences in delayed inflammatory or immune renal responses since transcriptions of, e.g., Interferon-γ, Tumor necrosis factor-α, Interleukin-1β, Monocyte chemoattractant protein-1, Toll-like receptor-2, Toll-like receptor-4 and Vascular endothelial growth factor were similarly altered in TLV and Control vs Sham.

Conclusions: Ultrafast cooling with TLV is renal-protective after cardiac arrest and resuscitation, which could increase kidney availability for organ donation.

Introduction

Institution of therapeutic hypothermia has been well demonstrated to improve both survival and neurological outcome in patients resuscitated after out-of-hospital cardiac arrest [1,2]. Beyond this neuroprotective effect, it is also important to investigate the effect of hypothermia on the multivisceral dysfunction and the so-called "post-cardiac syndrome" [3]. As example, acute kidney injury affects ~12% of the survivors after cardiac arrest and could worsen the prognosis [4]. In this setting, Susantitaphong et al. recently analyzed clinical studies reporting kidney-related outcomes and demonstrated that therapeutic hypothermia neither prevented the development of acute kidney injury nor dialysis requirement [5]. In animal models of cardiac arrest, the benefit afforded by hypothermia however directly depends upon its rapidity of institution after cardiopulmonary resuscitation [6]. This benefit was investigated regarding cardiac and neurological outcomes [6-10] while renal function was not precisely investigated. Therefore, we hypothesized that hypothermia can also exert a renal-protective effect after cardiac arrest if applied very rapidly after the no-flow episode.

An original strategy providing ultrafast cooling is total liquid ventilation (TLV) of the lungs with temperature-controlled perfluorocarbons [11]. TLV can indeed use the lung as a heat exchanger and cool the body while maintaining gas exchanges [10,12-14]. As compared to conventional cooling with combined cold blankets and fluid administration, TLV provided a potent protection on brain and heart in rabbits submitted to equal or less than 10 min of cardiac arrest [10,15]. Here, we propose to use more severe experimental conditions inducing kidney dysfunction after 15 min of cardiac arrest in rabbits. We hypothesised that ultrafast cooling with TLV can increase kidney resistance to the post-cardiac arrest syndrome. Our endpoints were kidney function biomarkers, morphological appearance and transcriptomic responses.

Materials and Methods

The experiments were conducted in accordance with French official regulations, after approval by the institutional Animal Care Committee (ComEth “Anses/ENVA/UPEC” n°16, Maisons-Alfort; protocol 13/12/11-5). It conformed with the guidelines laid out in the Guide for the Care and Use of Laboratory Animals from the National Academy of Science.

Animal preparation

New Zealand rabbits (3.0-3.5 kg) were anesthetized using zolazepam, tiletamine and pentobarbital (all 20-30 mg/kg i.v.). They were intubated and mechanically ventilated (FiO2=100%). After administration of pancuronium bromide (200 µg/kg i.v.), two electrodes were implanted upon the chest and inserted into the esophagus for subsequent induction of ventricular fibrillation. Rectal, esophageal and tympanic temperatures were continuously monitored using thermal probes (Harvard Apparatus, Paris, France). Throughout the protocol, external electrocardiogram was recorded, as well as arterial blood pressure from a catheter implanted into the ear artery. Data were digitalized and analyzed using the data acquisition software HEM v3.5 (Notocord, Croissy-sur-Seine, France).

Experimental protocol

As illustrated in Figure 1, the animals were randomly assigned after a period of stabilization to the Sham group or two groups submitted to cardiac arrest (Control and TLV groups). In these two groups, cardiac arrest was induced by ventricular fibrillation by passing an alternating current (10 V, 4 mA) between the implanted electrodes. After 15 min of untreated fibrillation, cardiopulmonary resuscitation was started using cardiac massage (~ 200 compressions/min), electric attempts of defibrillation (5-10 J/kg) and intravenous administration of epinephrine (15 µg/kg i.v.). After resumption of spontaneous circulation (ROSC), administration of epinephrine was still permitted with an infusion pump to maintain mean arterial pressure at ~80 mmHg. In the TLV group, the animals were cooled to 32°C using TLV after ROSC. The lungs were filled with 10 ml/kg of perfluorocarbon (Fluorinert,

3M, Cergy, France) and the endotracheal tube was connected to our prototype of liquid ventilator (tidal volume = 7-10 ml/kg; respiratory rate = 6 breaths/min). The temperature of the perfluorocarbon was adjusted to maintain esophageal temperature at a target temperature of ~32°C. After 20 min of TLV and achievement of the hypothermic target temperature, the perfluorocarbon was evacuated from the lungs and the endotracheal tube was again connected to a conventional mechanical ventilator. Hypothermia was maintained externally at 32°C during the subsequent entire follow-up. In all groups, the animals were followed during a total duration of 6 hours after cardiac arrest. Blood samples were withdrawn at baseline, 15, 60, 180 and 360 minutes for the assessment of blood creatinin levels and blood gases partial pressure. Urine production was also measured, as well as urinary creatinin concentration for calculation of the corresponding clearance. We also assessed blood and/or urinary osmolarity (Freezing point depression osmometer; Roebling osmometer, Burladingen, Germany) and levels of sodium, glucose, creatin phosphokinase (Hitachi/Roche Cobas, Roche Diagnostic, Meylan, France), N-acetyl-β-(D)-glucosaminidase (proximal tubule lysosomal enzyme; Roche Diagnostic, Mannheim, Germany), γ-glutamyltransferase (marker of acute renal injury; Roche Diagnostic, Mannheim, Germany) and β2-microtubulin (marker of proximal tubule dysfunction; Roche Diagnostic, Mannheim, Germany). Fractional sodium excretion was calculated from blood and urinary sodium levels. At the end of the follow-up, animals were euthanized and kidneys were sampled for electron microscopy, conventional histology and molecular biology.

Electron microscopy and histological analyses

Kidneys samples were processed for transmission electron microscopy, as previously described [16]. Briefly, tissue sections of 1 mm^3 were fixed in glutaraldehyde (3%; 2h at 4°C), washed and postfixed in osmium tetroxide (1%; 1h at 4°C). They were dehydrated in graded series of aceton and embedded in araldite. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and were examined under an electron microscope (JEOL

1010, Tokyo, Japan). Mitochondria integrity (membrane damage and crest reduction), cellular oedema, loss of brush bordure, cellular vacuolization, lyses of intracellular organelles were evaluated. The degree of histological lesions was determined in the cortex and medulla using a semi-quantitative graded scale from 0 to 10 according to lesions extension among the kidney samples (0: no alterations; 1, mild lesions < 10% of the kidney; 2, lesions affecting 11-20%; 3, 21-30%; 4, 31-40%; 5, 41-50%; 6, 51-60%; 7, 61-70%; 8, 71-80%; 9, 81-90%; 10, >91%). Scores were blindly attributed by two independent observers after examination of at least 10 different sections.

Kidneys slices were also fixed in formaldehyde (4%) for conventional histology after hematoxylin-eosin-saffron staining. We used a 0-5 score system to blindly quantify the lesions severity in the cortex and the medulla (0: normal appearance; 5: extensive necrosis). For each animal, the sum of these two scores (cortex and medulla) led to an overall score from 0 to 10. Detection of macrophages was performed on paraffin tissue sections using the RAM11 antibody against rabbit macrophages (Dako, Trappes, France), as previously described [17]. The brush border integrity was also evaluated by immunochemistry staining using the CD10 antibody (Dako).

Real-Time Quantitative polymerase chain reaction

In all animals, kidney samples were fixed immediately after organ removal using liquid nitrogen. For cortical tissue RNA extraction, we used a commercial kit (Macherey Nagel, Hoerdt, France). Genomic DNA was removed using DNA-free kit (Applied Biosystems, Saint Aubin, France) and first-strand reverse transcription (Applied Biosystems) was performed. Real-Time polymerase chain reaction assays were performed on a RotorGene Q (Qiagen,Courtaboeuf, France) following the manufacturer's recommendations. Rabbit DNA primers were designed using OligoPerfect™ (Invitrogen, Carlsbad, NM, USA), QuantPrim (Universität Potsdam, Max-Planck-Gesellschaft) and OligoAnalyser (Integrated DNA Technologies, Coralville, IO, USA), with the sequences detailed in Supplemental Digital

Content 1 (Table). Finally, messenger RNA expression level, relative to expression in healthy kidneys, was quantified with the Pfaffl method (expressed as Relative Fold Change), using ribosomal L19, β Actine and RPLPO as gene references.

Statistics

Data were expressed as mean±SEM. Statistical analyses were performed using a statistical software (SigmaStat 3.5, Systat Software Inc., Chicago, IL, USA). Hemodynamic and biochemical parameters were compared between groups using a two-way ANOVA for repeated measures. Post-hoc analyses were performed between groups at each time-point using a Student t-test with Bonferonni correction (two-tailed). Values were not compared between the different time-points in order to avoid multiple comparisons. Histological scores and molecular biology markers were compared between groups using a Kruskall-Wallis non parametric test. Significant differences were determined at $P \leq 0.05$.

Results

Twenty-four animals were included in the different groups (n=8 / group), with no missing data. In Control and TLV groups, ROSC was obtained in 4.1±0.7 and 3.7±0.5 min after cardiac arrest, respectively.

TLV affords a very rapid cooling and preserves hemodynamic

As illustrated in Figure 1B, a mild and reversible decrease in esophageal and rectal temperatures was observed in the Control group after cardiac arrest. In comparison, temperatures decreased very rapidly in the TLV group and achieved 32°C within 5 min after the onset of TLV in the esophagus. As shown in Table 1, this was associated with a strong decrease in heart rate in the TLV group as compared to Control and Sham groups (e.g, -32% in TLV vs Control groups at t=360 min after cardiac arrest). Only minor changes were observed regarding mean blood pressure as our goal was to support values of ~80 mmHg using epinephrine infusion. The total doses administered to achieve this goal was significantly higher in Control vsTLV groups (990±179 and 361±23 µg/kg, respectively), showing a favourable hemodynamic effects of hypothermia. Despite epinephrine administration, blood pressure was moreover significantly decreased in the Control group at the end of the follow-up (t=360 min after cardiac arrest) as compared to the TLV group. In these two groups, we also observed a dramatic increase in glucose and lactate blood levels, as well as acidosis and decrease in blood oxygen partial pressure (Table 1). During the TLV episode (15 min after cardiac arrest), blood carbon dioxide partial pressure was also significantly higher in the TLV group as compared to Control and Sham. Creatinin phosphokinase blood levels were similarly increased in TLV and Control vs Sham.

TLV limits kidney injury after cardiac arrest

As shown in Table 2, blood creatinin levels were significantly increased at the end of the follow-up (360 min) in both groups submitted to cardiac arrest as compared to the Sham group. They were significantly higher in the Control group as compared to TLV. Creatinin

clearance was also significantly reduced in both Control and TLV groups when compared to Sham. This reduction tended to be more important in the Control vs TLV group (+50%) but this did not achieve statistical significance. Total urine output also non-significantly decreased in Control as compared to Sham and TLV groups. As shown in Table 3, the beneficial effect of TLV on tubule function was evidenced by preserved fractional sodium excretion and urine concentration capacity. We also observed a limited glucose urinary excretion in TLV as compared to Control animals, despite similar blood glucose levels (Table 1). The urinary concentrations of selective markers of tubular damages, *i.e.*, N-acetyl-β-(D)-glucosaminidase, β2-microtubulin and γ-glutamyl transferase, were also significantly decreased in TLV vs Control despite not completely normalized as compared to Sham animals.

These functional alterations were supported by kidney lesions in animals submitted to cardiac arrest as compared to Sham animals (Figures 2 and 3). As shown in Figure 2, electron microscopy revealed altered microvilli (brush border) and loss in cytosolic and mitochondrial crest density in the cortex and medulla in the Control group (Figures 2C and 2G). In comparison, cortex and medulla appearance was better preserved in the TLV group (Figure 2D and 2H). In Sham animals, the appearance was normal at electron microscopy (Figures 2B and 2F). This led to a significant decrease in lesion score in TLV vs Control group with a significant increase for both groups when compared to Sham (Figure 2A). The glomerular apparatus was preserved in all groups (Figure 2E). These differences were confirmed using conventional histology (Figure 3A). The injuries were indeed particularly marked in the cortex of the Control group as illustrated by a tubular necrosis in Figure 3C. In the TLV group, lesions were attenuated with “only” dilation of the proximal tubes (Figure 3D). Virtually no macrophages infiltrations were detected using the RAM-11 antibody (Figure 3E-3H), even in territories with extensive necrosis in the Control group (Figure 3F). Figure 3G illustrates one of the rare macrophages in a territory with a normal appearance in this same

group. Immunochemistry marking of the brush border membrane showed extensive degradation in Control animals (Figure 3J) as compared to Sham animals (3I). These alterations were not prevented in all animals in the TLV group. Some kidneys indeed showed normal appearance (Figure 3K) while others had clear brush border membrane alteration (Figure 3L).

Cardiac arrest strongly upregulates hypoxia and inflammation markers in both Control and TLV groups

As shown in Figure 4A, we observed a dramatic increase in the expression of hypoxia markers in both Control and TLV groups as compared to Sham (Heme Oxygenase-1 (HO-1), Erythropoïetin, Hypoxia-Inducible Factor 1α and Vascular Endothelial Growth Factor. The expression of the apoptotic marker Fas and the mobility marker RhoA were also similarly expressed in TLV and Control groups (Figure 4B). As illustrated in Figures 4C and 4D, the expression of endothelial activation and innate immunity markers was also not different between these two groups, including E-selectin, Vascular Cell Adhesion Molecule 1, Interleukin (IL)-10, IL-1β, Interferon-γ, Tumor Necrosis Factor-α, IL-18, Monocyte Chemoattractant Protein-1 and Toll-Like Receptors (TLR) 2 and 4.

Discussion

In the present study, we demonstrate that ultrafast cooling induced by TLV protects kidneys in a severe model of cardiac arrest in rabbits. This was strongly supported by improved renal function and preserved morphology using electron microscopy and conventional histology. Transcriptomic profiles were not much affected by TLV regarding numerous genes involved in innate immunity and hypoxic responses.

We previously showed that ultrafast hypothermic TLV can strongly prevent both cardiovascular and neurological dysfunctions after cardiac arrest [10]. These investigations were conducted in rabbits after shorter duration of cardiac arrest comprised between 5 and 10 min [10]. In these conditions, we observed dramatic cardiac and neurological dysfunctions while renal dysfunction was very mild. Here, we used a prolonged episode of 15 min of no-flow to obtain acute renal dysfunction and likely very poor neurological prognosis. TLV preserved urine output and significantly limited the rise in blood creatinin levels. The latter benefit was importantly not related to a decreased release of creatinin with hypothermia since creatinin phosphokinase blood levels were similarly enhanced in TLV and Control groups. Surprisingly, we did not observe any significant improvement in creatinin urinary clearance in TLV vs Control. It is rather difficult to analyze this result according to the complex effect of hypothermia on urine production. After out-of-hospital cardiac arrest, Zeiner et al. also showed a delayed recovery in creatinin clearance with therapeutic hypothermia (32-34°C) [18]. One could also argue that hypothermia directly decreased urine production or conversely increased the production of diluted urine as previously showed in hypothermic patients treated for acute ischemic stroke [19] or with profound hypothermia [20,21]. This was not the case in our experimental conditions as hypothermia preserved urine production and osmolarity in the TLV group as compared to Control. Importantly, the functional benefit afforded by TLV was also supported by a significant improvement of all markers of tubular damage, including N-acetyl-β-(D)-glucosaminidase, β2-microtubulin and γ-glutamyl

transferase. It would also be relevant to investigate original and specific biomarkers such as Neutrophil gelatinase-associated lipocalin (NGAL) and Kidney injury molecule-1 [22,23].

Morphological data definitely confirm the renal-protective effect of TLV in our study using either conventional histology or electron microscopy. Such a beneficial effect of moderate hypothermia has also been shown in animal models of regional renal ischemia-reperfusion in rats [24]. In the latter study, body temperature during ischemia dramatically affected the severity of injury, again showing the importance of per-ischemic and early cooling. Intra-ischemic hypothermia is however difficult to achieve during cardiac arrest, especially regarding abdominal and renal temperatures. In a recent meta-analysis including 19 studies and 2218 patients, Susantitaphong et al. showed that therapeutic hypothermia neither prevented the development of acute kidney injury nor dialysis requirement but was associated with lower mortality [5]. The present study suggests that the benefit of hypothermia could be still evidenced in very severe experimental conditions when it is induced very rapidly.

In the present study, the severity of the ischemic insult was also supported by kidney transcriptomic alterations. As an example, hypoxic stress led to a 30-50 fold increase in HO-1 expression, as well as a 10 and 6 fold increases for Erythropoïetin and Hypoxia-Inducible Factor 1α, respectively. These expressions were not different between TLV and Control groups, suggesting that the renal-protective effect of TLV was not directly mediated through these pathways. However, one could speculate that the potent upregulation of Erythropoïetin and HO-1 could be associated with a facilitation of tissue repair, as previously suggested after tubulointerstitial injury and progressive nephritis [25]. We also did not observe any significant difference in transcriptomic profiles of cellular mobility, endothelial activation and innate immunity markers between Control and TLV groups. A non-significant decrease in TLR2 and pro-inflammatory Tumor Necrosis Factor-α and Interferon-γ markers was however observed in the TLV group, as well as a mild and non-significant upregulation of the

regulatory IL-10 cytokine. Interestingly, the latter cytokine was shown to mediate delayed protection afforded by remote ischemic preconditioning against myocardial ischemia-reperfusion injury [26]. Conversely, TLR2 and TLR4 expression are well known to mediate kidney ischemia-reperfusion damages [27,28]. The lack of differences between groups could be a consequence of the early time-point of organ removal (6 h after cardiac arrest). This also suggests that the protective effect of TLV is at least in part related to other mechanisms, likely an early anti-necrotic effect through ultrafast hypothermia. Improvement in the hemodynamic status could also participate in the renal-protective effect.

The main limitation of the present study was therefore probably the short duration of follow-up before organ sampling and analyses after cardiac arrest. Animals were followed during only 6 h as it was difficult to maintain animals alive for much longer duration (*e.g.*, 24 h) according to the severity of the cardiac arrest insult (15 min of no-flow). In future studies, it could be relevant to analyse kidney function at later time-points, *e.g.* after transplantation in recipient animals. Hypothermic TLV could indeed offers quite new and promising therapeutic perspectives for uncontrolled organ donation after cardiac arrest or for controlled organ donation after brain death in initially resuscitated patients [29]. It was not possible to conduct transplantation experiments in rabbits in the present experimental conditions but we currently are working on a new technology of liquid ventilation that could be used in a relevant porcine model for a definitive proof-of-concept using organ transplantation [30]. There is indeed a high degree of proximity between human and pig kidneys with multilobular, multipapillary architecture, while mice, rats, dogs and rabbits have unilobular, unipapillary kidneys [31]. In dogs and rodents, segmental arteries are bypassed due to the lack of multiple medullary pyramids, while in humans and pigs an elaborated system of interlobar and segmental arteries is present to supply the numerous kidney lobes [30].

In conclusion, ultrafast cooling with TLV is renal-protective after cardiac arrest and resuscitation. Further experiments with organ transplantation might be relevant to afford a proof-of-concept in this setting.

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Table 1: Heart rate, mean arterial pressure and blood biochemical parameters

	Baseline	After cardiac arrest (min)			
		15	60	180	360
Heart rate (beats/min)					
Sham	253±12	234±8	239±12	244±11	235±8
Control	254±8	204±15*	184±9*	187±5*	203±11*
TLV	250±7	151±6†	131±3†	137±2†	139±5†
Mean blood pressure (mmHg))					
Sham	75±3	72±3	80±3	87±3	83±5
Control	81±4	84±4*	80±5	84±3	63±5*
TLV	78±3	98±3†	84±4	74±2†	74±5†
Lactates blood levels (mmol/L)					
Sham	3.0±0.3	2.8±0.5	2.6±0.3	2.2±0.3	2.2±0.5
Control	2.3±0.4	13.0±1.7*	12.9±1.3*	13.1±0.8*	11. 6±1.3*
TLV	2.7±0.2	9.6±0.9†	12.1±1.0*	10.6±0.8*	9.8±1.0*
Glucose blood levels (mg/dL)					
Sham	183±23	180±19	130±15	133±6	126±4
Control	184±31	480±27*	536±35*	700±28*	550±47*
TLV	153±58	375±64*	460±76*	477±88†	474±82*
Creatin phosphokinase blood levels (mg/Dl)					
Sham	42±1	-	-	-	56±1
Control	43±1	-	-	-	69±2*
TLV	44±1	-	-	-	67±1*
Blood pH					
Sham	7.41±0.02	7.42±0.03	-	7.44±0.02	7.45±0.02
Control	7.42±0.02	7.05±0.05*	-	7.06±0.08*	7.05±0.07*
TLV	7.42±0.05	6.92±0.06†	-	7.11±0.05*	7.04±0.05*
Blood pCO2 (mmHg)					
Sham	46±3	37±4	-	37±3	38±2
Control	46±5	44±3	-	43±3	43±6
TLV	42±3	77±8†	-	34±7	32±4
Blood pO_2 (mmHg)					
Sham	532±31	558±34	-	550±29	559±38
Control	504±34	185±41*	-	269±72*	250±65*
TLV	520±57	245±61*	-	310±58*	201±71*

TLV, total liquid ventilation; *, $p<0.05$ vs corresponding Sham group; †, $p<0.05$ vs corresponding Sham and Control groups.

Table 2: Blood creatinin levels, urine production and creatinin clearance

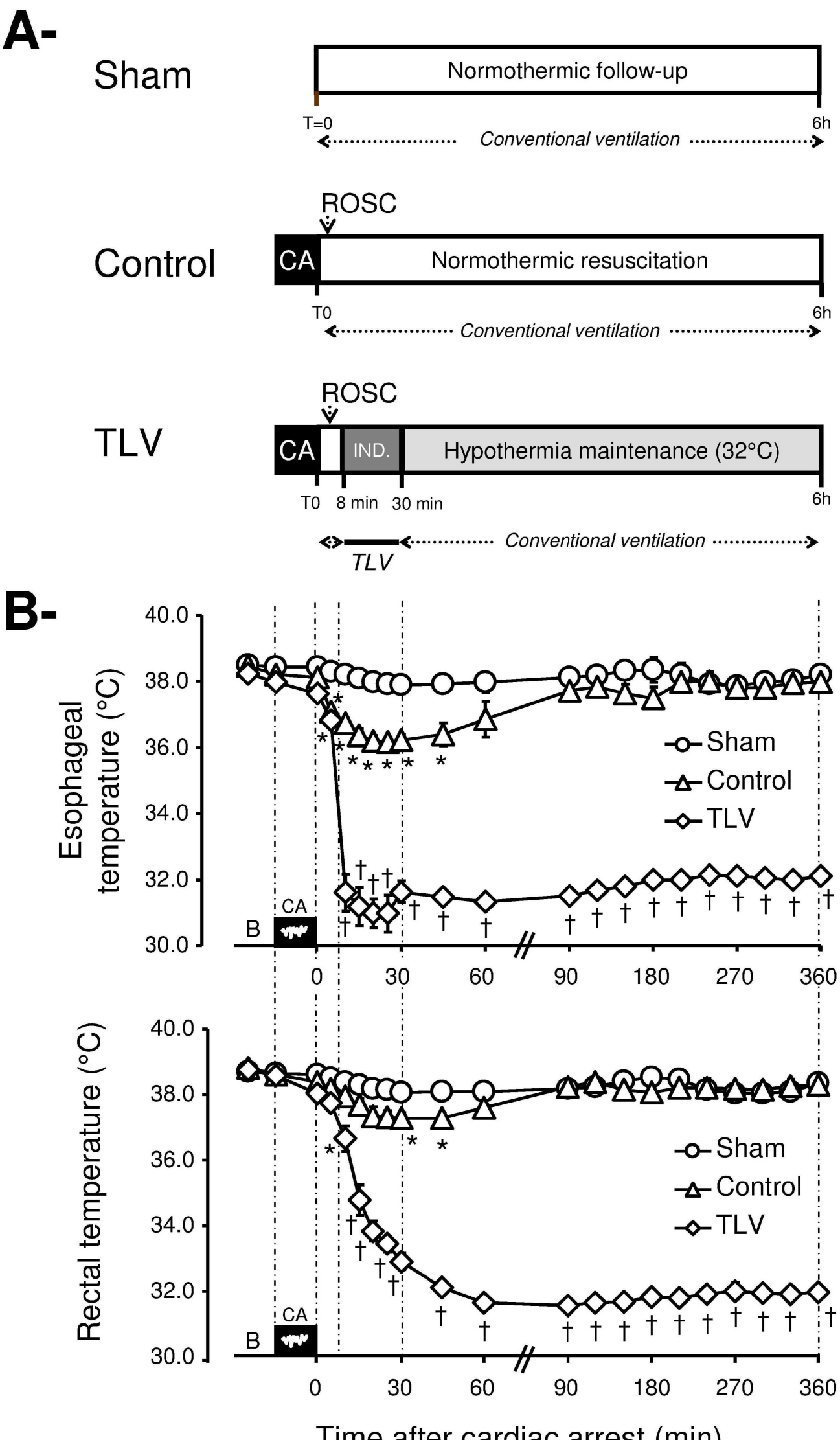
	Baseline	After cardiac arrest T=360 min
Blood creatinin levels (mg/L)		
Sham	7.5±0.4	7.8±0.8
Control	7.4±0.6	16.1±1.8*
TLV	6.9±0.7	12.3±1.2†
Total urine output (ml/h)		
Sham	-	6.3±1.6
Control	-	3.5±0.6
TLV	-	7.1±1.1
Creatinin clearance (ml/min/kg)		
Sham	-	2.8±0.5
Control	-	0.6±0.2*
TLV	-	0.9±0.2*

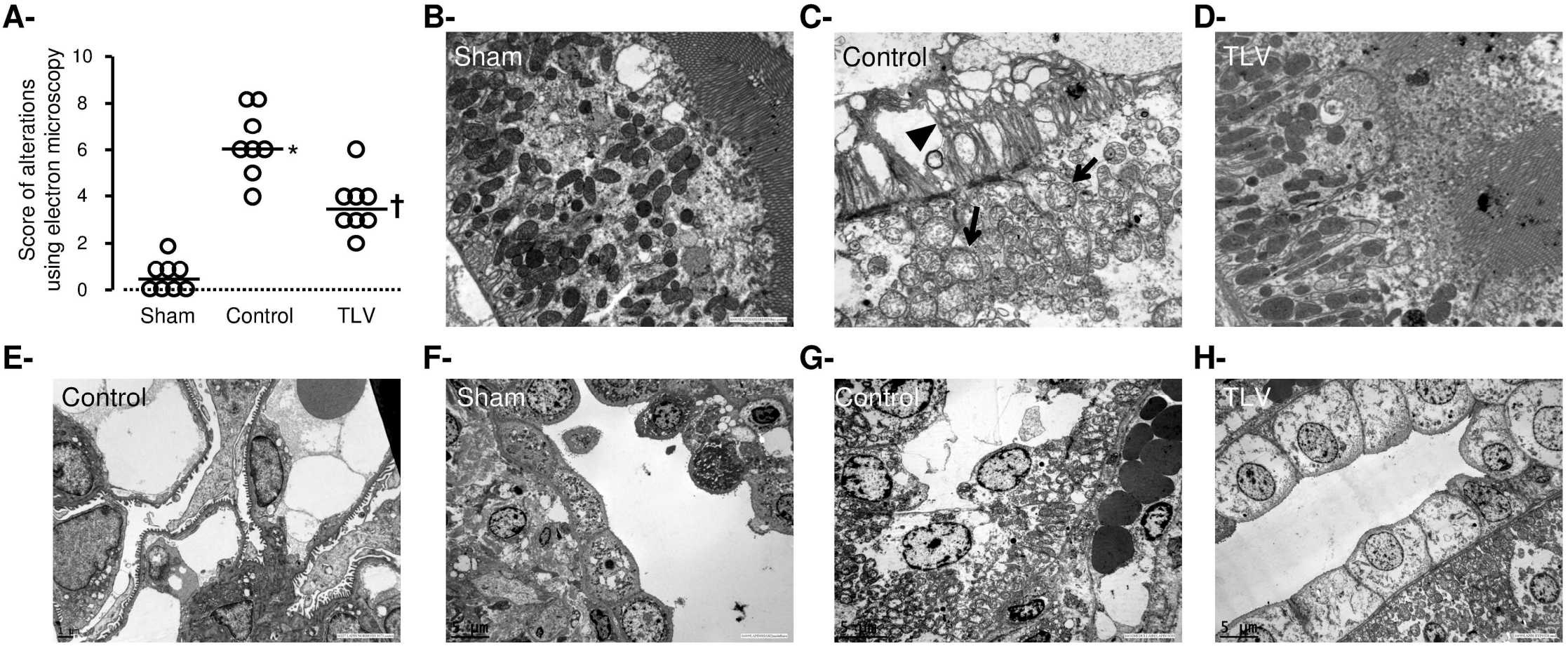
TLV. total liquid ventilation; *, $p<0.05$ vs corresponding Sham group; †, $p<0.05$ vs corresponding Sham and Control groups.

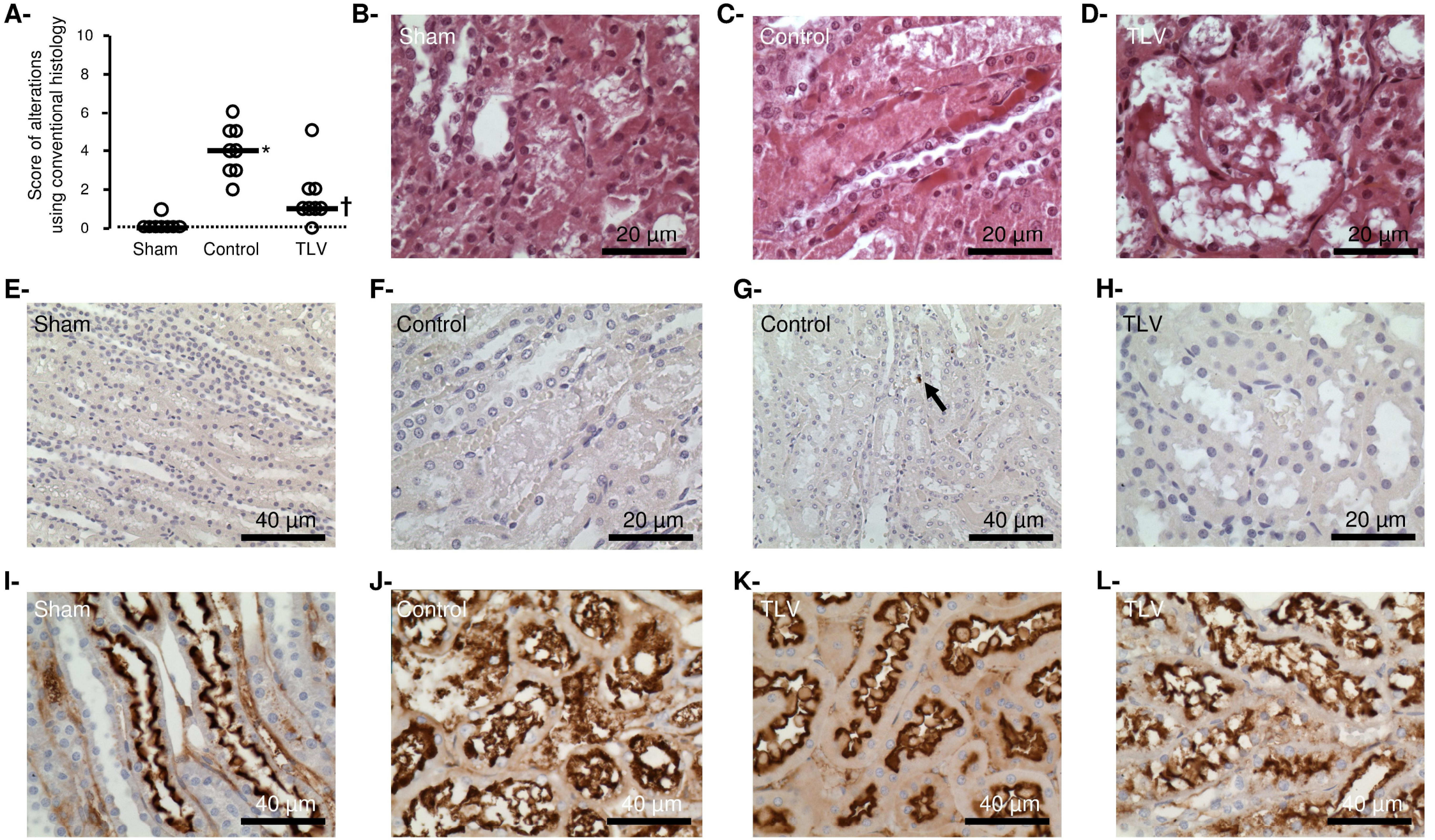
Table 3: Urinary markers of renal function

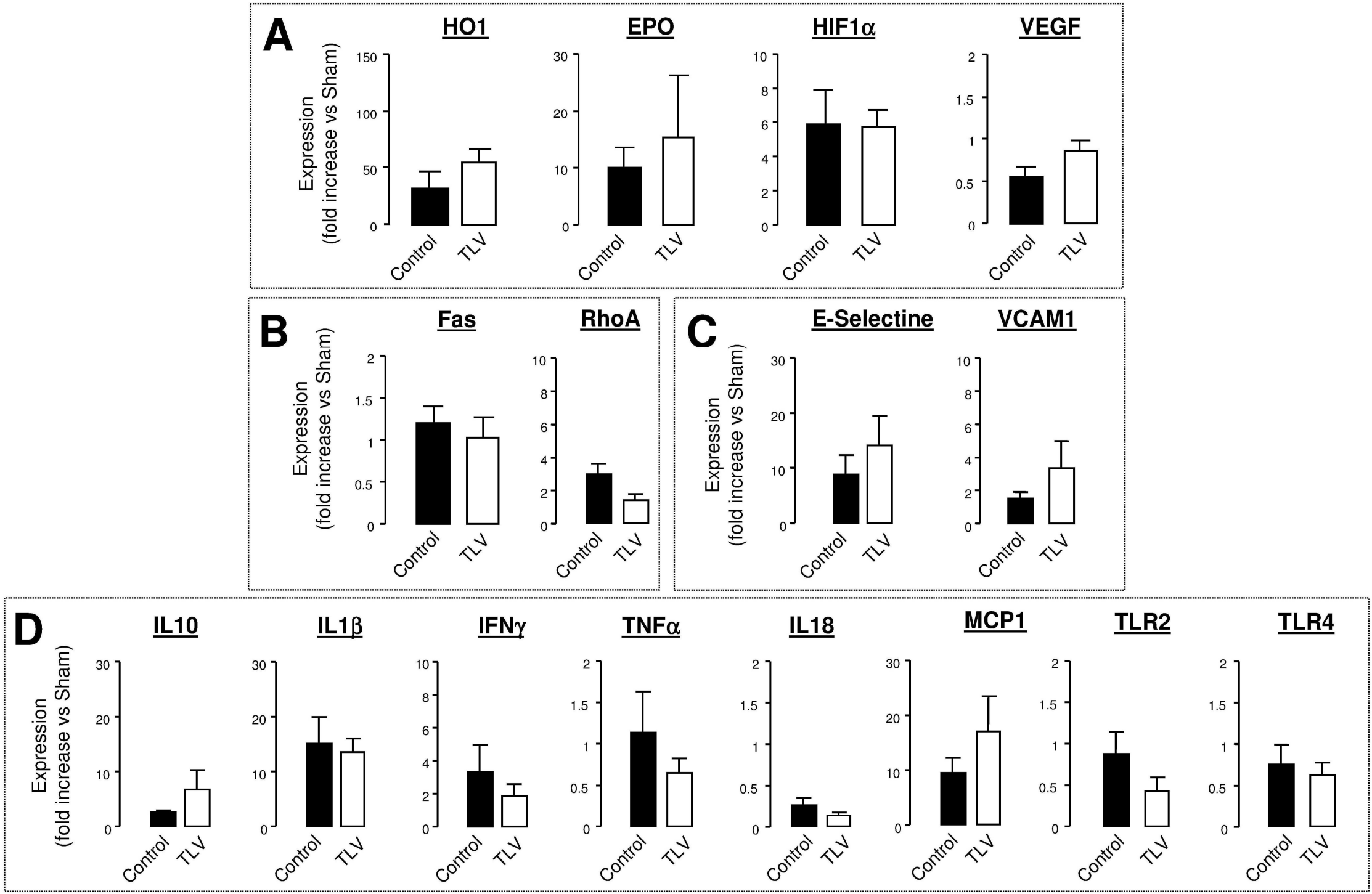
	Baseline	After cardiac arrest T=360 min
Fractional sodium excretion (%)		
Sham	1.04±0.03	0.84±0.03
Control	1.01±0.03	4.58±0.67*
TLV	1.04±0.02	2.09±0.11†
Ratio between plasmatic and urinary osmolarity		
Sham	0.93±0.01	0.91±0.01
Control	0.94±0.01	1.31±0.02*
TLV	0.96±0.01	1.01±0.00†
Glucose urinary levels (g/L)		
Sham	0.06±0.01	0.10±0.02
Control	0.06±0.01	1.27±0.02*
TLV	0.06±0.01	0.40±0.03†
N-acetyl-β-(D)-glucosaminidase urinary levels (Unit / mol creatinin)		
Sham	0.84±0.05	0.87±0.07
Control	0.94±0.04	3.07±0.10*
TLV	1.0±0.03	1.70±0.11†
β2-microtubulin urinary levels (Unit / mol creatinin)		
Sham	0.18±0.01	0.20±0.01
Control	0.17±0.01	1.12±0.04*
TLV	0.17±0.01	0.44±0.01†
γ-glutamyl transferase urinary levels (Unit / mol creatinin)		
Sham	5.31±0.59	5.18±0.39
Control	4.69±0.34	12.96±0.44*
TLV	4.82±0.49	8.36±0.29†

TLV. total liquid ventilation; *, $p<0.05$ vs corresponding Sham group; †, $p<0.05$ vs corresponding Sham and Control groups.


A-
Sham
Normothermic follow-up
T=0
6h
Conventional ventilation
ROSC
Control
CA
Normothermic resuscitation
T0
6h
Conventional ventilation
ROSC
TLV
CA
IND.
Hypothermia maintenance (32°C)
T0
8 min
30 min
6h
Conventional ventilation
TLV
B-
Esophageal temperature (°C)
40.0
38.0
36.0
34.0
32.0
30.0
Sham
Control
TLV
CA
B
0
30
60
90
180
270
360
Rectal temperature (°C)
40.0
38.0
36.0
34.0
32.0
30.0
Sham
Control
TLV
CA
B
0
30
60
90
180
270
360
Time after cardiac arrest (min)


A-
Score of alterations using electron microscopy
10
8
6
4
2
0
*
†
Sham
Control
TLV
B-
Sham
C-
Control
D-
TLV
E-
Control
1 µm
F-
Sham
5 µm
G-
Control
5 µm
H-
TLV
5 µm


A-
Score of alterations using conventional histology
10
8
6
4
2
0
*
†
Sham
Control
TLV
B-
Sham
20 µm
C-
Control
20 µm
D-
TLV
20 µm
E-
Sham
40 µm
F-
Control
20 µm
G-
Control
40 µm
H-
TLV
20 µm
I-
Sham
40 µm
J-
Control
40 µm
K-
TLV
40 µm
L-
TLV
40 µm


A
HO1
EPO
HIF1α
VEGF
Expression
(fold increase vs Sham)
Control
TLV
B
Fas
RhoA
C
E-Selectine
VCAM1
D
IL10
IL1β
IFNγ
TNFα
IL18
MCP1
TLR2
TLR4