Objective: As adenosine monophosphate (AMP)-activated protein kinase both controls cytoskeleton organization in endothelial cells and exerts anti-inflammatory effects, we here postulated that it could influence vascular permeability and inflammation, thereby counteracting cardiac wall edema during sepsis.

Design: Controlled animal study.

Settings: University research laboratory.

Subjects: C57BL/6J, α1 AMPK –/–, and α1 AMPK +/+ mice.

Intervention: Sepsis was triggered in vivo using a sublethal injection of lipopolysaccharide (O55B5, 10 mg/kg), inducing systolic left ventricular dysfunction. Left ventricular function, edema, vascular permeability, and inflammation were assessed in vivo in both wild-type mice (α1 AMPK +/+ ) and α1 AMPK-activated protein kinase–deficient mice (α1 AMPK –/– ). The 5-aminoimidazole-4-carboxamide riboside served to study the impact of AMP-activated protein kinase activation on vascular permeability in vivo. The integrity of endothelial cell monolayers was also examined in vitro after lipopolysaccharide challenge in the presence of aminoimidazole-4-carboxamide and/or after α1 AMP-activated protein kinase silencing.

Measurements and Main Results: α1 AMPK deficiency dramatically impaired tolerance to lipopolysaccharide challenge. Indeed, α1 AMPK –/– exhibited heightened cardiac vascular permeability after lipopolysaccharide challenge compared with α1 AMPK +/+ . Consequently, an increase in left ventricular mass corresponding to exaggerated wall edema occurred in α1 AMPK –/– , without any further decrease in systolic function. Mechanistically, the lipopolysaccharide-induced α1 AMPK +/+ cardiac phenotype could not be attributed to major changes in the systemic inflammatory response but was due to an increased disruption of interendothelial tight junctions. Accordingly, AMP-activated protein kinase activation by aminoimidazole-4-carboxamide riboside...
counteracted lipopolysaccharide-induced hyperpermeability in wild-type mice in vivo as well as in endothelial cells in vitro. This effect was associated with a potent protection of zonula occludens-1 linear border pattern in endothelial cells.

**Conclusions:** Our results demonstrate for the first time the involvement of a signaling pathway in the control of left ventricular wall edema during sepsis. AMP-activated protein kinase exerts a protective action through the preservation of interendothelial tight junctions. Interestingly, exaggerated left ventricular wall edema was not coupled with aggravated systolic dysfunction. However, it could contribute to diastolic dysfunction in patients with sepsis. (Crit Care Med 2014; 42:00–00)

**Key Words:** capillary permeability; edema; heart; mice knockout; sepsis; signal transduction

Septic cardiomyopathy is a well-recognized cardiovascular complication in patients with severe sepsis, characterized by a reversible decrease in systolic and/or diastolic left ventricular (LV) function (1, 2). Although its impact on outcome remains to be defined, it at least contributes to hemodynamic impairment in patients with sepsis (3). In contrast to ischemic cardiomyopathy, LV dysfunction does not result from hypoperfusion as coronary blood flow is enhanced during sepsis, and no myocardial necrosis is discerned by histological analysis (4). Currently, the pathophysiologic mechanisms of depressed LV function remain to be identified. Lipopolysaccharide (LPS) from infective agents can directly affect heart function via toll-like receptor 4 (TLR4) expressed by cardiomyocytes. However, it mainly results from the release of various proinflammatory cytokines or mediators because of TLR4 activation in immune cells and especially macrophages (5, 6). Among these cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-1β mainly contribute to cardiac dysfunction (7, 8).

Increased microcirculatory permeability, a major complication in sepsis due to endothelial barrier disruption, contributes to end-organ dysfunction. This is particularly the case in the lungs where enhanced permeability induces lung edema causing acute respiratory distress syndrome (ARDS) (9). The endothelial barrier displays junctional complexes connected to the actin cytoskeleton, that is, tight and adherens junctions. Increased permeability could result from either disturbances of junctional complexes or cytoskeleton remodeling and contraction (10). Whether vascular permeability increases in the heart, as is the case in other tissues, and whether it contributes to LV dysfunction during sepsis remain incompletely explored.

Initially described for its metabolic role, adenosine monophosphate (AMP)-activated protein kinase (AMPK) also phosphorylates cytoskeletal proteins in many cell types, including epithelial and endothelial cells (EC) (11, 12). Our group has demonstrated that α,AMPK isoform leads to myosin light chain (MLC) phosphorylation in epithelial cells (11, 13). Vasodilator-stimulated phosphoprotein (VASP) is another cytoskeletal substrate of AMPK in EC (12). As both VASP and MLC are substrate of AMPK in EC (12). As both VASP and MLC are phosphorylated by α,AMPK on tight junction organization in EC.

**MATERIALS AND METHODS**

**Animals, Materials, and Reagents**

Experiments were performed on male C57BL/6J mice (age 8 wk; Janvier Laboratories, Le Genest Saint Isle, France) and wild-type α,AMPK or knockout α,AMPK male mice (age 8 wk; Institut Cochin, Paris, France). The animals were maintained under a 12:12-hour light-dark cycle with free access to food and water. Animal handling was approved by the Animal Research Committee at Université catholique de Louvain (UCL) (2012/UCL/MD/003) and conformed to American Heart Association Guidelines for the Use of Animals in Research.

Materials and reagents were obtained from the sources indicated in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A703). Primers and standards for IL-6, IL-1β, and Regulated on Activation Normal T Cell Expressed and Secreted (RANTES) were kindly provided by Prof. Th. Michiels (UCL, Institut de Duve, Brussels, Belgium) (15). Primers and standards for chemokine (C-X-C motif) ligand 1 (CXCL1) and chemokine (C-X-C motif) ligand 2 (CXCL2) were generously supplied by Prof. J.-C. Renauld (UCL, Institut de Duve).

**In Vivo Model of Endotoxemia and Measurements of Cardiac Permeability**

Endotoxemia was induced in mice by injecting intraperitoneally (IP) LPS (or saline vehicle). Evans blue dye (EBD) was considered a marker of albumin extravasation, as described previously (16). EBD 20 mg/kg was administered IP with LPS or saline vehicle. After 6 or 24 hours, the animals were euthanized with pentobarbital 300 mg/kg IP. The chest was opened, and 30 mL of saline solution was flushed through the LV. The heart was removed and frozen. Vascular leakage, corresponding to the amount of dye in the extravascular compartment, was quantified by image J software (Wayne Rasband, National Institutes of Health, Bethesda, MD) as the relative surface of fluorescence (594 nm) on frozen sections (7-μm thick).

**Echocardiographic Assessment and MRI**

At baseline and 6 and 24 hours after LPS injection, the mice were anesthetized by inhaled isoflurane, followed by echocardiography (Vevo 2100 Imaging System; Visual Sonics, Toronto, ON, Canada). Parasternal long- and short-axis views, four-chamber view, and mitral pulse wave Doppler were recorded. M-mode data were obtained from the parasternal long-axis view. LV dimensions were measured at end diastole and at end systole. The data were analyzed in a blinded manner by two
Cell Culture and Treatment

Human coronary arterial endothelial cells (HCAECs) and human dermal microvascular endothelial cells (HDMECs) were purchased from Cell Applications (San Diego, CA) and Promocell (Heidelberg, Germany), respectively, and cultured in the manufacturers’ recommended medium designated as meso endocell growth medium for HCAECs and EC growth medium 2MV for HDMECs. For cell junction studies, cells (5 × 10^5) between 2 and 6 passages were plated onto 24-well slides in complete medium containing 5% fetal bovine serum (FBS), for 24 hours at 37°C in a humidified atmosphere with 5% CO₂. They were starved in basal medium supplemented with 1% FBS for 6 hours prior to treatment with LPS. For in vitro transwell assay, cells (1.5 × 10^5) were placed on 24-transwell plates for 24 hours and treated as above for the times indicated in the figure legends. For endothelial α-AMPK knockdown, cells were transiently transfected with 30 nM of small interfering RNA (siRNA) in the presence of lipofectamine for 48 hours, before treatment.

Western Blotting

Proteins were separated, as described (11). The membranes were probed with AMPK antibodies (1:1000). Bound antibodies were detected by chemiluminescence. Loading was controlled with anti-eukaryotic elongation factor 2.

In Vitro Transwell Assay

EBD 1% was added in the upper transwell chamber for 10 minutes. The upper part of the transwell plates was removed, and EBD that diffused to the bottom chamber was quantified by plate reader at optical density 620 nm.

Multiplex Cytokine Assay

At 6 and 24 hours, mice were euthanized, and plasma obtained by venipuncture was analyzed for TNF-α, IL-6, IL-1β, CXCL1, and RANTES by cytometric bead array-based immunoassay, with a dual-laser flow cytometer (FACS CantoII; BD Biosciences, Erembodegem, Belgium) and FCAP array software (BD Biosciences), following the manufacturer’s instructions.

Myeloperoxidase Activity

At 24 hours after LPS challenge, lungs were harvested and myeloperoxidase (MPO) activity was measured as described previously (17).

In Vitro and In Vivo Histological Examination and Immunofluorescence Staining

Cells were seeded on cover slides and fixed with 2% paraformaldehyde 24 hours later, once confluence was reached. They were washed, treated with blocking solution (5% bovine serum albumin-saponin 0.01% in phosphate-buffered saline), and stained with anti-zonula occludens-1 (1:50) for 1 hour at room temperature. The cells were then washed and incubated with Alexafluor 488. Slices were examined under fluorescence with a Zeiss Axio Imager microscope (Zeiss, Wetzlar, Germany).

For in vivo histology, hearts were frozen in Optimum Cutting Temperature compound compound. Heart sections (7-μm thick) were fixed with ice-cold acetone or paraformaldehyde 4%. Tissues were permeabilized in 0.1% Triton X-100 and blocked with 5% albumin solution. Tissues were stained with anti-ZO-1 (1:50) or antibodies recognizing leukocyte antigens (CD45, 1:25 and F4/80, 1:100).

RNA Extraction and Real-Time Reverse Transcription-Quantitative Polymerase Chain Reaction

Samples were prepared with RNeasy Mini Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer’s protocol. Messenger RNAs were quantified with NanoDrop (Thermo Scientific, Erembodegem, Belgium). Reverse transcription was performed with iScript cDNA Synthesis Kit and 500 ng RNA. Reverse transcription-quantitative polymerase chain reaction (PCR) was undertaken in an IQ5 apparatus (Bio-Rad, Hemel Hempstead Herts, UK) with qPCR Core Kit for Sybergreen (Eurogentec, Seraing, Belgium). Standards consisted of 10-fold dilutions of known plasmid concentrations carrying the PCR fragment of interest (pCR4-Topo, Invitrogen, Merelbeke, Belgium). Primer sequences and PCR conditions are presented in Supplemental Table S1 (Supplemental Digital Content 1, http://links.lww.com/CCM/A703). The results were normalized to 60S ribosomal protein L32 gene expression (18).

Statistical Analyses

Analyses were performed using SPSS software, version 20 (IBM, Chicago, IL) and Graphpad Prism 5.0 (GraphPad Software, La Jolla, CA). Continuous variables were checked for normality using the Kolmogorov-Smirnov test, and homogeneity of variance was assessed using the Bartlett’s test. Means or medians were compared between groups using one-way analysis of variance with post hoc test or with Kruskal-Wallis test. Linear and quadratic time trends were assessed and compared between groups using mixed models with the group as a fixed factor and time as a random repeated factor with unequal spacing. The Bonferroni correction was applied to p values in multiple comparisons. Kaplan-Meier method was used to estimate survival, and survival curves were compared using the log-rank test. A p value of less than 0.05 was considered significant.

RESULTS

Impaired LV Function Is Associated With Increased Vascular Permeability

C57BL/6j mice were treated with escalating doses of LPS ranging from 0.1 to 40 mg/kg. At the lowest concentrations, we
did not discern any effect on mortality and LV function, but 10 mg/kg LPS slightly increased mortality, whereas higher doses (20–40 mg/kg) killed nearly all mice (Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/CCM/A703). At 10 mg/kg, a systematic decrease in ejection fraction (EF) was observed within 6 hours (Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/CCM/A703). Interestingly, 10 mg/kg LPS also induced EBD extravasation within 6 hours after treatment (Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/CCM/A703). Increased vascular leakage in the myocardial wall therefore occurred at the same time as LV dysfunction, indicating that this phenomenon could participate in septic cardiodepression.

In subsequent experiments, 10 mg/kg LPS was chosen because it systematically depressed LV function and concomitantly augmented microvascular permeability into the myocardium without excess mortality (sublethal dose).

**Absence of α-AMPK Attenuates Tolerance to Endotoxemia and Increases Microvascular Permeability, Resulting in Myocardial Edema**

α-AMPK−/− mice were treated with 10 mg/kg LPS and compared with wild-type controls. The absence of α-AMPK dramatically impaired tolerance to LPS challenge (Fig. 1A). The survival rate after 24 hours was 30% in LPS-treated α-AMPK−/− mice compared with 93% in LPS-treated α-AMPK+/+. Microvascular permeability was measured by EBD leakage in the heart. Under basal conditions, leakage was already apparent in α-AMPK−/− mice. The 10 mg/kg LPS mediated more pronounced leakage in α-AMPK−/− than in α-AMPK+/+ mice (Fig. 1, B and C), demonstrating increased vascular permeability in vivo.

Echocardiographic measurements were recorded at baseline, 6 hours, and 24 hours after injection of 10 mg/kg LPS. α-AMPK−/− and α-AMPK+/+ presented similar decreases in LV systolic function (EF) at 6 and 24 hours (Table 1). Nevertheless, α-AMPK−/−-treated mice displayed increased LV mass after 24 hours (Fig. 2, A and B). LV end-diastolic volume was inversely correlated with LV mass (Fig. 2C). As hypertrophic remodeling is impossible within 24 hours, we speculated that the increase in LV mass corresponded to myocardial edema onset. To test this hypothesis, mice underwent MRI for 24 hours after LPS injection, which confirmed the presence of diffuse and massive myocardial wall edema in α-AMPK−/− compared with α-AMPK+/+ mice (Fig. 2D). It is noteworthy that edema was also observed in α-AMPK+/+ animals, suggesting that it is part of the pathologic landscape of septic cardiomyopathy.

**Systemic and Myocardial Inflammation in Response to LPS Stimulation**

As ARDS (enhanced pulmonary permeability and neutrophil accumulation) represents major end-organ damage ascribed to systemic inflammation, we postulated that changes in the inflammatory response might be responsible for the altered vascular permeability. We therefore quantified neutrophil infiltration in mice subjected to 10 mg/kg LPS by measuring MPO activity in lung extracts. Exposure to LPS for 24 hours led to a dramatic increase in neutrophil accumulation. However, ARDS in α-AMPK−/− mice was similar to that in α-AMPK+/+ mice (Fig. 3A). Cytokines were then measured in plasma at 6 and 24 hours after LPS injection, when heightened myocardial vascular permeability was evident. As expected, LPS strikingly augmented cytokine release, that is, IL-1β, IL-6, TNF-α, RANTES, and CXCL1. After LPS stimulation, plasma cytokine concentrations were virtually similar in α-AMPK−/− and α-AMPK+/+ mice, except for IL-6 (Fig. 3 B–F).
Indeed, a slight but significant 1.6-fold increment was observed 6 hours after LPS challenge. Such comparable cytokine production and ARDS indicate that exaggerated myocardial wall edema in the α₁AMPK–/– group was not correlated with major changes in systemic inflammation. However, an anti-inflammatory effect of AMPK has been described. In particular, in vitro inhibition of AMPK expression in macrophages dramatically elevated IL-6 expression in response to LPS (19). Therefore, one may wonder whether α₁AMPK in macrophages can still control systemic inflammation and influence cardiac vascular permeability. To rule out this hypothesis, we studied mice in which α₁AMPK was specifically invalidated in macrophages (macrophage-α₁AMPK–/–), as macrophages play a critical role in the systemic inflammatory response to LPS (20). These mice were challenged by LPS, and cardiac vascular permeability was measured. The LPS-induced increase in cardiac vascular permeability was similar in macro-α₁AMPK–/– and macro-α₁AMPK+/+ mice (Supplemental Fig. S2, Supplemental Digital Content 1, http://links.lww.com/CCM/A703), which strongly suggests that the cardiac phenotype of LPS-treated α₁AMPK–/– mice cannot be attributed to the absence of AMPK in macrophages.

In ischemia-reperfusion models, exaggerated vascular permeability leads to inflammatory cell extravasation (21). WBC infiltration was characterized in heart sections of α₁AMPK–/– and α₁AMPK+/+ mice subjected to LPS challenge. In contrast to ischemia-reperfusion, LPS did not provoke massive infiltration.

### TABLE 1. Echocardiographic Measurements at Baseline, 6 Hours, and 24 Hours After Lipopolysaccharide Injection

<table>
<thead>
<tr>
<th>Variable</th>
<th>α₁AMPK+/+</th>
<th>α₁AMPK–/–</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-diastolic interventricular septal thickness (mm)</td>
<td>0.51±0.02</td>
<td>0.53±0.01</td>
</tr>
<tr>
<td>End-systolic interventricular septal thickness (mm)</td>
<td>0.74±0.04</td>
<td>0.64±0.02</td>
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<tr>
<td>End-diastolic left ventricular internal diameter (mm)</td>
<td>3.6±0.1</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>End-systolic left ventricular internal diameter (mm)</td>
<td>2.9±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>End-diastolic left ventricular posterior wall thickness (mm)</td>
<td>0.52±0.02</td>
<td>0.53±0.01</td>
</tr>
<tr>
<td>End-systolic left ventricular posterior wall thickness (mm)</td>
<td>0.65±0.08</td>
<td>0.60±0.03</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>20±1</td>
<td>10±1</td>
</tr>
</tbody>
</table>

2D-parasternal long axis

<table>
<thead>
<tr>
<th>Variable</th>
<th>α₁AMPK+/+</th>
<th>α₁AMPK–/–</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular end-diastolic volume (µL)</td>
<td>50±3</td>
<td>65±5</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume (µL)</td>
<td>27±2</td>
<td>50±5</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>46±1</td>
<td>23±2</td>
</tr>
<tr>
<td>Left ventricular mass (mg)</td>
<td>75±3</td>
<td>82±3</td>
</tr>
</tbody>
</table>

Pulse Doppler

<table>
<thead>
<tr>
<th>Variable</th>
<th>α₁AMPK+/+</th>
<th>α₁AMPK–/–</th>
</tr>
</thead>
<tbody>
<tr>
<td>E (mm/s)</td>
<td>519±27</td>
<td>516±50</td>
</tr>
<tr>
<td>A (mm/s)</td>
<td>293±15</td>
<td>ND</td>
</tr>
<tr>
<td>E/A</td>
<td>1.78±0.14</td>
<td>ND</td>
</tr>
<tr>
<td>E’ (mm/s)</td>
<td>10.9±1.6†</td>
<td>4.8±1.1a</td>
</tr>
<tr>
<td>E/E’</td>
<td>54±12</td>
<td>136±31</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>482±10</td>
<td>420±16</td>
</tr>
</tbody>
</table>

α₁AMPK = α₁ adenosine monophosphate-activated protein kinase, LPS = lipopolysaccharide, ND = not determined.

†p < 0.05 vs baseline.

a p < 0.05 vs LPS (6 hr).

b p < 0.05 vs LPS (24 hr).

c p < 0.05 AMPK+ vs AMPK–.
of immune cells in α,AMPK+/− or α,AMPK+/+ hearts (Fig. 4A–C). Histological studies did not reveal any increase in lymphocytes (Fig. 4D), neutrophils (Fig. 4E and F), and macrophages (Fig. 4G) in the myocardium. More surprisingly and despite the absence of leukocyte infiltration, drastically different in the expression of some myocardial cytokines and chemokines (IL-6, IL-1β, TNF-α, CXCL1, and CXCL2) were detected in α,AMPK−/− compared with α,AMPK+/− hearts, after LPS stimulation (Supplemental Fig. S3, Supplemental Digital Content 1, http://links.lww.com/CCM/A703). Considered altogether, AMPK locally reduced LPS-induced myocardial wall edema and tissue cytokine expression by not acting only on inflammatory cells.

AMPK Activation by 5-Aminomidazole-4-Carboxamide Riboside Prevents Cardiac Vascular Hyperpermeability During Endotoxemia

The 5-aminomidazole-4-carboxamide riboside (AICAr) allosterically activates AMPK after its phosphorylation into AICAr ibotide in many cell types (including EC), except in cardiomyocytes (22). In C57BL/6J mice, AICAr treatment countered LPS-induced EBD leakage (Fig. 5, A and B). AICAr did not decrease vascular permeability in the heart of α,AMPK−/− mice during endotoxemia, which implies AMPK in its protective effect (Fig. 5C).

**DISCUSSION**

In this study, we describe, for the first time, an experimental model (α,AMPK−/− mice) exhibiting exaggerated myocardial wall edema during sepsis. Increased wall edema did not result in a further decline of systolic LV function and, more interestingly, was not associated with inflammatory cell infiltration. This abnormal response could be attributed to the role of
Figure 3. Systemic inflammation in α₁ adenosine monophosphate (AMP) K (α₁AMPK⁺/⁺) mice after endotoxia. A, Myeloperoxidase (MPO) activity in lung extracts 24 hr after 10 mg/kg lipopolysaccharide (LPS) challenge (black bars) or saline vehicle (white bars). The data are mean ± SEM, n = 5–12 per group. B–F, Plasma cytokine and chemokine levels in α₁AMPK⁺/⁺ (white boxes) and α₁AMPK⁻/⁻ mice (gray boxes) 6 and 24 hr after 10 mg/kg LPS or saline vehicle. The data are expressed as box plots representing the 75–25% range of cytokine concentrations: the horizontal line within the boxes denotes the median (n = 5–8/group). Bars above and below the boxes depict maximum and minimum values. * indicates values statistically different from the saline group. $ represents values statistically different from the saline group. NS = nonsignificant, CXCL-1 = chemokine (C-X-C motif) ligand 1, CXCL-2 = chemokine (C-X-C motif) ligand 2, RANTES = Regulated on Activation Normal T Cell Expressed and Secreted.
α₁AMPK in controlling endothelial tight junctions, maintaining endothelial barrier integrity in septic hearts.

Increased vascular permeability in response to sepsis is extensively reported in the literature (23, 24). Classically, heightened endothelial permeability is responsible for ARDS characterized by lung edema and accumulation of inflammatory cells. However, the contribution of sepsis-increased endothelial permeability to dysfunction of other organs is not fully elucidated. This is particularly the case in the heart. Myocardial wall edema has been suggested previously, based on histological analysis of heart cross-sections from patients who died from septic shock (25). Most of these samples were taken after long ICU stay and were not representative of the acute sepsis phase, when cardiodepression is seen (26). In addition, LV function was not assessed at this time point. Therefore, no correlation between myocardial edema and function could be

![Figure 4. Myocardial inflammatory cell infiltration in α₁ adenosine monophosphate (AMP) K (α₁AMPK)−/− mice during endotoxemia. Leukocytes (A, B) and macrophages (G) detected by immunohistological staining with anti-CD45 and anti-F4/80 antibody, respectively. A, Arrows indicate positive cells. B, The spleen (gray bar) served as positive control. Leukocyte (C), lymphocyte (D), and neutrophil (F) infiltration assessed by quantifying Cluster of Differentiation 11b, T-cell specific factor 7, and myeloperoxidase (MPO) gene expression in heart extracts, respectively, by reverse transcription-quantitative polymerase chain reaction. E, Neutrophils quantified by measuring MPO activity in heart extracts. Hearts from α₁AMPK+/+ and α₁AMPK−/− mice were harvested 24 hr after injection of 10 mg/kg lipopolysaccharide (LPS) (black bars) or saline vehicle (white bars). The data are mean ± SEM, n = 5–12 per group. α₁AMPK = α₁AMP-activated protein kinase, NS = nonsignificant, cDNA = complementary DNA.](image-url)
ascertained. Our data clearly demonstrated that the extent of myocardial wall edema did not correlate with LV systolic function depression. Indeed, in α₁AMPK⁻/⁻ animals, edema was clearly observed after 24 hours but did not correspond to a further decrease in LV systolic function. In contrast, an inverse relationship between LV end-diastolic volume and wall thickness suggests, but does not demonstrate, that it could correspond to decreased diastolic function, as described recently in a large series of patients with sepsis (27). It is interesting that, even in LPS-treated α₁AMPK⁺/⁺ mice, mild edema has been observed by MRI.

Endothelium impairment promotes the passage of leukocytes and plasma into the interstitial compartment. In the heart, this phenomenon is well-recognized after ischemia-reperfusion, in experimental models as well as in humans (21, 28). Factors inducing increased vascular permeability (e.g., angiopeitin-like 4 deficiency) are associated with deleterious, persistent and expanding inflammatory responses, tissue injury, and poor LV function recovery (21). Altered vascular integrity is coupled with leukocyte infiltration (CD45-positive cells). A similar paradigm is thought to occur in septic hearts, mainly based on postmortem histological analysis of patients who died after long ICU stay (29). We provided strong evidence that this is actually not the case because the hearts of α₁AMPK⁻/⁻ mice did not contain more inflammatory cells, whatever the method deployed to detect them. Taking a similar approach, Lee et al (30) obtained analogous findings in the kidneys. In contrast to ischemia-reperfusion-induced acute kidney injury (30), no major inflammatory cell infiltration (macrophages and neutrophils) was detected in acute septic kidney injury.

Among other factors, the systemic inflammatory response connects infection to systemic complications, including vascular hyperpermeability. One may argue that AMPK acts as an anti-inflammatory signal. Macrophages are a key element mediating systemic inflammation in response to LPS (20). In these cells, the role of AMPK in the control of inflammation remains controversial. On one hand, α₁AMPK deletion in murine bone marrow–derived macrophages amplifies the inflammatory response to LPS, that is, increased TNF-α and IL-6 expression (19). On the other hand, α₁AMPK is an activating kinase of tumor growth factor-β-activated kinase in human monocytes (31). Sublethal doses of LPS injection in α₁AMPK⁻/⁻ did not dramatically elevate plasma proinflammatory cytokine levels, compared with AMPK⁺/⁺. Deletion of α₁AMPK in macrophages was not sufficient to modulate permeability after 10 mg/kg LPS. Furthermore, ARDS (reflecting systemic inflammation) was comparable in α₁AMPK⁻/⁻ and α₁AMPK⁺/⁺. Taken together, our data suggest that the observed phenotype cannot be explained by inflammation control and required an additional mechanism. The absence of α₁AMPK was linked with disassembled interendothelial junctions and, more particularly, tight junctions, as demonstrated by ZO-1 staining in vivo. The loss of endothelial tight junction integrity could be due to a defect in tight junction assembly resulting from the absence of α₁AMPK. In Madin-Darby Canine Kidney epithelial cells, AMPK favors tight junction assembly and transepithelial electric resistance.
establishment after calcium switch (32, 33). This is in line with a perturbed ZO-1 linear pattern in α1AMPK−/− hearts (in vivo) and in α1AMPK deficient EC (in vitro), in basal state. However, the role of AMPK is not restricted to tight junction assembly as it has been described in pulmonary microvascular EC (34). Indeed, in C57BL/6J mice in vivo, AMPK activation by AICAr preserved cardiac microvascular tight junctions and permeability under LPS stimulation. These data were confirmed in vitro. We showed that EC exposed to LPS in culture conserved the ZO-1 linear pattern if they were pretreated with AICAr, suggesting that AMPK contributes not only to the assembly but also to the maintenance of tight junctions. We postulated that AMPK acts through cytoskeleton protection against LPS injury. The molecular mechanism remains to be elucidated, but it is MLC independent (data not reported). Previous reports have shown that VASP may protect the endothelial barrier through a prevention of stress fiber formation (35). As the AMPK-mediated phosphorylation of VASP-Thr278 interferes with F-actin accumulation (36), VASP is a likely candidate to explain the protective action of AMPK on cell permeability.

Two limitations have to be pointed out. First, our data demonstrated increased mortality and vascular permeability in α1AMPK−/− mice subjected to endotoxemia. However, we provided no evidence that both phenomena are linked. The causes of death remain to be investigated but could be attributed to more pronounced vasodilatation inducing severe hypotension. The latter is unlikely because the absence of AMPK should potentiate smooth muscle cell contraction in response to vasoconstrictors (37). Second, our Doppler variables did not prove that diastolic dysfunction was associated with increased wall thickness. It was difficult to perfectly control filling pressures and volumes under LPS challenge. Pressure-volume loops should be helpful in such assessments.

CONCLUSIONS

α1AMPK counteracts cardiac vascular hyperpermeability and exerts a protective action against myocardial edema induced by endotoxemia through preservation of endothelial tight junctions. AMPK activators are currently administered in clinical practice for type 2 diabetes treatment (38). It remains to be determined whether these activators could be helpful during sepsis by protecting against hyperpermeability leading to organ failure.

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Figure 7. Effect of adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation on endotoxin-induced endothelial monolayer permeability. A. Time-course of lipopolysaccharide (LPS)-induced human dermal microvascular endothelial cells (HDMEC) hyperpermeability. Cells were pretreated with 1 mM 5-aminoimidazole-4-carboxamide riboside (AICAr) (gray bars) or saline vehicle (black bars) before 50 µg/mL LPS challenge for 2, 6, or 12 hr. B. HDMEC monolayers permeability were measured after α₁AMPK silencing. Cells were transfected with α₁AMPK or scramble small interfering RNA (siRNA). They were then pretreated with 1 mM AICAr (gray bars) or saline vehicle (black bars) before 50 µg/mL LPS challenge for 6 hr. Cell permeability is expressed as fold increase compared with samples without LPS and AICAr. The data are mean ± SEM. Experiments were performed three times in duplicate. Control group was treated with saline vehicle (white bars). * indicates values statistically different from the corresponding controls without LPS. § denotes values statistically different from LPS without AICAr pretreatment. NS = nonsignificant.