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High versus standard-volume haemofiltration in hyperdynamic porcine peritonitis: effects beyond haemodynamics?

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Abstract Objective: The role of haemofiltration as an adjunctive treatment of sepsis remains a contentious issue. To address the role of dose and to explore the biological effects of haemofiltration we compared the effects of standard and high-volume haemofiltration (HVHF) in a peritonitis-induced model of porcine septic shock. **Design and setting:** Randomized, controlled experimental study. **Subjects:** Twenty-one anesthetized and mechanically ventilated pigs. **Interventions:** After 12 h of hyperdynamic peritonitis, animals were randomized to receive either supportive treatment (Control, $n = 7$) or standard haemofiltration (HF 35 ml/kg per h, $n = 7$) or HVHF (100 ml/kg per hour, $n = 7$). **Measurements and results:** Systemic and hepatosplanchnic haemodynamics, oxygen exchange, energy metabolism (lactate/pyruvate, ketone body ratios), ileal and renal cortex microcirculation and systemic inflammation (TNF- α , IL-6), nitrosative/oxidative stress (TBARS, nitrates, GSH/GSSG) and endothelial/coagulation dysfunction (von Willebrand factor, asymmetric dimethylarginine, platelet count) were assessed before, 12, 18, and 22 h of peritonitis. Although

fewer haemofiltration-treated animals required noradrenaline support (86, 43 and 29% animals in the control, HF and HVHF groups, respectively), neither of haemofiltration doses reversed hyperdynamic circulation, lung dysfunction and ameliorated alterations in gut and kidney microvascular perfusion. Both HF and HVHF failed to attenuate sepsis-induced alterations in surrogate markers of cellular energetics, nitrosative/oxidative stress, endothelial injury or systemic inflammation. **Conclusions:** In this porcine model of septic shock early HVHF proved superior in preventing the development of septic hypotension. However, neither of haemofiltration doses was capable of reversing the progressive disturbances in microvascular, metabolic, endothelial and lung function, at least within the timeframe of the study and severity of the model.

Keywords Sepsis · Haemofiltration · High volume haemofiltration · Microcirculation · Energy metabolism · Oxidative stress

Introduction

Extracorporeal blood purification techniques as an adjunctive treatment of sepsis in critically ill patients remain a conceptually sound but still unresolved issue [1–3]. Haemofiltration, which is nowadays one of the most widely used hemopurification method in the ICU, failed to significantly reduce plasma cytokine concentrations and ameliorate organ dysfunction in septic patients when low ultrafiltration doses (2 l/h of plasma water exchange) were used [4]. In contrast, the importance of dialysis intensity to improve the outcome of critically ill patients with acute renal failure has been demonstrated [5–7]. Nonetheless, although the minimal ultrafiltration rate of 35 ml/kg per hour has been recommended [1, 3], recent large multicenter trial, in which 63% of patients were septic, challenged the issue of a dosage-outcome relationship in patients with acute kidney injury [8]. In addition, Ronco et al. [5] suggested a beneficial trend in survival when further increasing the dose of haemofiltration up to 45 ml/kg per hour in a small subgroup of septic patients. Although limited in their robustness and methodological aspects, several human [9–15] and animal [16–20] studies further support the concept of high-volume haemofiltration (HVHF) in the treatment of endotoxemia or septic shock, mainly by demonstrating a reduced need for vasopressors dose and better hemodynamic stability. However, human studies were either uncontrolled or retrospective, thereby limiting the inference that could be drawn [21]. Similarly, the clinical relevance of some experimental studies focusing predominantly on haemodynamics, gas exchange, plasma cytokine levels, immunoparalysis and short-term survival, is limited by the fact that (1) haemofiltration was started before, during or very early after the septic or endotoxemic insult [21]; (2) the treatment effects were investigated mostly in *hypodynamic* models characterized by hypotension and low cardiac output, which is in contrast to the *hyperdynamic* pattern commonly seen in patients with septic shock. Finally, all studies compared the effects of HVHF either with untreated subjects or with low volume haemofiltration (i.e. 20 ml/kg per hour); hence, it is unknown whether increasing the dose of plasma water exchange confers an additional benefit over currently accepted standard haemofiltration dose (i.e. 35 ml/kg per hour). Therefore, to further explore the biological effects of haemofiltration and the role of its dose in sepsis, we investigated (1) the impact of haemofiltration on regional and microcirculatory perfusion, energy metabolism, oxidative stress, endothelial dysfunction and systemic inflammatory response in a porcine model of hyperdynamic fecal peritonitis-induced septic shock that integrates standard day-to-day care resuscitative measures; (2) whether increasing haemofiltration dose from

standard (35 ml/kg per hour) to high volume (100 ml/kg per hour) provides an additional benefit.

Material and methods

The experiment was performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. The study protocol was approved by the University Animal Care Committee. Twenty-one anesthetized and ventilated pigs of either gender with a body weight [median (quartiles): 38 kg (34, 42)] were investigated. The anesthetic procedure, surgical instrumentation, and physiological measurements were performed as recently described (see the Electronic supplement material for details) [22–24]. Hemodynamic parameters recorded over a period of 22 h comprised mean arterial pressure, cardiac output, intrathoracic blood volume (ITBV), filling pressures, portal venous, hepatic and renal arterial blood flows. Microvascular measurements included ileal mucosal and renal cortex microvascular perfusion [laser Doppler flowmetry and the Side-stream Dark Field (SDF) imaging] [22–26]. Arterial, portal, hepatic and renal venous lactate (L) and pyruvate (P) concentrations, arterial serum levels of alanine aminotransferase (ALT), the hepatic venous ketone body ratio (KBR, acetoacetate/ β -hydroxybutyrate), arterial thiobarbituric acid reactive species (TBARS), reduced and oxidized glutathione (GSH/GSSG), and nitrate + nitrite (NO_x) concentrations were measured. From arterial blood samples we also assessed von Willebrand factor (vWF), platelet count, asymmetric dimethylarginine (ADMA), tumor necrosis factor alpha (TNF- α), and interleukin 6 (IL-6). To correct for dilutional effects resulting from volume resuscitation NO_x, TBARS, ADMA, ALT, vWF, IL-6 and TNF- α levels were normalized for plasma protein content [22–24]. Finally, ultrafiltrate TNF- α and IL-6 concentrations were analyzed.

Haemofiltration setting

The zero-balanced haemofiltration was performed on the MultiFiltrate system (Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany) with a synthetic membrane (Ultraflux AV600S; the high-flux polysulphon filter, surface 1.4 m², cut off up to 30,000 Da, Fresenius). Blood flow rate was set at 200 ml/min. Bicarbonate buffered (35 mmol/l) replacement fluid (Medisol Bi-4, solution for haemofiltration, Medites Pharma, Roznov pod Radhostem, Czech Republic) was administered 50% in predilution and 50% in postdilution fashion. The replacement fluid was warmed to 39°C in the MultiFiltrate, and the HOTLINE[®] Blood and Fluid Warmer

(Smiths Medical, UK) was inserted on the venous return line to prevent an extracorporeal circuit-induced decrease in body temperature. Anticoagulation was achieved with an initial i.v. bolus 0.2 ml/10 kg of nadroparinum (Fraxiparine, Glaxo Group Ltd, Greenford, Middlesex, UK) for all experimental animals, including the control group.

Protocol

After baseline data measurements, peritonitis was induced by inoculating 0.5 g/kg of autologous feces suspended in 200 ml saline into the abdominal cavity through the drainage tubes. A second set of measurements was obtained after 12 h of peritonitis induction. Then, the pigs were randomly divided into three groups: control animals (CON $n = 7$), animals receiving continuous haemofiltration at standard ultrafiltration rate of 35 ml/kg per hour (HF $n = 7$) and animals treated by HVHF at an ultrafiltration rate of 100 ml/kg per hour (HVHF $n = 7$). Further measurements were recorded at 18 and 22 h of peritonitis. In addition to the Plasma Lyte solution, 6% hydroxyethyl starch 130 kDa/0.4 (Voluven 6%, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) was infused at a rate of 10 ml/kg per hour; (7 ml/kg per hour if CVP or PAOP ≥ 18 mmHg) to maintain cardiac filling pressures ≥ 12 mmHg. Continuous i.v. noradrenaline was administered if mean arterial pressure (MAP) fell below 70 mmHg. The dose of noradrenaline was adjusted to maintain MAP 70–75 mmHg. Additional five sham-operated animals were subjected to the haemofiltration. When the last set of data had been obtained, the animals were euthanized by KCl injection under deep anaesthesia. The protocol of the study is illustrated in electronic supplemental material (Fig. 1 ESM).

Statistical analysis

All values shown are median and interquartile range. After the exclusion of normality using the Kolmogorov–Smirnov test, differences within each group between values obtained before and after the induction of peritonitis were tested using the Friedman ANOVA on ranks and, subsequently, Dunn's test for multiple comparisons with the Bonferroni's correction. Differences between the groups were analyzed using the Kruskal–Wallis one-way analysis of variance on ranks.

Results

Systemic haemodynamics, oxygen exchange, acid–base data and metabolism

The cumulative amount of colloid infusion to maintain a comparable cardiac preload was not statistically different

in the three groups [control group 167 (154; 186), HF group 194 (160; 210), HVHF 194 (159; 202) ml/kg]. Fluid resuscitation maintained ITBV and significantly increased filling pressures (data not shown) without intergroup differences. All pigs developed hyperdynamic circulation with high cardiac output and low systemic vascular resistance, not reversed by the treatment. Although not statistically significant, six animals (86%) in the control group received noradrenaline infusion, while three (43%) and two (29%) pigs required vasopressor support in the HF and HVHF groups, respectively (Fig. 1). In animals requiring vasopressor support, the median time to development of arterial hypotension was not different in all groups (control group 19 [18, 20], HF group 17 [17, 20], HVHF group 18 h [17, 19]) (Fig. 1). The increased cardiac output resulted in a significant rise of systemic oxygen delivery without intergroup differences, while systemic oxygen consumption did not change in any of the three groups over time. The decline in arterial pH and BE was less pronounced in both haemofiltration groups, although the intergroup difference did not reach statistical difference. A significant progressive deterioration of the PaO₂/FiO₂ ratio was observed in all groups, without intergroup differences. The arterial L/P ratio increased progressively in all three groups, again without intergroup differences. There were no significant differences in body core temperature between the groups over time ($P = 0.079$ at 22 h) (Table 1).

Regional haemodynamics, microcirculation, metabolism and organ function

While liver blood flow and liver oxygen delivery increased significantly in all three groups, liver oxygen uptake remained unchanged. Renal artery blood flow, oxygen delivery and consumption did not change over time in any of the experimental groups. Despite well-maintained

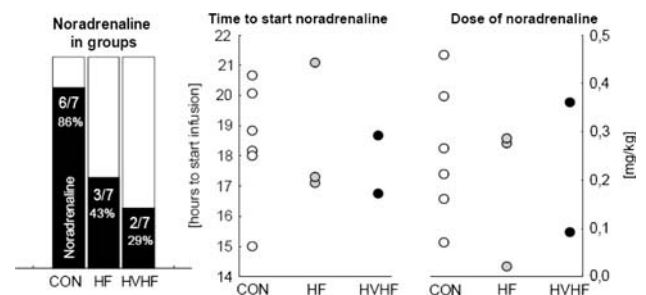


Fig. 1 Noradrenaline support. *Left* proportion of animals treated with noradrenaline in each group (*black part of slopes*); *middle* time to start of noradrenaline infusion after the induction of peritonitis; *right* total dose of administered noradrenaline; *white plots* designate control group (CON), *grey plots* designate standard haemofiltration group (HF) and *black plots* designate high-volume haemofiltration group (HVHF)

Table 1 Systemic haemodynamics, oxygen exchange, metabolism and temperature

	Baseline	12 h	18 h	22 h
MAP (mmHg)				
CON	94 (89; 104)	93 (85; 102)	85 (80; 102)	76 (74; 92) ^a
HF	84 (82; 96)	88 (84; 94)	92 (88; 103)	78 (71; 81)
HVHF	94 (87; 100)	98 (84; 113)	91 (76; 98)	81 (72; 91)
CO (ml/kg)				
CON	70 (61; 88)	108 (94; 122) ^a	114 (107; 128) ^a	147 (99; 183) ^a
HF	73 (72; 85)	125 (102; 131) ^a	129 (112; 134) ^a	136 (126.162) ^a
HVHF	74 (68; 80)	106 (92; 117) ^a	111 (107; 135) ^a	131 (126.155) ^a
MPAP (mmHg)				
CON	26 (23; 27)	34 (30; 36) ^a	35 (34; 40) ^a	38 (36; 43) ^a
HF	27 (25; 47)	30 (29; 48) ^a	34 (31; 67) ^a	34 (32; 59) ^a
HVHF	25 (22; 29)	29 (27; 39) ^a	34 (31; 37) ^a	40 (31; 47) ^a
SVR (dyne s/cm ⁻⁵)				
CON	2774 (2524; 2839)	1717 (1467; 1956) ^a	1361 (1068; 1837) ^a	851 (719; 1514) ^a
HF	2201 (1914; 2889)	1275 (1191; 1964) ^a	1276 (1172; 1669) ^a	929 (858; 1276) ^a
HVHF	2447 (2070; 2734)	1782 (1589; 2014)	1340 (1158; 1583) ^a	1161 (830; 1326) ^a
ITBV (ml/kg)				
CON	22 (19; 33)	25 (19; 31)	26 (21; 28)	22 (18; 30)
HF	25 (20; 29)	30 (24; 30)	25 (21; 25)	26 (25; 27)
HVHF	19 (17; 25)	24 (22; 26)	26 (21; 28)	27 (25; 28)
DO ₂ (ml/min per kg)				
CON	9 (8; 13)	15 (14; 18) ^a	16 (15; 20) ^a	24 (16; 24) ^a
HF	10 (8; 11)	18 (12; 18) ^a	17 (16; 18) ^a	16 (15; 17) ^a
HVHF	10 (9; 11)	15 (13; 15) ^a	14 (13; 15) ^a	15 (13; 16) ^a
VO ₂ (ml/min per kg)				
CON	5 (4; 6)	6 (5; 9)	6 (5; 7)	7 (6; 9)
HF	5 (4; 6)	6 (4; 7)	6 (5; 7)	5 (5; 6)
HVHF	4 (4; 5)	6 (5; 6)	5 (5; 6)	5 (5; 6)
Arterial pH				
CON	7.52 (7.50; 7.54)	7.47 (7.44; 7.49) ^a	7.45 (7.43; 7.50) ^a	7.36 (7.32; 7.41) ^a
HF	7.50 (7.48; 7.54)	7.49 (7.46; 7.50)	7.48 (7.46; 7.49)	7.45 (7.40; 7.48)
HVHF	7.53 (7.50; 7.55)	7.46 (7.43; 7.52) ^a	7.45 (7.43; 7.47) ^a	7.40 (7.36; 7.48) ^a
Base excess (mmol/l)				
CON	5.7 (4.5; 6.6)	5.5 (4.7; 5.9)	4.2 (2.5; 5.9)	1.4 (-1.5; 1.4) ^a
HF	6.8 (5.2; 8.6)	7.2 (5.1; 8)	5.3 (3.4; 6.6)	4.4 (0.8; 5.8) ^a
HVHF	7.9 (5.5; 8.4)	5.5 (3.9; 6.2)	4.3 (3.2; 4.9)	2 (1.3; 6.4) ^a
Core temperature (°C)				
CON	38.0 (37.0; 38.3)	40.2 (39.1; 41.3) ^a	39.6 (38.7; 40.3)	39.5 (38.0; 40.4)
HF	38.2 (37.2; 39.3)	39.7 (39.0; 40.5) ^a	38.7 (38.0; 38.8)	38.5 (38.0; 38.8)
HVHF	37.6 (35.3; 38.0)	39.6 (37.8; 39.8) ^a	37.7 (37.4; 38.1)	37.5 (37.4; 38.1)
Oxygenation index (PaO ₂ /FiO ₂)				
CON	416 (345; 475)	409 (289; 490)	283 (267; 396) ^a	160 (94; 271) ^a
HF	491 (439; 509)	365 (292; 445) ^a	349 (259; 454) ^a	238 (174; 294) ^a
HVHF	467 (396; 495)	437 (360; 499)	289 (230; 347) ^a	156 (72; 321) ^a
Arterial lactate/pyruvate ratio				
CON	11 (7; 19)	18 (11; 22)	17 (15; 36)	31 (20; 46) ^a
HF	12 (11; 14)	10 (7; 14)	9 (8; 20)	28 (19; 56) ^a
HVHF	13 (11; 17)	15 (8; 29)	22 (14; 53) ^a	60 (29; 87) ^a

MAP mean arterial pressure, CO cardiac output, MPAP mean pulmonary artery pressure, SVR systemic vascular resistance, ITBV intrathoracic blood volume, DO₂ systemic oxygen delivery, VO₂ systemic oxygen uptake, CON control group, HF standard haemofiltration group, HVHF high-volume haemofiltration group

^a Significant difference within each group versus baseline ($P < 0.05$). Data are median and 25th and 75th quartiles

regional blood flow both ileal mucosal and renal cortex microvascular perfusion, as assessed by laser Doppler flowmetry, fell progressively in the control and HVHF groups (Fig. 2). Although the decline in renal cortex perfusion did not reach statistical significance in the HF group, there were no significant intergroup differences between all groups at the end of the experiment (Fig. 2). Direct visualization of the ileal mucosal microcirculation

was consistent with laser Doppler flowmetry: Side-stream Dark Field imaging showed well perfused villi before the induction of peritonitis, while the mean flow index deteriorated over time in all experimental groups without intergroup differences. Whereas the decrease in renal and hepatic venous pH was less pronounced in treated animals, neither of the haemofiltration doses significantly prevented sepsis-induced changes in the hepato-splanchnic L/P ratios

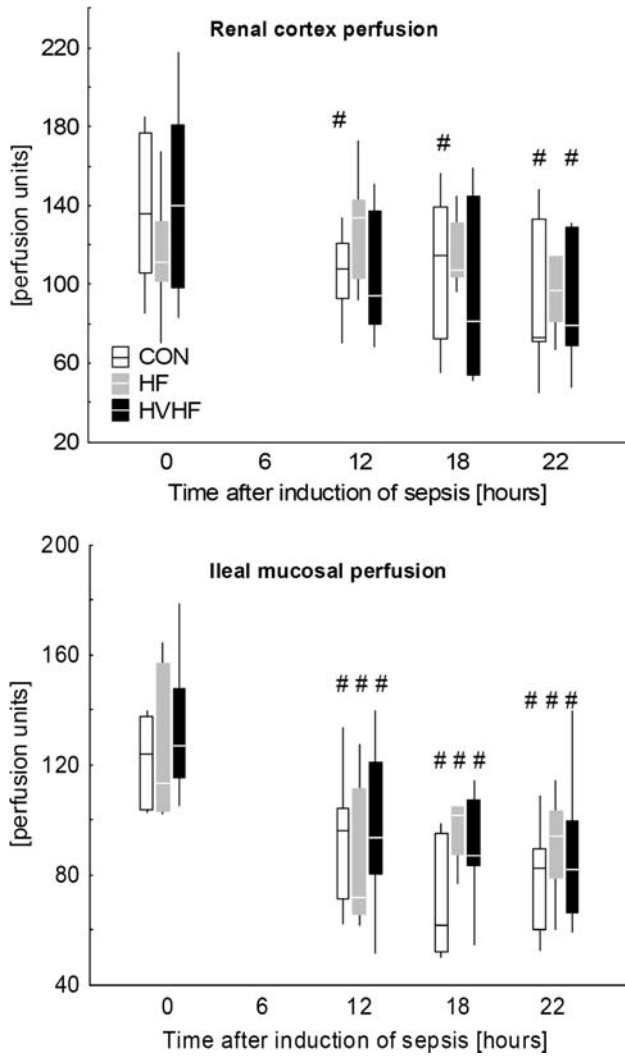


Fig. 2 Renal cortex and ileal mucosal microcirculation in the control (white plots), standard haemofiltration (HF grey plots), and high-volume haemofiltration (HVHF black plots) groups. Upper renal cortex perfusion (laser Doppler flowmetry). Lower ileal mucosal perfusion (laser Doppler flowmetry). Data are median (quartiles/range). #Significant difference within each group versus baseline ($P < 0.05$)

and KBR (Fig. 3). The significant increase in serum ALT activity occurring in the control and HF animals did not reach any intergroup significance. As expected, both treatment modalities presented with lower levels in serum creatinine (Table 2).

Inflammation, oxidative/nitrosative stress and endothelial dysfunction (Table 3, Fig. 2 ESM)

Peritonitis-induced septic shock resulted in gradually increased plasma levels of TNF- α and IL-6 (Table 3) and overproduction of nitric oxide, as documented by a significant increase in arterial NO $_x$ levels (Fig. 2 ESM).

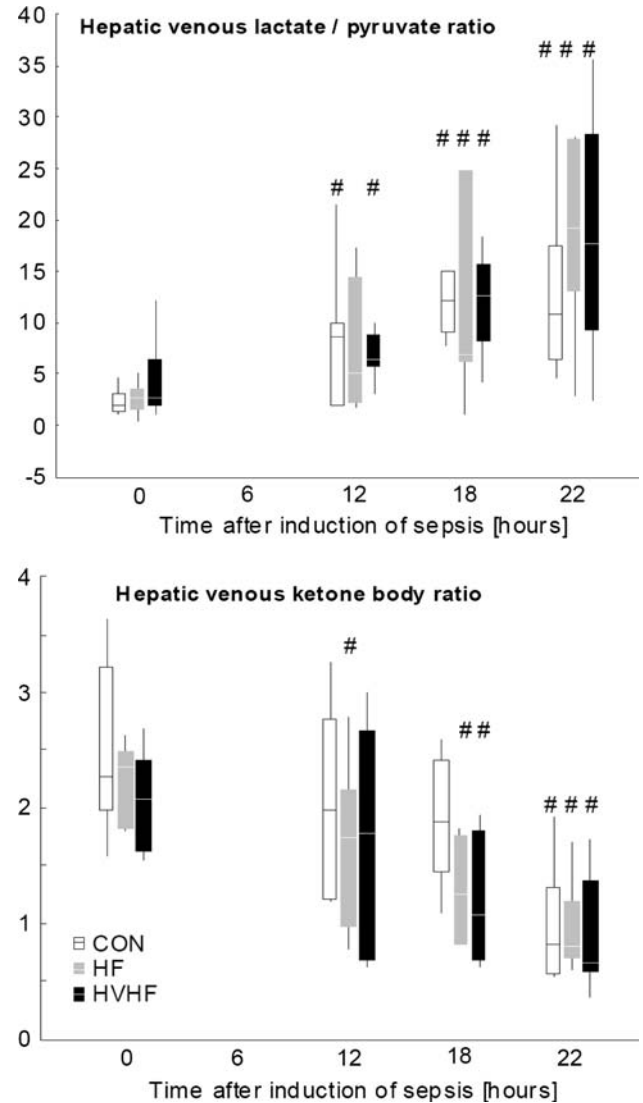


Fig. 3 Hepatic venous lactate/pyruvate ratios (upper), hepatic venous ketone body ratios (lower) in the control (white plots), standard haemofiltration (HF grey plots) and high-volume haemofiltration (HVHF black plots) groups. Data are median (quartiles/range). #Significant differences within each group versus baseline ($P < 0.05$)

These changes were accompanied by a several-fold increase in TBARS levels and GSH/GSSG ratio (Table 3) providing evidence for oxidative stress and an altered redox status. Conversely, ADMA concentrations decreased during the course of peritonitis (Table 3). Oxidative/nitrosative stress was paralleled by a marked increase in vWf suggesting altered endothelial function (Fig. 2 ESM). Both HF and HVHF did not affect the plasma levels of IL-6. Although HVHF tended to blunt the sepsis-induced increase in TNF- α , none of haemofiltration doses influenced the surrogate markers of oxidative/nitrosative stress and endothelial dysfunction. Likewise, platelet count fell progressively in response to

Table 2 Regional haemodynamics, oxygen exchange, acid–base status and organ function

	Baseline	12 h	18 h	22 h
Q liver (ml/kg per min)				
CON	27 (21; 28)	35 (31; 37) ^a	28 (27; 33)	24 (19; 28)
HF	20 (20; 23)	30 (26; 38)	33 (26; 40)	29 (18; 38)
HVHF	27 (24; 29)	34 (27; 38)	34 (31; 42)	36 (26; 38)
Liver DO₂ (ml/min per kg)				
CON	1.8 (1.6; 2.2)	4.4 (4.1; 4.6) ^a	4.1 (3.6; 4.7) ^a	3.4 (2.9; 4.1) ^a
HF	1.3 (1.0; 1.7)	4.1 (2.8; 4.4) ^a	3.9 (3.0; 4.7) ^a	3.3 (2.6; 4.1) ^a
HVHF	1.9 (1.7; 2.2)	3.4 (3.3; 4.4) ^a	4.0 (3.5; 4.4) ^a	3.1 (2.9; 3.8) ^a
Liver VO₂ (ml/min per kg)				
CON	1.0 (0.8; 1.1)	1.0 (0.6; 1.5)	1.1 (0.8; 1.2)	1.1 (1.0; 1.3)
HF	0.8 (0.5; 0.9)	0.9 (0.7; 1.0)	0.9 (0.7; 1.1)	1.0 (0.8; 1.3)
HVHF	0.7 (0.6; 0.8)	1.0 (0.8; 1.2)	0.8 (0.6; 1.0)	0.8 (0.7; 1.4)
pH HV				
CON	7.44 (7.43; 7.46)	7.38 (7.37; 7.42) ^a	7.39 (7.37; 7.41) ^a	7.31 (7.28; 7.36) ^a
HF	7.42 (7.39; 7.47)	7.43 (7.40; 7.43)	7.41 (7.34; 7.43)	7.41 (7.35; 7.42)
HVHF	7.45 (7.42; 7.49)	7.36 (7.34; 7.48)	7.40 (7.38; 7.42)	7.41 (7.35; 7.41)
Serum ALT levels (nkat/g protein)				
CON	12 (12; 15)	11 (11; 16)	12 (12; 18) ^a	19 (12; 19) ^a
HF	12 (10; 13)	12 (11; 15)	15 (12; 16) ^a	14 (12; 17) ^a
HVHF	15 (9; 16)	12 (11; 16)	13 (11; 16)	15 (10; 17)
Q renal artery (ml/kg per min)				
CON	6 (4; 6)	7 (5; 8)	7 (5; 7)	4 (4; 6)
HF	5 (4; 6)	7 (6; 10)	6 (5; 8)	6 (4; 9)
HVHF	7 (3; 9)	6 (3; 8)	4 (3; 9)	4 (2; 10)
Renal DO₂ (ml/min per kg)				
CON	0.8 (0.6; 0.9)	1.1 (0.8; 1.2)	0.8 (0.7; 1.1)	0.6 (0.5; 1.1)
HF	0.7 (0.5; 0.8)	1.0 (0.7; 1.4)	0.8 (0.7; 1.0)	0.7 (0.5; 1.1)
HVHF	0.7 (0.4; 1.4)	0.8 (0.4; 1.1)	0.5 (0.4; 1.0)	0.5 (0.2; 1.2)
Renal VO₂ (ml/min per kg)				
CON	0.2 (0.1; 0.2)	0.2 (0.1; 0.2)	0.2 (0.2; 0.2)	0.2 (0.1; 0.2)
HF	0.2 (0.1; 0.3)	0.2 (0.2; 0.2)	0.2 (0.1; 0.2)	0.2 (0.2; 0.3)
HVHF	0.1 (0.1; 0.2)	0.2 (0.1; 0.2)	0.1 (0.1; 0.2)	0.1 (0.04; 0.2)
pH RV				
CON	7.50 (7.49; 7.51)	7.42 (7.41; 7.47) ^a	7.46 (7.43; 7.47) ^a	7.35 (7.33; 7.39) ^a
HF	7.49 (7.41; 7.51)	7.45 (7.44; 7.48)	7.46 (7.40; 7.48)	7.44 (7.37; 7.46) ^a
HVHF	7.54 (7.53; 7.54)	7.47 (7.44; 7.51) ^a	7.43 (7.43; 7.44) ^a	7.40 (7.31; 7.44) ^a
Creatinine (μmol/l)				
CON	91 (91; 97)	95 (81; 118)	98 (88; 129)	129 (94; 130)
HF	86 (79; 98)	84 (75; 95)	75 (62; 77) ^b	72 (67; 77) ^b
HVHF	88 (78; 96)	85 (75; 95)	62 (56; 82) ^b	62 (51; 78) ^b
Ileal mucosal microcirculation (MFI)				
CON	3.0 (2.9; 3.0)	2.9 (2.2; 3.0) ^a	2.4 (2.1; 2.7) ^a	2.2 (2.0; 2.3) ^a
HF	3.0 (2.9; 3.0)	2.4 (1.9; 2.8) ^a	2.8 (2.5; 3.0)	2.5 (2.4; 2.7) ^a
HVHF	3.0 (3.0; 3.0)	3.0 (2.9; 3.0) ^a	2.3 (2.0; 2.5) ^a	2.5 (2.3; 2.9) ^a

Q liver liver blood flow, *liver DO₂*, liver oxygen delivery, *liver VO₂*, liver oxygen uptake, *pH HV* hepatic venous pH, *ALT* alanine aminotransferase, *Q renal artery* renal artery blood flow, *renal DO₂* renal oxygen delivery, *renal VO₂*, renal oxygen uptake, *pH RV* renal venous pH, *creatinine* serum creatinine levels, *MFI* mean flow

index, *CON* control group, *HF* standard haemofiltration group, *HVHF* high-volume haemofiltration group

^a Significant difference within each group vs. baseline ($P < 0.05$)

^b significant intergroup differences at the same time ($p < 0.05$). Data are median and 25th and 75th quartiles

peritonitis in all groups, without intergroup differences (Fig. 2 ESM). In contrast to high plasma levels of both cytokines, the concentrations of IL-6 and of TNF- α in hemofiltrates from both conventional and HVHFs were close to zero (data not shown).

In sham-operated animals haemofiltration did not affect hemodynamic, microcirculatory, and metabolic data nor markers of inflammation, oxidative/nitrosative stress and endothelial dysfunction (data not shown).

Discussion

This experimental study evaluated the effects of continuous conventional and HVHF in various biological systems related to multiple pathophysiological pathways in a porcine model of septic shock. The main findings of the present study were that (1) haemofiltration prevented the progression of septic hypotension within the study period, with the effect being more pronounced in the HVHF

Table 3 Inflammation and oxidative/nitrosative stress

	Baseline	12 h	18 h	22 h
Plasma IL-6 levels (nmol/g protein)				
CON	115 (70; 205)	555 (361; 650) ^a	1298 (504; 2405) ^a	2329 (821; 9805) ^a
HF	72 (22; 187)	292 (262; 426) ^a	1432 (487; 2256) ^a	978 (786; 4048) ^a
HV FH	148 (110; 265)	317 (173; 901) ^a	567 (312; 2259) ^a	985 (543; 10771) ^a
Plasma TNF- α levels (nmol/g protein)				
CON	1 (1; 1)	5 (5; 9) ^a	11 (6; 18) ^a	18 (6; 23) ^a
HF	1 (0; 2)	5 (3; 7) ^a	6 (3; 10) ^a	13 (5; 18) ^a
HV FH	2 (0; 2)	3 (2; 8) ^a	5 (3; 7) ^a	6 (4; 9) ^a
Plasma TBARS levels (nmol/g protein)				
CON	19 (15; 22)	47 (38; 60) ^a	77 (55; 89) ^a	66 (54; 98) ^a
HF	17 (16; 22)	50 (45; 51) ^a	60 (49; 71) ^a	72 (53; 88) ^a
HV FH	17 (15; 19)	45 (35; 71) ^a	55 (43; 85) ^a	53 (42; 80) ^a
Plasma ADMA levels (nmol/g protein)				
CON	54 (46; 68)	87 (73; 94)	118 (56; 141)	53 (46; 120)
HF	50 (42; 57)	53 (38; 79)	41 (36; 97)	61 (45; 128)
HV FH	48 (42; 59)	80 (71; 93)	75 (46; 128)	97 (50; 117)
GSH/GSSG				
CON	9 (7; 10)	15 (12; 25)	25 (13; 33)	142 (30; 375) ^a
HF	14 (10; 19)	18 (15; 47)	41 (23; 201)	148 (56; 497) ^a
HV FH	10 (8; 12)	32 (13; 33)	28 (22; 63)	106 (28; 204) ^a

^a Significant difference within each group versus baseline ($P < 0.05$)

IL-6 interleukin-6, TNF- α tumor necrosis factor alpha, TBARS plasma thiobarbituric acid reactive species, ADMA asymmetric dimethylarginine, GSH/GSSG reduced/oxidized glutathione ratio,

CON control group, HF standard haemofiltration group, HV FH high-volume haemofiltration group

^a Significant difference within each group versus baseline ($P < 0.05$)

group; (2) despite favorable effects on vasopressor requirements, neither of the haemofiltration doses beneficially modulated the sepsis-induced alterations in microcirculatory perfusion and energy metabolism, endothelial dysfunction and oxidative stress.

The reduced need for vasopressor support observed in both haemofiltration groups is consistent with previous experimental [16, 19, 20, 27] and human studies [9, 11, 13, 14]. The unchanged cardiac performance and comparable cardiac preload suggest an influence of haemofiltration on pathways implicated in the control of vascular tone, such as cytokine-driven iNOS-generated overproduction of nitric oxide or opening of ATP-sensitive potassium channels [28, 29]. The former pathway, however, is unlikely since we were unable to demonstrate a clear-cut removal of pro-inflammatory cytokines nor a suppression of excessive NO production, as documented by the absence of an effect on plasma nitrate/nitrite levels. In addition, reactive nitrogen/oxygen species were recently reported to be capable of inducing vascular hyporeactivity and vasodilatory hypotension [30–32]. Nevertheless, as haemofiltration failed to affect surrogate markers of sepsis-induced oxidative/nitrosative stress, it seems conceivable to speculate that the better hemodynamic stability was not related to changes in these downstream pathways. One could also argue that the vasopressor-sparing effect of haemofiltration might simply be the result of cooling [33] and/or better acid–base control [34, 35] rather than a mediator-based mechanism. However, we did not observe any obvious differences in body core temperature and systemic pH in septic animals treated

with or without haemofiltration. Admittedly, the contribution of cooling effect to the reduced need for vasopressor support cannot be entirely neglected. In support of this notion, we could recently demonstrate that even a mild haemofiltration-induced decrease in core temperature, the extent of which was comparable to this study, had the potential to increase systemic vascular resistance in septic patients [33]. In contrast, Rogiers et al. [36] observed that a decrease in body temperature during haemofiltration resulted in a rapid development of hypotension and hypodynamic circulation in ovine septic shock model. It should be noted, however, that in the Rogiers study the body temperature fell below baseline with the lowest value of 36.1°C, which is in marked contrast to our results. Alternatively, cutting the plasma peak concentrations of cytokines [37] without a measurable effect on plasma cytokines levels or elimination of other unmeasured vasodilatory or inflammatory mediators [9, 38–40] could have explained the observed systemic hemodynamic effects of haemofiltration. Finally, removal of sedative drugs leading to an increased sympathetic drive of animals cannot be completely excluded.

The apparent dissociation between the salutary effects of haemofiltration on vasopressor requirement and the totally absent improvement in other biological systems is a striking and key finding of this study. In this context, our experiment is the first and only study so far to assess the effects of haemofiltration on sepsis-mediated disturbances of microvascular perfusion and energy metabolism in different vital organs. Using laser Doppler flowmetry we

did not observe any positive impact of haemofiltration on ileal mucosal and renal cortex microvascular perfusion. Since direct visualization of the ileal mucosal microcirculation gave similar results, it is unlikely that we overlooked major microvascular effects. These findings might be of particular importance given the fact that early microcirculatory alterations are related to a poor prognosis in human sepsis [41]. Furthermore, taking into account a hypothesis that haemofiltration may have reduced the burden of various mediators which, in fact, affect cellular metabolism, a concordant amelioration in markers reflecting metabolic stress would have been expected. Nonetheless, the progressive changes in hepato-splanchnic lactate/pyruvate and ketone bodies ratios indicating a deranged cytosolic and mitochondrial redox potential, respectively [42], clearly argue against an improvement in cellular energy balance with haemofiltration. Our results are in sharp contrast to data from Li et al. [43] who demonstrated that HVHF (100 ml/kg per hour) improved myocardial mitochondrial complex I activity in porcine peritonitis. These apparent dissociations are most likely due to the substantial differences in the model and study design used. While animals in the above mentioned study exhibited severe hypodynamic hypotensive shock and were studied within 12 h since the induction of peritonitis, we evaluated the effects of haemofiltration over 22 h of progressive hyperdynamic sepsis. Moreover, the prophylactic timing of intervention (1 h after the insult) and avoidance of vasopressors in the study by Li et al. make both studies difficult to compare. Given the limitations of our methodology, we were unable to determine whether or not the failure of haemofiltration to counteract the sepsis-induced microvascular and metabolic derangements is attributable to the limited capacity of plasma water exchange to affect the tissue or even cellular concentrations of sepsis mediators [44–46].

The endothelial cells represent a crucial target organ for the action of inflammatory mediators and are believed to be one of the key elements in the pathophysiology of sepsis [47, 48]. To the best of our knowledge, no data is available to document the capacity of haemofiltration to modulate sepsis-induced endothelial and coagulation dysfunction. In our study haemofiltration did not ameliorate the peritonitis-induced increase in plasma vWf, a generally accepted marker of endothelial injury [49]. Likewise, the progressive fall in platelet count, probably as a result of disseminated intravascular coagulation, was not influenced by haemofiltration. The contribution of the extracorporeal circuit to these changes seems unlikely as haemofiltration did not influence the studied variables in sham-operated animals. These results are corroborated by our clinical study demonstrating that continuous hemodiafiltration neither modified plasma vWf levels nor affected platelet count [50].

While all previous experimental studies compared the effects of HVHF either with untreated subjects or with

low-volume haemofiltration (20 ml/kg per hour), our study is the first to seeking whether increasing the dose of plasma water exchange confers an additional benefit over the currently accepted standard haemofiltration dose, i.e. 35 ml/kg per hour. Although our observation corroborates recent reports, both from animal [16, 19, 20, 27] and clinical [9–14] trials, demonstrating a favorable effect on systemic haemodynamic, an ultrafiltration rate of 100 ml/kg per hour failed to be superior over 35 ml/kg per hour in its capability to ameliorate disturbances in other mutually independent biological systems. These results could indicate, among other things, that the value of haemodynamic improvement as a surrogate marker for efficacy of haemofiltration might be ambiguous. Albeit indirectly, our findings also argue in support of the notion that haemofiltration appears to be the most effective when applied as pre-treatment or concomitantly with the septic or endotoxemic insult [21, 51, 52], which might limit the clinical applicability.

Although our model replicates many of the features of human sepsis, there are some limitations that should be discussed. Antibiotic therapy that would bring the model closer to clinical standard was not used in this model. However, our model was designed to induce hyperdynamic sepsis with increasing severity over time. The antibiotic therapy was expected to blunt the host response to peritonitis, thereby attenuating the development of full manifestation of a true clinical septic response over the 22-h period as used in our experiment. Moreover, the study was not designed to evaluate survival rate but, rather, to focus on different and independent putative biological targets of haemofiltration. Furthermore, we can only speculate whether the observation study time was sufficient for detecting significant changes in the physiological end-points measured. Finally, since the severity of septic shock could be classified as moderate in our model, we cannot exclude different magnitude of the treatment response in more profound shock state.

In summary, in this model of septic shock secondary to peritonitis early HVHF proved superior to standard modality in preventing the development of septic hypotension. Nevertheless, the favorable effects of haemofiltration on vasopressor requirement did not translate into improved microvascular, metabolic and endothelial function, at least within the timeframe of the study and severity of the model.

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