INTRODUCTION

Sepsis is one of the top 10 leading causes of death worldwide. The in-hospital mortality for septic shock is between 40% and 50%. Gram-negative bacteria have replaced Gram-positive bacteria as the most common group of sepsis pathogens, and are associated with higher mortality. Lipopolysaccharide (LPS, endotoxin), situated on the outer membrane of Gram-negative bacteria, is recognized by toll-like receptors (TLRs), mainly TLR-4.
example, tumor necrosis factor-α (TNF-α) and interleukins (IL), can lead to endothelial dysfunction, characterized by increased capillary permeability and vasodilatation,\textsuperscript{8} tissue hypoxia and organ failure.\textsuperscript{9}

Intravenous (IV) fluids and vasoressors are among the most important and frequently used evidence based interventions in sepsis. Amounts, infusion rates, and types of fluids are still a topic for discussion. Most studies find no difference between albumin solutions and crystalloids concerning mortality,\textsuperscript{10-12} among these a Cochrane review from 2011 summarizing 38 randomized controlled studies.\textsuperscript{13} The Surviving Sepsis Campaign 2016 Guidelines, however, recommend albumin in addition to crystalloids, for patients who require very large amounts of crystalloids.\textsuperscript{14,15}

Positive effects of albumin solutions not only attributed to expansion of plasma volume have been proposed. There has been evidence that albumin can act as a scavenger of nitric oxide, thereby countering peripheral vasodilatation.\textsuperscript{16} Albumin has also been suggested to have anti-oxidative as well as anti-inflammatory effects.\textsuperscript{17}

Adverse effects of albumin are less studied. Perdomo-Morales and colleagues demonstrated contamination of (1,3)-\(\beta\)-glucan derived from filters during the production process of human albumin solutions (HAS).\textsuperscript{18,19} The (1,3)-\(\beta\)-glucan contributes to an increase in IL-6 release by monocytes,\textsuperscript{18} and other studies have suggested a synergistic effect with endotoxins and (1,3)-\(\beta\)-glucan leading to more intense pyrogenic reactions in human models.\textsuperscript{19,20}

Plasma proteins can indeed affect host response to bacterial products. M1 protein, a PAMP released by Streptococcus pyogenes, increases IL-6 production and decreases the contractile response to noradrenaline.\textsuperscript{10} Likewise, the plasma protein lipopolysaccharide-binding protein (LBP) enhances the inflammatory response to LPS by presenting it to CD14 and TLR4.\textsuperscript{21}

In order to enable identification of subgroups of sepsis patients that may respond positively or negatively to treatment with albumin solutions, in the present study we investigated if preparations of albumin for medical use could affect LPS-induced inflammatory response of the vascular wall and monocytes. Since immunoglobulin G and fibrinogen are main components of plasma and therefore potential contaminants in commercial albumin solutions, for comparison we also explored the effect of these on the response of human tissue to LPS.

2 | MATERIALS AND METHODS

2.1 | Human arteries

The project was approved by the local research ethics committee (LU 18-93 and 2015/801). After written informed consent, pieces of omentum majus were obtained from six patients undergoing abdominal surgery for pancreatic, ovarian, or uterine cancer. Omental arteries were dissected free from fat and connective tissues and cut into eight 2-4 mm long segments.

2.2 | Incubations of arteries

Arterial segments were incubated at 37°C in Dulbecco’s modified Eagle’s medium without phenol red (Gibco) in the presence of L-arginine (1 mmol L\(^{-1}\)), penicillin (2000 U mL\(^{-1}\)), and streptomycin (0.2 mg mL\(^{-1}\), all from Sigma-Aldrich, “DMEM”), aerated by 8% CO\(_2\) in O\(_2\) for 5 minutes. Pilot experiments demonstrated that incubation with LPS for 48 hours resulted in a marked and consistent inflammatory response reflected in cytokine release and decreased contractile response to noradrenaline.

2.3 | Measurement of smooth muscle contraction in human arteries

In the first set of experiments, the following combinations of substances were added to the incubation medium: (a) control without additives, (b) control with albumin (4 mg mL\(^{-1}\), equivalent to 10% of normal plasma albumin concentration), from Octapharma; Albunorm 200 g L\(^{-1}\); MTrn 27080; Batch L647A6663; Vnr 05 43 76 (called A1 below), (c) LPS 100 EU mL\(^{-1}\), from Sigma-Aldrich or (d) LPS 100 EU mL\(^{-1}\) with albumin A1 4 mg mL\(^{-1}\). The concentration of LPS used (100 EU mL\(^{-1}\)) was determined from pilot experiment showing that incubation with LPS at 100 EU mL\(^{-1}\) resulted in a clear but submaximal decrease in vascular smooth muscle contraction compared to control.

After incubation, the segments were thread onto two L-shaped hooks in 2-mL tissue baths containing Krebs-Ringer solution (composition in mmol L\(^{-1}\): Na\(^+\) 143, K\(^+\) 4.6, Cl\(^{-}\) 126, Ca\(^{2+}\) 2.5, HCO\(_3\)\(^{-}\) 25, Mg\(^{2+}\) 0.79, SO\(_4\)\(^{2-}\) 0.79, H\(_2\)PO\(_4\)\(^{-}\) 1.2, glucose 5.5 and ethylene diamine tetra-acetic acid, EDTA, 0.024) aerated with 8% CO\(_2\) in O\(_2\). One of the hooks was attached to a Grass FTO3C force-displacement transducer for measurement of isometric force. The force was recorded on a Grass polygraph model 7b (Grass Instrument Corp.). The vessels equilibrated for 1 hour during which the final pretension was adjusted to 4-8 mN.\textsuperscript{22} Potassium chloride (83 mmol L\(^{-1}\)) was then added and a resulting smooth muscle contraction confirmed viability. After wash-out, noradrenaline (NA, 10\(^{-15}\)-10\(^{-6}\) mol L\(^{-1}\)) was added cumulatively in 0.5 \(_{10}\)log units. The resulting contraction was registered and concentration-response curves were constructed.
After measurement each artery segment was weighed and contraction was expressed in mN mg⁻¹.

2.4 | Measurement of cytokine and nitrite/nitrate production by human arteries

In another set of experiments, similar incubations as above were used except for that LPS at 50 EU mL⁻¹ was chosen since this gave a sufficient submaximal cytokine production in pilot experiments. Furthermore, additional vessel segments were incubated with immunoglobulin G (1.1 mg mL⁻¹, Sigma-Aldrich) or fibrinogen (0.3 mg mL⁻¹, Sigma-Aldrich) instead of albumin. After incubation the artery segments were removed and weighed, and the incubation medium was centrifuged at 5000 × g for 5 minutes. The supernatant was removed and kept at −20°C until subsequent measurement of cytokine and nitrite/nitrate levels. Concentrations of IL-1β, IL-6, IL-8, IL-10, and TNF-α in the incubation medium were measured with cytometric bead array (CBA) enhanced sensitivity flex set (BD Biosciences) using fluorescence-activated cell sorting (FACS Verse, BD). Nitrite/nitrate was measured as previously described.²³

2.5 | Isolation of monocytes

Venous blood from healthy volunteers was collected into heparin coated glass vials. Peripheral blood mononuclear cells (PBMC) were separated with density gradient centrifugation using Lymphoprep™ (Axis-Shield PoC AS, 01-63-12-001-A) according to the manufacturer’s instructions. Cells were washed twice by centrifugation, resuspended in sterile phosphate buffered saline (PBS) and counted in a Bürker chamber.

Cells (300 000) were put into wells of a 96-well sterile cell culture plate (ThermoNuclon Delta Surface) containing 150 μL DMEM supplemented with 10% fetal bovine serum (F2442, Sigma-Aldrich). After 2 hours of incubation at 37°C in an atmosphere containing 5% CO₂, the medium was changed to remove non-adherent cells. 24 Cells were then equilibrated for another 20 hours.

2.6 | Incubations of monocytes

After equilibration, wells were rinsed twice using sterile PBS and 150 μL DMEM without fetal bovine serum was added. The following combination of substances were added, followed by another 20-hour incubation as described above: (a) control with no additives, (b) Albumin 4 mg mL⁻¹, (c) LPS 25 EU mL⁻¹ (in pilot experiments giving a clear but submaximal TNF-α production), or (d) LPS plus albumin 4 mg mL⁻¹. The following albumin solutions were tested:

A1: Albunorm 200 g L⁻¹; OctapharmaMTnr 27080; Batch P750B6662; Vnr 05 43 76.
B: Albumin Behring 200 g L⁻¹; CSL Behring; MTnr 09701S; Batch G2644411A.
C: Alburex 200 g L⁻¹; CSL Behring; MTnr 50243; Lot 4376400015; Vnr 078216.
D: Albumin Baxalta 200 g L⁻¹; Shire Sweden; MTnr: 237785E; Lot PAA15224; Vnr 048043.

2.7 | Cytokine production by monocytes

Following incubation, TNF-α concentration in the DMEM was measured using enzyme-linked immunosorbent assay (ELISA, R&D DuoSet™, DY210-05) according to the manufacturer’s instructions. Standard curves were generated diluting TNF-α to known concentrations using the different media employed, that is, DMEM alone as well as DMEM containing 4 mg mL⁻¹ albumin solution A1, A2, B, C, or D, respectively.

In a separate set of experiments, monocytes were incubated with LPS as above with or without albumin solution A1 at 2, 4, or 8 mg mL⁻¹. Following incubation, TNF-α levels were measured in all incubations. In the incubations without or with albumin at 4 mg mL⁻¹, IL-1β, IL-6, IL-8, and IL-10 were measured with CBA as described.

2.8 | Endotoxin levels in albumin solutions

Albumin solutions were diluted × 5 in endotoxin-free PBS and analyzed using a high sensitivity assay based on Limulus amoebocyte lysate as previously described.²⁵ A standard curve was constructed by analysis of healthy plasma with known concentrations of endotoxin. Detection level in albumin solutions (200 mg mL⁻¹) was 0.15 EU mL⁻¹ (approximately corresponding to 20 pg mL⁻¹).

2.9 | Electrophoresis

Albumin solutions diluted ×100 and ×1000 in DMEM, plasma from a healthy volunteer and recombinant human lipopolysaccharide-binding protein (LPB, R&D Systems, 870-LP-025) were separated in gradient gels 4%-15%, (Bio-Rad 456-1083) using standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie brilliant blue (Novex).

2.10 | In-gel digestion and mass spectrometry

A band of interest (corresponding to 130 kDa in albumin solution B) was excised from the gel and digested with trypsin. The peptide analysis was performed on a Q Exactive HFX mass spectrometer (Thermo Fisher Scientific) connected to an Ultimate 3000 ultra-high-performance liquid chromatography system (Thermo Fisher Scientific), Peptides were loaded on a precolumn (Thermo Fisher
Science; ID 75 μm x 2 cm, column temperature 35°C) and then separated on an EASY-Spray column (Thermo Fisher Scientific; ID 75 μm x 25 cm, column temperature 45°C). Separation was performed at a flow rate of 300 nL min\(^{-1}\) in 45 minutes with a non-linear gradient ranging from 4% to 55% acetonitrile containing 0.1% formic acid. Raw data were analyzed with Proteome Discoverer version 2.2 (Thermo Fisher Scientific). Peptides were identified using SEQUEST HT against UniProtKB Human database (release 20180911). The search was performed with the following parameters applied: static modification: cysteine carbamidomethylation and dynamic modifications: N-terminal acetylation and methionine oxidation. Precursor tolerance was set to 10 ppm and fragment tolerance was set to 0.02 ppm. Up to two missed cleavages were allowed and Percolator was used for peptide validation at a q-value of maximum 0.01.

2.11 Measurement of (1-3)-β-glucan in albumin solutions

Levels of (1-3)-β-glucan in the five different albumin solutions were measured using enzyme-linked immunosorbent assay (QuickDetect™ 1-3-β-glucan ELISA Kit, BioVision, E4446-100) according to the manufacturer’s instructions. The detection level was 0.8 pg mL\(^{-1}\).

2.12 Western blot

Proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P Transfer membrane, IPVH00010) in tris-glycine-ethanol (wet method) at 100 V for 1 hour. Membranes were then blocked in 5% skim milk (Difco™, 232100) in PBS with 0.05% Tween 20 (Medicago AB, 09-8902-100) for 2 hours and exposed to a primary polyclonal goat IgG anti-human LBP antibody, (0.1 Tween 20 (Medicago AB, 09-8902-100)) for 2 hours and exposed to a then blocked in 5% skim milk (Difco™, 232100) in PBS with 0.05% albumin solutions. The shift was reflected in a statistically significant decrease in NA was shifted to the right, similarly in the absence and presence of albumin. The shift was reflected in a statistically significant decrease in median pEC\(_{50}\)-value compared to control (6.45 [6.21-6.58], 6.06 [5.72-6.21] and 6.07 [5.73-6.17] (P < .05) for control, after incubation with LPS and after incubation with LPS together with albumin, respectively Figure 1).

After incubation with LPS, the concentration-response curve for NA was shifted to the right, similarly in the absence and presence of albumin. The shift was reflected in a statistically significant decrease in median pEC\(_{50}\)-value compared to control (6.45 [6.21-6.58], 6.06 [5.72-6.21] and 6.07 [5.73-6.17] (P < .05) for control, after incubation with LPS and after incubation with LPS together with albumin, respectively Figure 1).

Median AUC after incubation in the presence of LPS with albumin tended to be lower than after incubation with LPS alone but the difference did not reach statistical significance (P = .061). Incubation with albumin alone did not affect the concentration-response curve for noradrenaline (not shown).

3.2 Cytokines and nitrite/nitrate released by blood vessels

After incubation with LPS alone there was a statistically non-significant tendency toward an increase in median release of IL-6 and IL-8 from human arteries compared to control (Figure 2). When LPS was combined with albumin, however, statistically significantly greater

### Analysis of mass spectrometry data

The raw DDA data were analyzed with Proteome Discoverer™ 2.2 (PD 2.2) Software (Thermo Fisher Scientific). Peptides were identified using SEQUEST HT against UniProtKB Human database (release 20180911). The search was performed with the following parameters applied: static modification: cysteine carbamidomethylation and dynamic modifications: N-terminal acetylation, methionine oxidation, and phosphorylation. Precursor tolerance was set to 10 ppm and fragment tolerance was set to 0.02 ppm. Up to two missed cleavages were allowed and Percolator was used for peptide validation at a q-value of maximum 0.01. Filter settings at the protein level: Master is equal to Master, Protein Unique Peptides is greater than or equal to 3.
amounts of IL-6 and IL-8 were released compared to control as well as to with LPS alone (Figure 2). Release of IL-1β, IL-10, and TNF-α from the blood vessels was around three orders of magnitude lower than release of IL-6 and IL-8 (Figure 3). Incubation with LPS alone did not affect cytokine release to any larger extent compared to control. Adding albumin to the incubation increased median release of IL-1β, IL-10, and TNF-α compared to incubation with LPS alone (Figure 3). Immunoglobulin G and fibrinogen did not affect LPS-induced cytokine release. Incubation with albumin, immunoglobulin G, or fibrinogen alone did not affect release of cytokines from the omental arteries (data not shown).

No detectable levels of nitrite/nitrate were observed in any of the incubations.

3.3 | Effect of different albumin solutions on cytokine release by monocytes

Due to the limited availability to human arteries, experiments with isolated human monocytes were included in order to investigate the effects of a larger array of different commercially available albumin solutions. LPS caused a significant increase in median release of TNF-α (3.3 [2.6-9.2] pg 100 000 cells⁻¹) compared to control (0.15 [0.0-0.30] pg 100 000 cells⁻¹; Wilcoxon signed rank test, \( P < .001 \), \( n = 11 \)).

Exposure to any of the albumin solutions alone did not affect TNF-α release compared to control (\( n = 8 \), not shown). When albumin solution A1, A2, B, or C was present during incubation with LPS, median TNF-α release was, however, statistically significantly greater than in the presence of LPS alone (\( P < .05 \), Figure 4). Albumin solution D did not affect median TNF-α release compared to incubation with LPS alone.

Median TNF-α release was increased by incubation with Albumin A1 at 2, 4, and 8 mg mL⁻¹ and LPS compared to incubation with LPS alone (\( P < .05 \), not shown). No concentration-dependent effect of albumin was detected.

Release of IL-6 and IL-10 was significantly higher during incubation with albumin and LPS compared to LPS alone (\( P < .05 \), Figure 5). Release of IL-1β and IL-8 during incubation with LPS differed from control neither in the presence nor in the absence of albumin (data not shown).

3.4 | Contents of the Albumin solutions

Electrophoretic separation of all albumin solutions diluted 100 times showed a prominent band with a molecular weight consistent to albumin. In all solutions a fainter band could be seen with a molecular weight approximately 130 kDa (Figure 6A). Diluting the albumin 1000 times narrowed the albumin band but no fainter bands were revealed next to it (Figure 6B).
In-gel digestion and mass spectrometry of the 130 kDa band showed that albumin was 100-fold more abundant than any other protein, which strongly suggests that this band consists of dimeric albumin.26

The two albumin solutions from manufacturer A were found to contain (1-3)-β-glucan at 9 and 17 pg mL$^{-1}$, respectively, while levels in all the other solutions were below detection level.

The level of endoxins was below detection level (0.15 EU mL$^{-1}$) in all albumin solutions except in solution A1 (close to detection level) and B (0.24 EU mL$^{-1}$).

3.5 | Western blot

LBP-like immunoreactivity was found in a band corresponding to the recombinant positive LBP control and in healthy human control plasma. No LBP-like immunoreactivity was found in any of the albumin solutions (Figure 7).27

4 | DISCUSSION

Albumin solutions, are frequently used to treat hypovolemia in patients with sepsis and septic shock. In addition to being a plasma expander, other positive effects, such as antioxidant and positive effects on vessel wall integrity, have been proposed.27 An animal study demonstrated that human albumin completely prevents both LPS-induced vascular hypo-reactivity to the vasconstrictor phenylephrine and endothelial dysfunction in the mouse.27 Interactions of albumin with bacteria, or components of bacteria, in human sepsis models are, however, largely unknown. In this in vitro study we show that commercially available solutions
of human albumin worsen the effect of LPS from Escherichia coli, resulting in diminished vascular contractile response to noradrenaline and significantly increased release of cytokines such as TNF-α, IL-6, IL-8, and/or IL-10 from the vascular wall and monocytes. The increase in cytokine release from both vascular wall, presumably from the endothelial cells, and monocytes suggests that albumin

**FIGURE 5** Interleukin (IL)-6 (A) and IL-10 (B) release into the medium after incubation of human monocytes with and without lipopolysaccharide (LPS, 25 EU mL⁻¹) in the absence or presence of albumin (4 mg mL⁻¹). The presence of albumin together with LPS caused a statistically significantly greater median IL-6 and IL-10 release compared to incubation with LPS alone (*, P < .05). Friedman repeated measures ANOVA on ranks followed by post hoc test vs group stimulated with LPS alone using Dunnett’s method. Horizontal lines are medians and boxes indicate interquartile range, n = 10

**FIGURE 6** SDS-PAGE electrophoresis of albumin solutions. When diluted 100 times (A) a prominent band between protein standards 55 and 70 kDa corresponding to albumin was present in all solutions. A fainter band at approximately 130 kDa could also be seen. When diluted 1000 times (B) the albumin band was narrower but no fainter bands were disclosed. Protein size (kDa) standards are shown to the left. SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

**FIGURE 7** Western blot of albumin solutions. Protein size (kDa) standards are shown to the left (green). Lipopolysaccharide-binding protein (LBP)-like immunoreactivity (red) was found in a band corresponding to the recombinant positive LBP control and in healthy human control plasma (P). No LBP-like immunoreactivity was found in any of the albumin solutions (B, C, A2, A1 or D)
affects several features of human innate immunity activated by LPS. Controls with vessels and monocytes incubated with albumin alone exclude that the albumin solutions themselves are responsible for the inflammatory response.

Possible contamination of the albumin solutions was investigated. We found levels of (1-3)-β-glucan consistently lower than the suggested cut-off level for contamination of albumin solutions, 20 pg mL⁻¹. 28 In some albumin solutions (1-3)-β-glucan was detected but levels did not correlate with the level of TNF-α release form monocytes in the presence of LPS, hence it is not likely that (1-3)-β-glucan contamination explains our results. In fact, background levels of (1-3)-β-glucan up to 51 pg mL⁻¹ are considered normal in human serum. 29 Furthermore, it is unlikely that endotoxin contamination of the albumin solutions could explain our findings. The highest endotoxin level in any albumin solution was 0.24 EU mL⁻¹, a concentration 100 times lower than the lowest used in the experiments. The Food and Drug Administration defines contamination in medical devices in contact with the cardiovascular system, such as albumin solutions, at levels above 0.5 EU mL⁻¹. 30

Human innate immunity has evolved to react in response to LPS. Therefore, we hypothesized that a component of donor plasma that directly potentiates LPS was present in the albumin solutions. SDS-PAGE did not reveal any major contamination except for a faint band around 130 kDa present in all albumin solutions tested. Abundant high molecular weight plasma proteins include IgG and fibrinogen. None of these proteins affected LPS-induced cytokine release from human arteries, precluding the possibility that contamination by these was responsible to the pro-inflammatory LPS effect. Identification of this band by mass spectrometry convincingly showed that it represents albumin dimer present in the commercial preparations.

LBP is a plasma protein well-known to enhance LPS activity. LBP binds to LPS leading to a cytokine response after interaction of the LPS-LBP complex with CD 14 and TLR 4. 31,32 Cytoplasmic domains then trigger activation of the transcription factor nuclear factor-κB, and a number of pro-inflammatory genes starts to produce cytokines. 33 During electrophoresis, a band with the molecular size of LBP (varying around 60 kDa) might have been hiding under the prominent albumin band. Western blot did, however, not detect LBP in any of the albumin solutions, at least at levels equal to or greater than that found in normal plasma.

Further studies will be needed to identify the compound or compounds in the solutions responsible for the enhancing effect of LPS-induced inflammation found, for example, mass spectrometry of a wider range of fractions of the albumin solutions. For example, low levels of pro-inflammatory cytokines, presumably originating from the donors, may contribute to priming the tissue to exaggerated sensitivity to LPS without having any detectable effect of its own.

One possible mechanism to the increased inflammatory effect observed in the present study, could be that albumin helps to create a more stable state of LPS, via binding of the lipid A portion, thereby to a greater extent extracting and transferring endotoxin to specific cellular targets such as TLR 4. This interaction has previously been described, but demonstrated to include LBP and CD14. 34,35 It cannot be excluded that albumin alone could be responsible for enhancing the response to LPS. In fact, a previous study has shown that human embryonic kidney 293 cells, expressing TLR 4, can be activated by an LPS-albumin complex, without involving LBP or CD 14. 35 Since TLR 4 are present on the surface of both monocytes and human endothelial cells, 35,36 this could be a likely mechanistic explanation to our results. The fact that albumin solution D did not affect the response to LPS speaks against this theory but it cannot be excluded that albumin solution D, for example, contains a component that prevents albumin from forming an LPS-albumin complex.

We have previously demonstrated that M1 protein from S pyogenes incubated with human arteries in combination with fibrinogen increases IL-6 and IL-8 secretion and weakens smooth muscle contraction. 37 Albumin solution did not affect the response to M1 protein 37 and in the present study fibrinogen was found not to affect the response to LPS, indicating that the interactions between each plasma protein preparation and bacterial compound are specific. The LPS-induced vascular hypo-responsiveness in human arteries does not seem to involve induction of nitric oxide synthase and subsequent increased levels of nitric oxide (NO) since we could not demonstrate elevated levels of the NO metabolites nitrite/nitrate despite enrichment of the NO precursor L-arginine in the incubation medium.

Limitations of this study include that the omental arteries come from patients with cancer disease, which increases cytokine levels. However, we have throughout compared contraction as well as cytokine release to the baseline level of every patient individually. Another limitation of the study is the in vitro setup and we do not know whether chosen LPS concentrations reflect the levels in patients with sepsis. Furthermore, even though we have tried to create an environment as close to the in vivo situation as possible; neither whole blood nor plasma were present in our experimental models. This artificial setting must be taken into account when interpreting the results and especially transferring them to the clinic.

Clinical effects were not investigated in this study and transferring the results to an in vivo situation should be done with caution. A plasma concentration of infused albumin and any possible contaminating compound corresponding to 4 mg mL⁻¹ mostly used in the present in vitro experiments would in an adult be achieved with infusion of as little as 250 mL 20% albumin solution, which would not be uncommon during sepsis treatment. On one hand, unless the infused albumin molecules are chemically modified in some way it is not likely that a 10% increase in albumin concentration would affect host response to endotoxin to any major extent. On the other hand, if the inflammation enhancing effect is caused by a contamination or enrichment of a plasma factor resulting from the manufacturing process of the albumin solutions, the effect may remain also during clinical use. The fact that albumin solution already at 2 mg mL⁻¹ already had an effect with no significant increase at higher concentrations speaks in favor of this hypothesis.
The ultimate question is whether the increase in host immune response by albumin solutions indicated in the present study might worsen outcome for patients with Gram-negative sepsis. If so, it would be important to identify patients with endotoxaemia as a subgroup which might experience negative effects from treatment with albumin solutions. Further studies ought to have priority since sepsis patients are exposed to albumin treatment on a daily basis in ICUs worldwide.

5 | CONCLUSION

We have shown that HAS triggers an aggravated LPS-induced pro-inflammatory cytokine activation in human omental arteries and monocytes as well as vasoplegia. A possible explanation could be that albumin forms complexes with LPS activating TLR 4 in an LBP independent manner.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTION

Viveka Björck was involved in laboratory work, statistical analysis, and drafting of manuscript. Mikael Bodelsson was involved in statistical analysis and drafting of manuscript. Linea Andersson and Lisa I. Påhlman were involved in laboratory work and scientific discussions.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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