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Vascular leakage and its associated phenomena vasodilation and endothelial activation are pathophysiological features of various diseases. Multiple drug candidates targeting these phenomena are in development, necessitating translational models to demonstrate proof-of-pharmacology and proof-of-mechanism in early-phase clinical trials. This single-center experimental study evaluated the intradermal lipopolysaccharide (id LPS) challenge model as a tool to induce and characterize vascular leakage in healthy participants. Eight participants (male:female = 4:4) received id LPS in the volar forearms, followed by serial pharmacodynamic assessments, including imaging and suction blister induction up to 9 hours after injection. Id LPS administration resulted in significant increases in skin perfusion ($P < 0.0001$), erythema ($P = 0.0013$), and skin volume ($P = 0.0008$), indicating initial stages of inflammation and fluid extravasation. Blister fluid analysis revealed elevated extravascular concentrations of albumin ($P = 0.0011$), total protein ($P < 0.0001$), and neutrophils ($P = 0.0342$), supporting the presence of vascular leakage. Moreover, the expression of endothelial activation markers VCAM-1 ($P = 0.0015$), ICAM-1 ($P = 0.0004$), ITGB1 ($P = 0.01$), and E-selectin ($P = 0.0218$) increased significantly. Disruption of endothelial cell–cell integrity was supported by increased expression of VE-cadherin ($P = 0.0002$) in blister fluid. These findings support the applicability of the id LPS model for the induction of vascular leakage in humans. This model holds potential as a translational tool for evaluating the pharmacodynamic responses of vascular leakage-targeting drugs in early clinical development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Vascular leakage, and its associated phenomena vasodilation and endothelial activation, are features of various infectious, inflammatory and malignant diseases. Registered drugs targeting these processes are scarce, but new drug candidates are in development. Therefore, the development of models to demonstrate proof-of-pharmacology and proof-of-mechanism for such drugs can be a useful translational tool in early-phase clinical studies.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study addresses whether the intradermal lipopolysaccharide (id LPS) challenge model can be applied as a unique translational tool in early-phase clinical research to induce and characterize vascular leakage in healthy participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study demonstrates that the id LPS model can indeed be applied for such purposes, since the model allows for the assessment of:

- Vasodilation: skin perfusion and erythema via non-invasive imaging
- Vascular leakage: skin volume via non-invasive imaging and extravasation of albumin and total protein in suction blister fluids
- Endothelial activation and integrity: markers such as VCAM-1, VE-cadherin, and neutrophil extravasation in suction blister fluids.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ By employing id LPS as a minimally invasive model to assess vascular leakage in humans, proof-of-mechanism and proof-of-pharmacology of vascular leakage-targeting drug candidates can be explored in future studies. Studying these proofs in early stages of the drug development process aids in the efficiency of drug development and allows for more informed dose selection in future clinical studies.

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The vascular endothelium is a dynamic, semipermeable layer lining all blood vessels that regulates barrier function and vasomotor tone. Vascular leakage is a hallmark feature of numerous acute and chronic conditions, including prevalent infectious, inflammatory and malignant diseases, such as sepsis and arthritis.[1] Within these conditions, endothelial barrier dysfunction and vascular integrity loss occur through a variety of mechanisms. Although vascular leakage significantly contributes to morbidity and disease progression, the development of drugs specifically targeting vascular leakage within these diseases remains highly challenging.[2–4]

Encouragingly, multiple vascular leakage-targeting drug candidates with various mechanisms of action are under investigation in clinical trials.[5] These drugs aim to reduce vascular leakage by stabilizing the endothelial barrier function. This effect is mainly induced through enhancing endothelial cell–cell junctions via the angiopoietin-Tie2 pathway, but various other mechanisms are also being explored.[3] Examples of such drugs in the clinical phase include razuprotafib (AKB-9778), which activates Tie2 via VE-PTP inhibition, and nesvacumab (REGN910), which binds and inactivates the Tie2 receptor ligand Ang2.[6–8] Furthermore, registered drugs are being investigated for repurposing for the treatment of vascular leakage. One such example is imatinib, which targets Abl-family kinases that mediate vascular permeability.[9]

Given the unmet medical need for vascular leakage-targeting drugs for a wide range of diseases,[3,4] development of methodology allowing evaluation of proof-of-pharmacology and proof-of-mechanism for these drugs in early-phase clinical studies would be valuable. Collecting such pharmacological knowledge aids the translation from preclinical to clinical settings, allows early dose optimization, and may help in the identification of potential safety concerns and therapeutic options. This ultimately leads to optimization of future clinical trial designs.[10,11]

Despite the availability of well-established animal models for vascular leakage,[12–17] experimental models applicable to humans focusing on this purpose are yet to be developed. The intradermal lipopolysaccharide (id LPS) challenge model could fill this gap. LPS, a Toll-like receptor 4 (TLR4) agonist, is an established clinical model used to induce acute and transient inflammation in the skin, and its cellular inflammatory profile has been extensively studied. The model allows direct assessment of immune responses via suction blister fluid and non-invasive imaging techniques. This method allows for serial sampling of tissue responses over time under highly controlled conditions. The relatively low burden for study participants enhances its practical feasibility and applicability.[18]

Besides an acute inflammatory response, LPS is also known to drive a vascular response in animal models, including increased expression of endothelial adhesion molecules, release of vasoactive mediators, and disruption of endothelial barrier integrity via TLR4 signaling.[19,20] Similarly, in the clinical setting, intravenously administered LPS activated biomarkers for endothelial and vascular activation, such as vascular cell adhesion molecule 1 (VCAM-1) and vascular endothelial growth factor (VEGF).[21,22] Currently, limited data are available on potential vascular effects induced by id LPS in humans, but it is known that locally increased perfusion and erythema as markers of vasodilation occur post-LPS injection, which suggests the involvement of endothelial barrier dysfunction at a microvascular level.[18] Given these data and the physiological interplay between inflammation and the vasculature, including neutrophil extravasation upon endothelial activation,[23] it can be envisioned that id LPS elicits vascular leakage in humans.

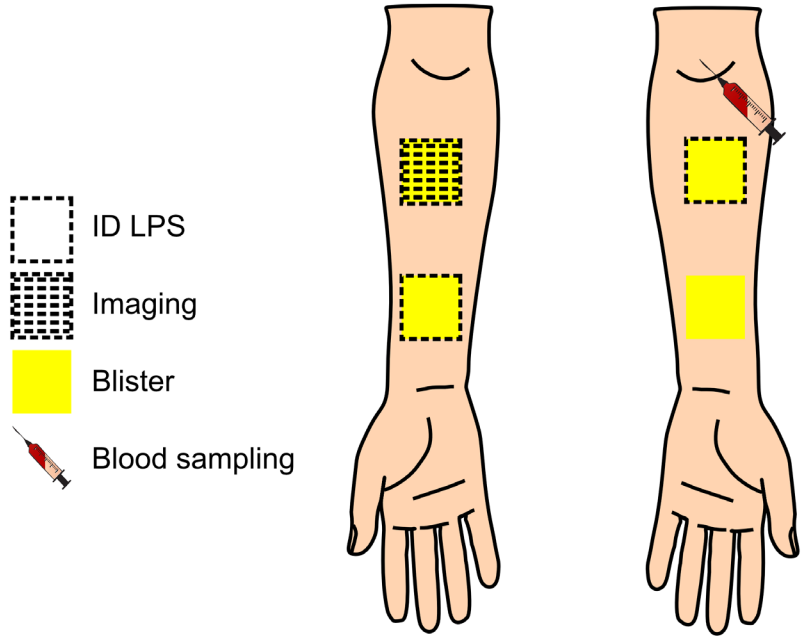
The objective of this study was to characterize id LPS as a human challenge model for vascular leakage, including key features, such as endothelial activation and plasma extravasation, with the aim of establishing its suitability for application in early-phase clinical research. By integrating data on clinical hallmarks of vascular activation and permeability (skin perfusion, erythema, and skin volume) with protein and neutrophil contents of interstitial fluid from LPS-inflamed regions, we aim to characterize the vascular leakage within this model and establish a unique translational tool that can be used to test drug candidates that target features of vascular leakage in a wide range of diseases and bridge preclinical and clinical pharmacological research.

METHODS

This study was conducted from June 2024 to February 2025 at the Centre for Human Drug Research (Leiden, The Netherlands) in accordance with the principles of the Declaration of Helsinki (as revised in 1983) and the Dutch Medical Research Involving Human Subjects Act (WMO). The protocol was approved by the independent Medical Ethics Committee of the Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO, Assen, The Netherlands) and was prospectively registered in the EU Clinical Trials Register (2024-512,377-27-00). All participants provided written informed consent prior to the initiation of any study-related procedures.

Participants

Healthy men and women, aged 18–45 years, were eligible for inclusion in this study if no clinically significant abnormal findings were obtained during a standardized screening procedure consisting of medical history, physical examination, 12-lead ECG, alcohol breath analysis, and clinical laboratory testing (i.e., serum chemistry, hematology, coagulation, urine drug screening, and urinalysis). Participants who previously participated in an LPS challenge study within the last year, and participants with a history of pathological scar formation, dermatologic conditions, or autoimmune diseases were excluded from participation. Furthermore, subjects with a Fitzpatrick skin type >III were excluded from participation given their increased risk of pathological scar formation after the invasive measurements conducted in this study. The use of vitamins was not permitted from 7 days (or five half-lives, whichever was longer)



	Baseline	0h	+0.25h	+0.5h	+0.75h	+1h	+1.5h	+2h	+3h	+4h	+6h	+9h
Id LPS		X										
Imaging	X		X	X	X	X	X	X	X	X	X	X
Blister	X			X						X		X
Blood sampling	X			X						X		

Figure 1 Schematic representation of the study design and schedule of assessments. In this experimental clinical study, eight healthy volunteers received a total of three id LPS injections to designated areas on the volar forearms. One id LPS-injected site on the arm served as area for non-invasive imaging prior to LPS injection (baseline) and repeatedly from 0.25 up to 9 hours thereafter. Imaging consisted of recording skin perfusion using laser speckle contrast imaging and capturing erythema and skin volume using multispectral imaging. For albumin, total protein and Olink protein analysis, blister fluid was collected from suction blisters induced at all areas on the arms at baseline and at 0.5, 4, and 9 hours after id LPS injection. Blood samples for albumin and total protein analysis were taken simultaneously with blister induction. h, hour; Id, intradermal; LPS, lipopolysaccharide.

prior to dosing until study completion. For concomitant medication, topical treatments, and vaccinations, a window of respectively 14 and 30 days prior to dosing until the end of the study was imposed. Throughout the study, participant safety was monitored through recording adverse events (AEs), vital signs (i.e., heart rate and blood pressure), and laboratory testing (including complete blood count and C-reactive protein).

Study design and treatments

This single-center experimental challenge study aimed to establish id LPS administration as a model for vascular leakage and included eight healthy volunteers in an equal male-to-female ratio (1:1). Participants received three id LPS injections of 5 ng each, administered to designated treatment areas on the volar forearms. An additional site on the volar forearms served as an untreated control (Figure 1). The LPS dose was chosen based on previous observations that at a dose of 5 ng there is an acute inflammatory response based on cytokine expression and neutrophil influx, with only mild systemic effects on leukocyte counts and tolerable, mild, transient topical AEs.[18,24] The inflammatory response at 5 ng has been shown to be modifiable by anti-inflammatory drugs.[25] Therefore, we argued that 5 ng LPS would induce sufficient inflammation to produce objectifiable vascular leakage within this model.

Non-invasive measures to assess features of vasodilation and vascular leakage

Dermal imaging—Vasodilation and fluid extravasation. Dermal imaging was conducted at regular intervals on a designated skin area on the forearms to characterize vasodilation by local assessments of skin perfusion and erythema, and to characterize fluid extravasation by assessing skin volume (Figure 1). All procedures were performed in temperature (20–24°C) and humidity (40–60%) controlled rooms and prior to any blister formation. The skin perfusion was quantified using laser speckle contrast imaging (PeriCam PSI NR System, Perimed AB, Järfälla, Sweden) and dedicated image capturing software (PimSoft, Perimed AB) at baseline and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 9 hours after LPS injection. Skin erythema and volume were measured using multispectral imaging (Antera 3D, Miravex, Ireland) at the same timepoints (Figure 1).

Invasive measures to assess features of vascular leakage

Blister fluid—Protein leakage. Suction blisters were induced on the designated areas on the forearms at baseline, 0.5 h, 4 h, and 9 h post-LPS injection (**Figure 1**) following the method described by Buters et al.[18] to determine the presence of vascular leakage based on extravascular albumin and total protein concentrations in interstitial fluid over time. After blister induction, blister fluid was collected in a Sarstedt tube containing 50 µl 3% sodium citrate (Sigma-Aldrich, St. Louis, MO, USA) in phosphate buffered saline (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and kept on ice. After the tube was centrifuged, the supernatant was collected and then frozen at −80°C for protein analysis at the clinical laboratory of the Leiden University Medical Center (Department of Clinical Chemistry and Laboratory Medicine, LUMC, Leiden, The Netherlands). All protein concentrations were measured using a Cobas® c502 automated analyzer (Roche Diagnostics International AG, Rotkreuz, Switzerland) with the applicable assays obtained from Roche Diagnostics. Albumin concentration was determined using the immunoturbidimetric Tina-quant Albumin Gen. 2 (ALBT2) assay (catalog number: 04469658190) in CSF mode (measuring range 0.036–4.8 g/L) upon manual four-fold dilution with 0.9% NaCl (Versylene Fresenius, Fresenius Kabi AG, Bad Homburg, Germany). Total protein concentration was determined using the turbidimetric Total Protein Urine/CSF Gen. 3 (TPUC3) assay (catalog number: 03333825190, measuring range 0.04–2.0 g/L) upon manual 12-fold dilution with 0.9% NaCl. Parallel blood samples were obtained in 3.5-mL SST tubes (BD, Franklin Lakes, NJ, USA) to assess corresponding serum protein levels using the colorimetric Albumin Gen. 2 (ALB2) assay (catalog number: 03183688122, measuring range 2.0–160 g/L) for albumin concentrations and the colorimetric Total Protein Gen. 2 (TP2) assay (catalog number: 03183734190, measuring range 2.0–120 g/L) for total protein concentrations.

Invasive measures to assess endothelial activation

Blister fluid – endothelial activation markers. The Olink Explore 384 Cardiometabolic Panel (Olink Proteomics AB, Uppsala, Sweden) was utilized to examine the relative changes in protein expression of a range of proteins related to endothelial activation and endothelial cell–cell integrity in the obtained blister fluids. This is a multiplex immunoassay based on proximity extension assay technology which reports normalized protein expression (NPX), an arbitrary unit on a $\log_2$ scale.[26]

Blister fluid—Neutrophil extravasation. Neutrophil extravasation was measured in cell pellets from centrifuged blister fluid. The living cells were stained with the following fluorescent antibodies to classify them as neutrophils: CD45, Siglec8, and CD66b (details in **Table S1**). Subsequently, the flow cytometry analysis was performed on a MACSQuant 16.3 analyzer (Miltenyi Biotec B.V., Leiden, The Netherlands).

Statistical analysis

Dermal imaging and protein leakage and neutrophil extravasation in blister fluid. No formal statistical considerations were applied to calculate the sample size in this study. However, based on prior experience in early-phase clinical pharmacology trials using *in vivo* challenge models, and given the application of these models in this setting, a sample size of eight subjects is considered adequate. Therefore, we adopted the same approach within this study.

Baseline characteristics were summarized using descriptive statistics: means and standard deviations (SDs) for continuous variables, and frequencies and percentages for categorical variables (e.g., sex and skin type). Pharmacodynamic end points were summarized over time (n, mean, and SD), and time profiles were graphically represented. Repeated pharmacodynamic measurements, defined as having more than one post-baseline measurement, were analyzed using a mixed-effects model with treatment, time, and their interaction (treatment × time) as fixed effects, and subject as a random effect. Least squares means (LSMs) were estimated for each timepoint and tested for statistical significance compared to baseline with a P value of 0.05 as the threshold. The contrast of interest calculated within the model was baseline vs. peak after id LPS injection. All statistical analyses were performed using SAS version 9.4 for Windows or newer (SAS Institute Inc., Cary, NC, USA).

Blister fluid—endothelial activation markers. Pharmacodynamic analyses of Olink protein expression in blister fluid were conducted using R statistical software version 4.4.2. Repeatedly measured end points for Olink protein expression were summarized using boxplots depicting NPX. The relative changes in expression over time were analyzed using the Skillings–Mack test followed by the Eisinga, Heskes, Pelzer & Te Grotenhuis post hoc test on data from subjects with complete observations ($n = 7$), defined as not yielding any panel-specific outlier samples for the Olink Cardiometabolic panel. Unless otherwise specified, the statistical significance threshold across all analyses was set at 0.05 and a Benjamini–Hochberg correction was applied to account for multiple testing.

RESULTS

Baseline characteristics & participant safety

A total of eight healthy volunteers (males: $n = 4$, females: $n = 4$), aged 19–31 years, were enrolled in this study. All participants had Fitzpatrick scores between I and III, and demonstrated normal vital signs and laboratory values, including hemoglobin levels and white blood cell counts. Detailed baseline characteristics are presented in **Table S2**. No relevant AEs or clinically significant abnormalities in vital signs and laboratory testing were observed in any of the participants, in line with previous studies using id LPS.[18,25]

Non-invasive measures to assess features of vasodilation and vascular leakage

Dermal imaging—Vasodilation. Skin perfusion increased after id LPS injection following a biphasic pattern, with an initial peak (56.5 ± 19.3 AU) at 1 hour and the highest observed peak (77.2 ± 19.8 AU) at 4 hours post-injection (**Figure 2d**). Erythema followed an ascending trend as well, albeit more variable than skin perfusion, reaching its peak (10.3 ± 2.0 AU) at 9 hours after LPS (**Figure 2e**). The observations of the peaks in skin perfusion and erythema were statistically significant when compared to baseline for both parameters (**Table 1**). Exemplary clinical figures reflecting these measurements over time are depicted in **Figure 2a,b**.

Dermal imaging—fluid extravasation. An increase in skin volume was seen immediately after administering the id LPS solution. Subsequently, a statistically significant second peak in skin volume (17.7 ± 18.4 mm^3) was observed 4 hours after injection, coinciding with the observed peak in skin perfusion (**Table 1**). The skin

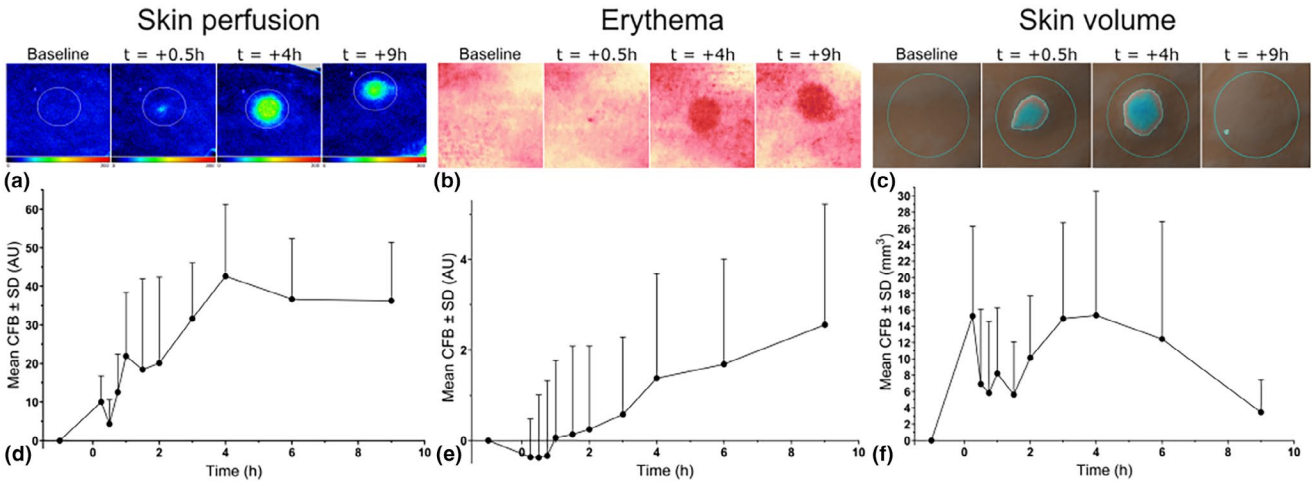


Figure 2 Non-invasive quantification of local dermal responses to id LPS to assess vasodilation and vascular leakage. Skin perfusion was non-invasively assessed using laser speckle contrast imaging, and erythema, and skin volume were evaluated using the Antera 3D camera. (**a–c**) Representative clinical imaging data from a participant demonstrating the observed local response in (**a**) skin perfusion, (**b**) erythema, and (**c**) skin volume at 0.5 h, 4 h, and 9 h after id LPS injection as compared to baseline. (**d–f**) Group summary data ($n = 8$) showing mean ± SD change from baseline in (**d**) skin perfusion, (**e**) erythema, and (**f**) skin volume over time after id LPS injection. AU, arbitrary unit; CFB, change from baseline; h, hours; LPS, lipopolysaccharide.

Table 1 Statistical analysis for the baseline vs. the peak of the non-invasive and invasive measurements of vasodilation, vascular leakage, and endothelial activation

	LSM estimate change from baseline	95% CI (lower, upper)	*P* value
Non-invasive measurements—vasodilation and fluid extravasation			
Skin perfusion (AU)[a]	42.6	29.6, 55.6	<0.0001
Erythema (AU)[b]	2.6	1.3, 3.9	0.0013
Skin volume (mm^3)[a]	15.3	7.4, 23.3	0.0008
Invasive measurements—proteins (g/L)			
Albumin[b]	5.9	2.8, 9.0	0.0011
Total protein[b]	12.1	8.0, 16.3	<0.0001
Invasive measurements—neutrophils (number of cells in 100 µL blister fluid)			
Neutrophils (CD45+/CD66b+/ Siglec8− cells)[b]	942	82, 1801	0.0342

AU, arbitrary units; CI, confidence interval; LSM, least squares means.
[a]Baseline vs. peak at 4 h after id LPS injection. [b]Baseline vs. peak at 9 h after id LPS injection.

volume then returned toward baseline, while skin perfusion reached a plateau (**Figure 2f**). Corresponding clinical figures are presented in **Figure 2c**.

Invasive measures to assess features of vascular leakage

Blister fluid—protein leakage. Albumin was measured in blister fluid as a marker for local vascular leakage. A time-dependent statistically significant increase in mean albumin contents of blister fluid, from 11.3 g/L at baseline to a maximum of 17.3 g/L, was observed 9 hours after dosing of LPS (**Figure 3a**, **Table 1**). Similar data and trends were observed for total protein concentrations in blister fluid (**Figure 3a**, **Table 1**). These increases were irrespective of albumin and total protein concentration in blood, as those concentrations did not vary over time (albumin least squares mean change from baseline −0.3, 95% CI (−0.7, 0.1) g/L, $P = 0.0879$, total protein least squares mean change from baseline −0.2, 95% CI (−1.1, 0.7) g/L, $P = 0.5919$) (**Figure 3b**).

A composite plot that comprehensively demonstrates the time courses of the effects of id LPS on vasodilation, fluid extravasation, and protein leakage can be found in **Figure S1**.

Invasive measures to assess endothelial activation

Blister fluid—endothelial activation markers. The Olink Cardiometabolic panel measured the expression of 369 proteins. Within this manuscript, we focus on the proteins indicating endothelial activation and proteins related to endothelial junctional integrity. The proteins indicating endothelial activation were vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), integrin β-1 (ITGB1), and E-selectin, all of which significantly increased over time after id LPS with a peak at 9 hours post-dose (**Figure 4a**). Endothelial cell–cell junctions were significantly altered after id LPS as well, illustrated by increased expression of VE-cadherin as an indicator for adherens junctions and a decreased expression of endothelial cell-selective adhesion molecule (ESAM) (**Figure 4b**).

Blister fluid—Neutrophil extravasation. The presence of neutrophils in blister fluid was assessed to underline the classical cascade of rolling, adhesion, crawling and transmigration, initiated upon interaction with the activated endothelial cells. In our study, LPS injection induced an influx of neutrophils into the blister fluid. This followed a similar pattern as the protein extravasation, with a peak at 9 h post-LPS injection (942, 95% CI (82, 1802) cells 100 µL of blister fluid, $P = 0.0342$) (**Figure 4c**, **Table 1**).

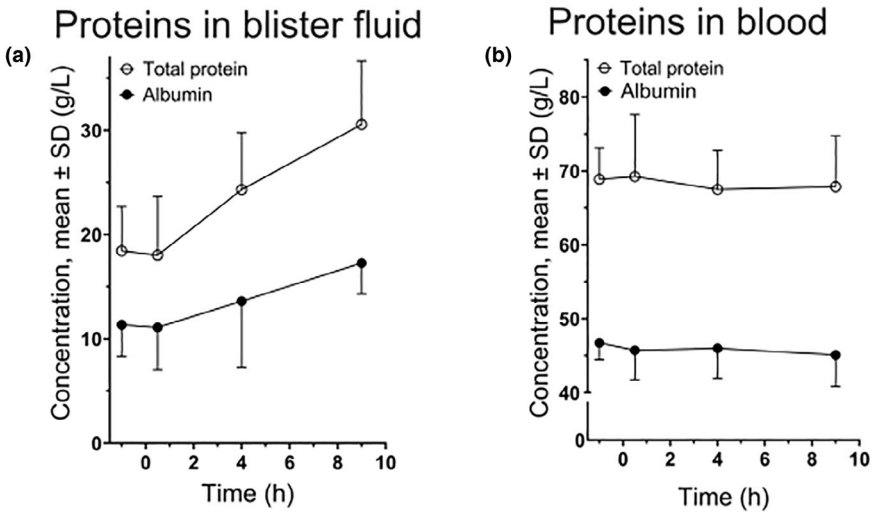


Figure 3 id LPS-induced increases of protein contents in local blister fluid as a measure of vascular leakage. (**a**) Concentrations of albumin and total protein (g/L, mean ± SD) in blister fluid at baseline prior to LPS injection, and at three LPS-injected skin areas at 0.5, 4, and 9 hours after id LPS injection. (**b**) Corresponding serum albumin and total protein concentrations (g/L, mean ± SD) measured from blood samples collected in parallel with the induction of the suction blisters. LPS, lipopolysaccharide.

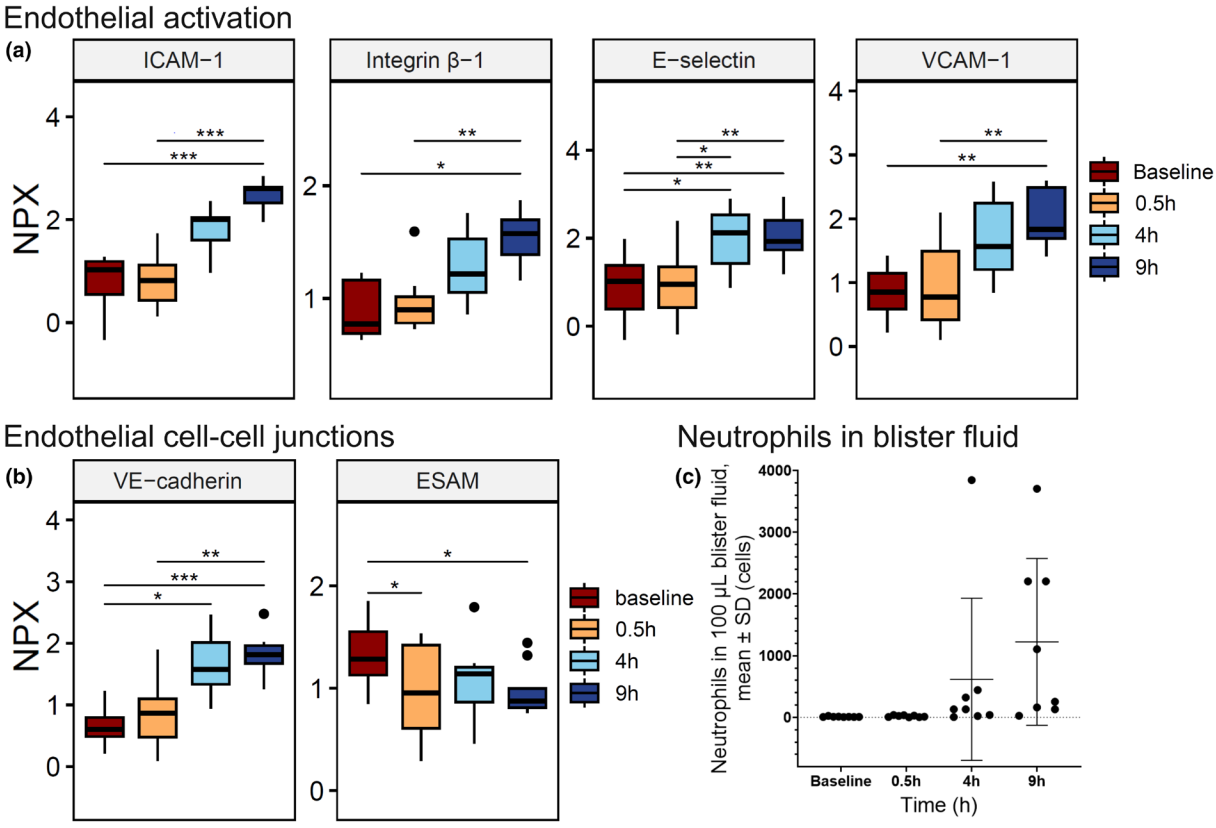


Figure 4 id LPS-induced alterations in endothelial activation and endothelial junction markers in local blister fluid. (**a**) NPX values representing the expression of proteins associated with endothelial activation (VCAM-1, ICAM-1, and E-selection) in blister fluid at baseline and at 0.5, 4, and 9 hours after id LPS injection. (**b**) NPX values for proteins associated with endothelial cell–cell interaction (VE-cadherin and endothelial surface adhesion marker (ESAM)) in blister fluid collected from skin suction blisters on a designated control area (baseline) and on three LPS-injected sites (0.5, 4, and 9 hours after injection) on the volar forearms. (**c**) Quantification (individual values and mean ± SD) of neutrophil infiltration at the designated skin area at baseline prior to LPS injection, and at three LPS-injected skin areas at 0.5, 4, and 9 hours after id LPS injection. Neutrophils were measured as the absolute number of CD45+/CD66b+/Siglec8− cells in 100 µL blister fluid using flow cytometry. Each boxplot in (**a**) and (**b**) represents the median, interquartile range, and 1.5*IQR (whiskers) NPX for each protein. Asterisks indicate levels of statistical significance as analyzed using the Eisinga, Heskes, Pelzer & Te Grotenhuis post hoc test. *$P<0.05$, **$P<0.01$, ***$P<0.001$. NPX, normalized protein expression.

DISCUSSION & CONCLUSION

In this study, we aimed to characterize the id LPS challenge in humans as a localized vascular leakage model. This application would be a valuable translational tool for early-phase clinical pharmacological studies evaluating the effects of vascular leakage-targeting compounds. By integrating data collected from dermal imaging techniques and analyses of biomarkers from blister fluid, we show that the id injection of LPS elicited endothelial activation and vascular leakage. Approximately 1 hour after the injection, clinical signs of increased skin perfusion and erythema marked the onset of the vascular response to LPS. This vascular response further increased over time, while skin volume as a measure of edema and protein leakage was also initiated and peaked at 9 hours post-injection, indicating the presence of additional LPS-induced vascular leakage. Olink data further substantiated these findings, revealing endothelial activation and loss of endothelial cell–cell integrity in response to id LPS in a similar time course.

It is known that increased perfusion and erythema, as measures of vasodilation, are consistent with early signs of inflammation and can contribute to capillary leakage by various mechanisms, for example, increasing the number of perfused vessels and local vascular pressure.[27,28] The local vascular responses in our study, characterized by increased skin perfusion and erythema, are in agreement with this knowledge and with observations of perfusion and erythema as previously reported from two other clinical studies that focused on the effects of id LPS on the inflammatory response.[18,25]

Our study adds to the available clinical data on id LPS by extensively profiling early effects of id LPS and by objectifying the presence of increased skin volume after id LPS. Importantly, the observation that skin volume decreased while perfusion remained elevated following their simultaneous peak suggests that skin volume is not solely attributable to enhanced perfusion. Rather, the increase and subsequent decrease in skin volume can be explained by the presence of vascular leakage after id LPS injection, as vascular barrier restoration and interstitial fluid clearance after an inflammatory stimulus reduce edema irrespective of skin perfusion.[29,30] An alternative explanation for the resolution of skin volume could be that the 3D multispectral imaging technique's sensitivity limits do not allow for detection of subtle changes. However, prior research has demonstrated relevant changes in a similar effect range over time using the same technique.[31,32]

Furthermore, blister fluid analysis provided direct biochemical evidence of loss of vascular barrier function and leakage, since albumin and total protein levels were significantly increased at LPS-challenged sites. Albumin leakage is a well-established marker of endothelial permeability in *in vivo* models for vascular leakage in rodent skin, lungs, and brain.[33–36] In humans, under normal circumstances, suction blister fluid contains protein concentrations comparable to those of interstitial fluid.[37] Fluid that actively leaks through a disrupted endothelial barrier contains high protein concentrations.[30] This highlights the relevance of our observations that protein concentrations increased after id LPS and supports the use of id LPS as a model of vascular leakage. Furthermore, we observed that the peaks in protein concentrations were not simultaneous with the peak in skin volume. This discrepancy is not unexpected, given that interstitial fluid is mainly reabsorbed within hours, while clearance of proteins is a much slower process, with proteins in interstitial fluid having a half-life of 20–29 hours (depending on the health status of the skin).[18,38–40]

An additional novel finding in our study was the relative increase in expression of endothelial activation markers, namely VCAM-1, ICAM-1, ITGB1 and E-selectin, and altered levels of cell–cell junction markers, namely VE-cadherin and ESAM, in the blister fluid. VCAM-1, ICAM-1, ITGB1 and E-selectin are proteins present on the luminal surface of endothelial cells. They promote leukocyte extravasation and are upregulated under inflammatory circumstances.[23,41] Furthermore, under these conditions, they may subsequently be shed and circulate in the bloodstream.[42,43] Therefore, the observed increase of these proteins in blister fluid combined with increased numbers of neutrophils suggests endothelial activation after id LPS. VE-cadherin is the main contributor to adherens junctions, while ESAM is part of the tight junctions between endothelial cells. The increased expression of VE-cadherin, which can also be shed under inflammatory conditions, serves as an indicator of disintegration of endothelial cell–cell interactions and a decline in endothelial integrity.[44–46] The effects of inflammation on ESAM expression and whether it is internalized or shed have not yet been elucidated in the literature. Finally, it is important to stress that these pathophysiological processes observed in our id LPS model are also observed in the pathophysiology of a wide range of vascular leakage-related diseases, including sepsis, arthritis and cancer,[47–50] which further supports the potential value of our model for future use in early-phase research with pharmaceuticals targeting vascular leakage in such diseases.

The intravenous LPS challenge is widely used as a human endotoxemia model, and as such there are data available on the systemic effects of LPS. However, there are only two studies describing outcome measures related to vascular leakage. These describe dose-dependent increases in E-selectin, VCAM-1 and ICAM-1 in a study administering 0.5–2 ng/kg LPS intravenously to healthy volunteers and VEGF increases in another clinical study administering 20 IU/kg LPS intravenously.[21,22] Our local dermal data are consistent with these observations. However, limitations of the systemic challenges are the impact on healthy volunteers, including temporary side effects, such as fever, nausea and headaches, and the fact that non-invasive imaging and sequential local interstitial fluid collection to study the direct effects of systemic LPS on the vasculature in a peripheral tissue is impossible.[51] Our id LPS model has the major advantage that it allows for controlled, high-precision localized assessment of vascular leakage over time, without the logistical and safety limitations of a systemic LPS challenge.

When comparing the imaging data with the data from blister fluid, there is a high degree of similarity in the observed trends and time course of the effects, indicating the interplay between the several aspects of vascular leakage. **Figure 5** presents the integration of these aspects based on theoretical knowledge and the findings from our study. The figure describes the vascular effects of the id LPS model and the outcomes that can be measured in the setting of clinical trials. While preclinical assays for vascular leakage, such as Evans blue-dyed protein extravasation in rodents and *in vitro* endothelial barrier models provide mechanistic insights into the mechanism of action of investigational drugs, these insights are

limited due to interspecies differences and the lack of presence of all aspects of human biology in culture systems. By collecting pharmacodynamic data in humans early in the clinical phase, an assessment of proof-of-pharmacology and proof-of-mechanism of new investigational vascular leakage-targeting drugs can be made, thereby bridging the gap between preclinical and clinical stages.

While our novel application of the id LPS model provides valuable insights into localized vascular leakage, several limitations must be acknowledged. First of all, the model reflects an acute innate immune stimulus and may not capture the complexity of chronic vascular inflammation or systemic microvascular pathology as present in chronic diseases. Secondly, while the 5 ng LPS dose, chosen based on prior knowledge on inflammation and tolerability, does provide insights into vascular leakage induced by LPS, this study did not evaluate an LPS dose–response relationship and we cannot exclude the possibility that a higher LPS dose might induce enhanced effects on vascular leakage. However, the safety and tolerability of study participants are of utmost importance and are safeguarded at

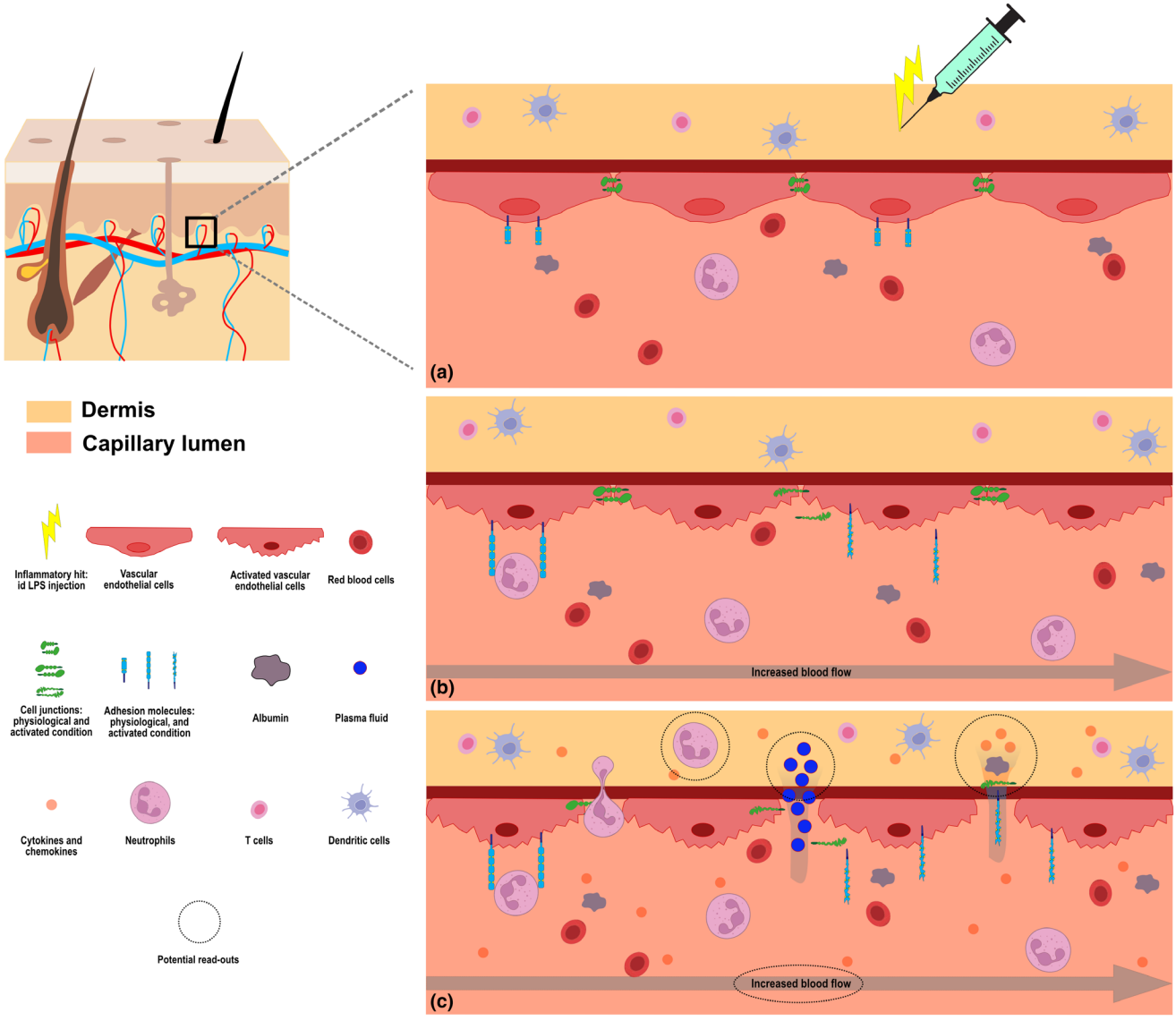


Figure 5 Schematic representation of the effects of an intradermal LPS challenge on vascular leakage and potential read-outs. This figure displays a cross section of part of the dermis, the vascular wall and part of the vascular lumen. (**a**) The inflammatory challenge is initiated by the intradermal injection of LPS into the skin of healthy volunteers. (**b**) The id LPS injection elicits an innate inflammatory response characterized by vasodilation (increased perfusion and erythema), leukocyte attraction, and endothelial activation. (**c**) Furthermore, the endothelium is activated (e.g., VCAM-1 and ICAM-1), leading to extravasation of neutrophils, and vascular integrity is altered by disintegration of tight junctions and the extravasation of fluids and proteins (e.g., albumin). Non-invasive available experimental read-outs for the described processes are imaging of vasodilation by laser speckle contrast imaging, and erythema and skin volume as measure of fluid extravasation by Antera 3D camera. Invasive experimental read-outs are analysis of local suction blister fluid by laboratory assays for albumin and total protein, Olink proteomics for endothelial markers, and flow cytometry for neutrophil extravasation. ICAM-1, intercellular adhesion molecule 1; LPS, lipopolysaccharide; VCAM-1, vascular cell adhesion molecule 1.

the 5 ng dose. Thirdly, it must be acknowledged that the variability the parameters display varies between the individual parameters, with some (perfusion and skin volume) yielding enough power to individually demonstrate treatment effects with small group sizes ($n = 6–10$) while others (erythema) do not. Despite this variability, the integration of the presented parameters and their complementary contribution to several aspects of vascular leakage aids in increasing the overall signal-to-noise ratio and the applicability of the model. Additionally, it would be beneficial to investigate the effects of a vascular leakage-targeting drug on this model. However, no such compounds have been registered yet, therefore preventing us to perform this benchmarking. For application of this model beyond early-phase clinical pharmacological trials, further studies expanding the knowledge on the effects of id LPS in a broader population should be conducted.

In conclusion, we demonstrated that the id LPS model is suitable for controlled induction of vascular leakage. A wide range of therapeutic agents targeting endothelial activation and vascular leakage, for example conventional immunosuppressants or Ang-Tie2 axis-, sphingosine-1-phosphate signalling-, and adherens junction integrity-targeting agents that have shown preventive effects on models of vascular leakage *in vitro* and *in vivo*, could be effectively evaluated using this framework.[3,52,53] Therefore, this unique model can be applied in proof-of-mechanism studies for pharmaceuticals targeting endothelial integrity and vascular barrier function.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.R., M.D., L.B., I.T., H.A., D.E., P.S., D.G., N.K., M.J., and M.M. wrote the manuscript. M.R., L.B., D.G., M.J., and M.M. designed the research. M.R., M.D., L.B., H.A., P.S., N.K., and M.J. performed the research. I.T. and D.E. analyzed the data.

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