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Impact of Hypothermic Perfusion on Immune Responses and Sterile Inflammation in a Preclinical Model of Pancreatic Transplantation

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Background. In organ transplantation, cold ischemia is associated with sterile inflammation that subsequently conditions adaptive immunity directed against the grafts during revascularization. This inflammation is responsible for venous thrombosis, which is the main postoperative complication affecting graft function. Our aim was to investigate the modulation of immune responses and endothelial function of pancreatic grafts during cold ischemia using different preservation modalities. **Methods.** According to a preclinical porcine model of controlled donation after circulatory death, pancreatic grafts were preserved under hypothermic conditions for 24 h according to 4 modalities: static cold storage, hypothermic machine perfusion, hypothermic oxygenated perfusion at 21%, and 100%. Biopsies of the head and tail of the pancreas were performed during preservation. The first step involved a broad screening of the gene expression profile (84 genes) during preservation on a limited number of grafts. In the second step, a confirmation test was performed in all 4 groups. **Results.** Vascular endothelial growth factor gene expression showed a decrease during preservation in the hypothermic oxygenated perfusion 21% and 100% groups compared with the static cold storage group. In contrast, thrombomodulin gene expression showed an increase during preservation in the hypothermic oxygenated perfusion 21% and 100% groups compared with the static cold storage and hypothermic machine perfusion groups. **Conclusions.** We demonstrated that compared with static cold storage, hypothermic oxygenated perfusion is an effective modality for modulating endothelial function by increasing thrombomodulin expression and decreasing ischemia and vascular endothelial growth factor expression.

(*Transplantation Direct* 2025;11: e1743; doi: 10.1097/TXD.0000000000001743.)

In organ transplantation, the interval between graft procurement from the donor and its revascularization in the recipient represents a critical phase. This is a period of ischemia with the cessation of the mitochondrial respiratory chain due to a lack of oxygen, the depletion of intracellular ATP stocks, and the implementation of the anaerobic pathway with the production of lactate. This cascade of events is responsible for acidosis, disruption of membrane ion pumps, cellular edema,

Received 24 July 2024. Revision received 25 September 2024.

Accepted 14 October 2024.

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Grants were received from the French National Agency (Agence de la biomedicine), the French Urological Association (Association Française d'Urologie), the Nantes University Hospital, and the Institut Georges Lopez.

B.M. received grant funding and technical support from Institut Georges Lopez and Hémarina. J.B. received grant funding and technical support from Institut Georges Lopez and Hémarina. L.B. provided expertise for Astellas and Bristol Myers Squibb. The other authors declare no conflicts of interest.

B.M. and J.B. participated in writing the article, research design, performing the research, and data analysis. S.B. and D.K. participated in performing the research and data analysis. S.L.B.-B. participated in research design. E.O. and T.P. participated in writing the article, research design, and data analysis. C.M. and J.H. participated in data analysis. D.M. and J.H. participated in performing the research. D.C., J.R., L.B., P.F., R.P., and G.B. participated in research design and data analysis.

The French Ministry of Research approved the research protocol (APAFiS No. 31507). All experiments were conducted in accordance with the ARRIVE 2.0 recommendations and the European Directive 2010/63/EU on animal experimentation.

The data that support the findings of this study are available from the corresponding author, J.B., upon reasonable request.

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001743

apoptosis, and ultimately loss of organ function. Furthermore, ischemic periods provoke innate inflammation within the grafts, which intensifies on reperfusion, accompanied by the recruitment of mediators of adaptive immunity. These events are initiated by releasing damage-associated molecular patterns and hypoxia-inducible factors (HIFs) from ischemic cells alongside the upregulation of adhesion molecules on cell surfaces, leading to the transcription of proinflammatory mediators. Endothelial damage induced by ischemia/reperfusion exacerbates vascular permeability, fostering immune cell infiltration into the organ via cytokines and chemokines that attract neutrophils and macrophages.[1]

In pancreatic transplantation, venous thrombosis of the graft remains a major postoperative complication. Historically, venous thrombosis of the graft was mainly due to technical defects of the surgical anastomosis. Today, despite the standardization of surgical techniques, a complication rate of 3%–10% of venous thrombosis of the graft is unlikely to be modifiable by technical development.[2] It is crucial to differentiate between thrombosis originating from the venous anastomosis and the portal vein and thrombosis developing within the pancreatic parenchyma, which is extensive and a consequence of ischemia/reperfusion injuries. Efforts are underway to mitigate the incidence of venous thrombosis associated with ischemia/reperfusion injury.

Data from kidney transplantation suggest that hypothermic perfusion of kidney grafts reduces the expression of inflammatory cytokines and hypoxia-inducible genes, leading to decreased long-term graft fibrosis[3] and 1-y rejection rates.[4] Additionally, hypothermic machine perfusion (HMP) may sustain shear stress and potentially activate endothelial protective genes such as the Kruppel-like factor.[5,6] Moreover, perfusion of certain molecules into the organ parenchyma during preservation (eg, polyethylene glycol) may confer protective effects on cell membranes through immunomodulatory properties.[7] Given these numerous properties, HMP could have a particular benefit in pancreatic transplantation. Some teams are working to develop this preservation modality for pancreas transplantation. However, the use of HMP in pancreatic transplantation is currently only preclinical, and consortia are aiming to determine the modalities of HMP in pancreatic transplantation.[8] To date, scientific data are lacking to evaluate HMP in clinical settings.

No study has specifically assessed the impact of HMP on the endothelial function of pancreatic grafts or immune responses. Thus, the objective of our study was to investigate the modulation of innate and adaptive immune responses, as well as endothelial function, during HMP and hypothermic oxygenated perfusion (HOPE), in comparison with static cold storage (SCS).

MATERIALS AND METHODS

Experimental Protocol

We conducted a preclinical study using a controlled donation after circulatory death porcine model (warm ischemia: 30 min; Figure 1). Sixteen pancreases were procured from male "large White" Sus scrofa pigs, with an average weight of 80 kg. After procurement, pancreatic grafts were preserved under hypothermic conditions for 24 h using 4 different modalities. Parenchymal biopsies were obtained at T0, 3 h, and 24 h to evaluate the modulation of the inflammatory response and endothelial function. The study protocol was approved by the French Ministry of Research (APAFiS No. 31507) and complied with the law on the protection of animals for scientific purposes (conditions of killing: articles R214-98 to R214-98-1). All experiments adhered to the ARRIVE 2.0 recommendations[9] and were conducted in accordance with the European Directive 2010/63/EU on animal experimentation.

Pancreas Procurement

Pancreas procurement followed procedures previously described by our team.[10,11] After premedication in a stall, the pigs were sedated under general anesthesia (using a mixture of isoflurane [2%], nitrous oxide [49%], and oxygen [49%]). A peripheral venous catheter was inserted into an ear vein for intraoperative analgesia, administering buprenorphine 0.05 mg/kg (Vétergesic, Sogeval, Laval, France) and paracetamol 25 mg/kg via intravenous injection. A xyphopubic incision was made to free the pancreas from its adherences, followed by approaching the retroperitoneal vessels (aorta and subrenal vena cava). Before placing the aortic and vena cava cannulas, a dose of unfractionated heparin (300 IU/kg) was administered intravenously. To induce in vivo warm ischemia of the pancreatic transplant, the thoracic aorta was clamped through a diaphragmatic window. Thirty minutes after aortic clamping, the abdominal organs were flushed via the aortic cannula with a fourth generation preservation solution cooled to 4 °C (osmotic agent: polyethylene glycol 35 1 g/L; IGL-1, Institut Georges Lopez, Lissieu, France). Organs were rinsed

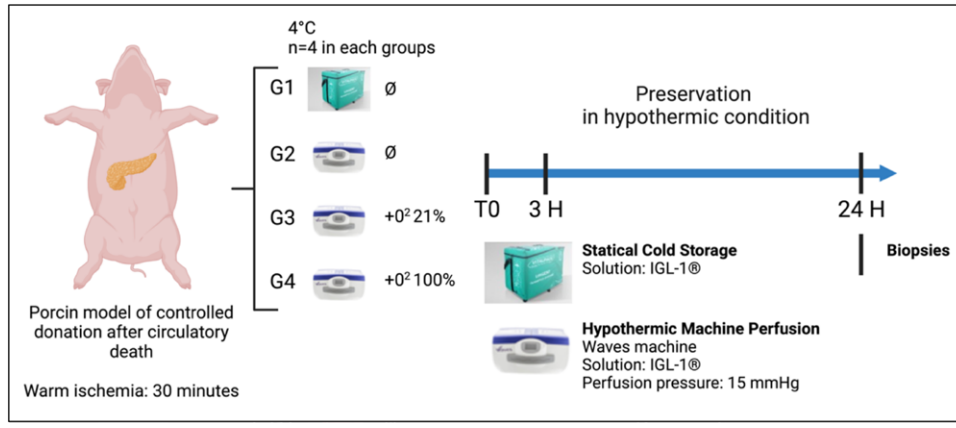

FIGURE 1. Experimental protocol.

at low pressure (20–40 cmH_2O) to minimize the risk of pancreatic edema. Crushed ice was added to the abdominal cavity to cool the organs. Once the organs were flushed and the return of the vena cava was clear, procurement was performed according to human multiorgan procurement standards, with the pancreas procured en bloc with the aorta.

Back-Table Preparation of Porcine Pancreases

The graft was prepared for perfusion, with a cannula inserted into the aorta. Leaks in the lumbar arteries, hepatic artery, splenic artery, and root of the mesentery were tied off until a clear return through the portal vein was obtained.

Preservation Modalities

Pancreatic grafts were preserved according to 4 modalities, with 4 grafts per group—group 1: SCS, group 2: HMP, group 3: HOPE 21%, and group 4: HOPE 100%. The preservation and perfusion solution used was IGL-1 (1 L; Institut Georges Lopez). For SCS, the pancreas was immersed in 1 L of IGL-1 and placed in a cooler with crushed ice. For the perfusion groups, the Waves perfusion machine (Institut Georges Lopez) was used. Perfusion was pulsed at a fixed pressure, with the flow rate adjusted by the machine to achieve the set pressure objective (15 mm Hg). Oxygenation of the perfusion solution was achieved via the oxygenation membrane included in each perfusion cassette, with an oxygenation rate of 2 L/min.

Data Collection and Analysis

Surgical biopsies (0.3 × 0.3 × 0.3 cm) of the head and tail of the pancreas were performed at T0, 3 h, and 24 h. Perfusate samples and biopsies were immediately frozen at −80 °C with dry ice pending analysis. The analysis of the expression profile of genes associated with innate and adaptive immune responses and the modulation of endothelial function was conducted in 2 stages.

The first step involved a broad screening of gene expression profiles during preservation using a limited number of samples (SCS [n = 2] and HOPE 100% [n = 2]) at 3 different time points (T0, 3 h, and 24 h) during hypothermic preservation. This evaluation was carried out by quantitative real-time polymerase chain reaction (RT-qPCR; RT-Profiler PCR Array, Qiagen, Hilden, Germany). The expression of 84 genes involved in immunity and endothelial physiology was assessed, including genes related to angiogenesis, vasoconstriction-vasodilation, inflammatory response, apoptosis, coagulation, and platelet activation (Table 1). Genes showing differential expression levels between the 2 preservation modalities (SCS versus HOPE 100%) and displaying strictly inverse evolution in these 2 groups during the entire preservation at each key point (T0 versus 3 and 24 h) were selected as genes of interest. The analysis for selecting genes of interest was qualitative, not quantitative. The expression pattern of each gene during preservation was individually evaluated, and genes were selected for the second phase only if they met all the criteria.

In the second step, a confirmation test was conducted on all 4 groups and all participants (n = 4 per group, 2 samples per participant and per biopsy) using only the identified genes of interest from step 1. For the second analysis, we included only biopsies performed at T0 and 24 h (technical reason). Gene expression at 24 h was compared with their respective levels at T0. The second analysis involved a statistical quantitative study of differential gene expression using preservation modalities. For this phase, all steps of the analysis were repeated.

Samples were homogenized with Ultra-Turrax (Ika, Staufen, Germany) in 400 µL of lysis buffer. Total pancreatic RNAs were extracted using the RNeasy MiniKit (ref No. 74106; Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Total RNAs were quantified spectrophotometrically using the NanoPhotometer (IMPLEN, Munich, Germany), and RNA purity was assessed by the A260/A280 ratio.

For each sample, reverse transcription (RT) into rDNA was performed from a volume containing 500 ng of total RNAs and supplemented with RNAase-free water (final volume 10 µL). RT was performed using the TURBO DNase (ref No. AM2238; Thermo Fisher, Waltham, MA). RNAs were mixed with 2 µL of RT Mix 1 consisting of 1 µL of 500 µg/mL RT primers (oligo(dT)12-18; Invitrogen, Fisher Scientific, Waltham, MA) and 1 µL of deoxynucleotide triphosphate (10 mM). The mixture was heated for 5 min at 65 °C and then immediately placed on ice. Next, 8 µL of Reverse Transcription

TABLE 1.

Screening of 84 genes involved in immunity and endothelial physiology

Physiopathology	Genes
Angiogenesis	*ANGPT1, CCL2 (MCP1), CCL5 (RANTES), CX3CL1, EDN1, EDNRA, ENG (EVI1), F3, FASLG (TNFSF6), FGF1, FGF2, FLT1 (VEGFR1), FN1, HIF1A, HMOX 1, IL1B, IL6, ITGA5, ITGAV, ITGB1, ITGB3, KDR (VEGFR3), KIT, MMP2, MMP9, NOS3 (eNOS), NPPB, NPR1, PGF, PLAU (uPA), PTGS2 (PGHS-2, COX2), SERPINE1 (PAI-1), SPHK1, TEK, THBS1 (TSP-1), VEGFA*
Vasoconstriction and vasodilatation	*ACE, AGT, AGTR1, ALOX5, APOE, CAV1, CX3CL1, EDN1, EDN2, EDNRA, F2R (PAR1), HMOX1, ICAM1, NOS3 (eNOS), NPPB, NPR1, PTGIS, PTGS2 (PGHS-2, COX2), SOD1*
Inflammatory response	*ACE, AGT, AGTR1, ALOX5, APOE, CCL2 (MCP1), CCL5 (RANTES), CX3CL1, CXCL2, CXCR5, EDNRA, F2R (PAR1), F3, FN1, HIF1A, HMOX1, IL1B, IL6, NPPB, PTGS2 (PGHS-2, COX2), SELE, SELP, SPHK1, TGFB1, THBS1 (TSP-1), TNF, VCAM1*
Apoptosis	*ANXA5, BAX, BCL2, BCL2L1 (BCL-X, BCLXL), CASP1, CASP3, CAV1, CCL2 (MCP-1), CCL5 (RANTES), CFLAR, CX3CL1, EDN1, EDNRA, FAS (TNFRSF6), FASLG (TNFSF6), FGF2, HIF1A, HMOX1, IL1B, IL6, IL7, OCLN, PTK2 (FAK), SPHK1, TEK, THBS1 (TSP-1), TNF, TNFSF10(TRAIL)*
Cellular adhesion	*ADAM17, AGT, BCL2, CDH5, COL18A1, CX3CL1, ENG (EVI-1), FGF1, FN1, ICAM1, IL1B, ITGA5, ITGAV, ITGB1, ITGB3, KDR(VEGFR3), PDGFRA, PECAM1, PLAU (uPA), PLG, PTK2 (FAK), SELE, SELL, SELP, SELPLG, SERPINE1 (PAI-1), TGFB1, THBS1 (TSP-1), TNF, VCAM1, VEGFA, VWF*
Coagulation	*ANXA5, CAV1, EDN1, F2R (PAR1), F2RL1, F3, FN1, KLKB1 (KLK3), MMP1, PECAM1, PLAT (tPA), PLAU (uPA), PLG, PROCR, PTK2 (FAK), SELL, SELPLG, SERPINE1 (PAI-1), TEK, TFPI, THBD, THBS1 (TSP-1), TIMP1, VWF*
Platelet activation	*APOE, CX3CL1, F2R (PAR1), FN1, IL11, IL6, ITGB3, NOS3 (eNOS), PDGFRA, PECAM1, PLG, SELP, SERPINE1 (PAI-1), SOD1, TGFB1, THBD, THBS1 (TSP-1), TIMP1, VEGFA, VWF*

Mix 2 was prepared and added to the previous mixture. RT Mix 2 was made by combining 4 µL of 5X first-strand buffer, 2 µL of 0.1 M dithiothreitol reducing agent, 1 µL of recombinant ribonuclease inhibitor (RNaseOUT, 40 U/µL) and 1 µL of Moloney murine leukemia virus reverse transcriptase. RT was then run in a thermal cycler at the following parameters: 50 min at 37 °C, followed by 15 min at 70 °C, and then terminal cooling to 4 °C.

For the first step of broad screening of gene expression, RT-qPCR was performed with RT2 Profiler PCR Arrays "Pig endothelial cell biology" (PASS-015ZC-12, ref No. 330231; Qiagen). To quantify each gene expression by qPCR, a total volume of 25 µL per well was prepared, containing 12.5 µL of PCR reagents (2X RT² SYBR Green Mastermix), 1 µL of sample (cDNA), and 11.5 µL of nuclease-free water. The qPCR was performed using the ViiA 7 Real-Time PCR System thermal cycler (Applied Biosystems, Thermo Fisher, Waltham, MA) under the following conditions: 10 min hold at 95 °C, 40 cycles consisting of a 15-s hold stage at 95 °C, followed by a 1-min PCR stage at 60 °C. The expression of each gene was normalized to the expression of housekeeping genes (*ACTB, B2M, GAPDH, HPRT1, RLP13A*) in each sample and then compared.

For the second step, to quantify each gene of interest for each sample by qPCR, a total volume of 10 µL per sample was prepared. This contained 0.50 µL of commercial probes (TaqMan Assay) 20X, 5 µL of PCR reagent (TaqMan Universal Master Mix II, No AmpErase UNG) 2X, 1 µL of sample (RT product), and 3.5 µL of nuclease-free water. The qPCR was performed using the ViiA 7 Real-Time PCR System thermal cycler (Applied Biosystems, Thermo Fisher) under the following conditions: 40 cycles consisting of a 20-s hold stage at 95 °C, followed by a 20-s PCR stage at 60 °C. All analyses were performed in duplicate.

The qPCR data (Ct: cycle threshold) were acquired using ViiATM 7 Software, and the relative amount of RNAs was determined using the 2-ΔΔCt (fold change) method.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 9.1.1). For the first step, results were presented in the form of a Volcano plot, with differential expression expressed as log(fold change) and statistical significance as -log10(*P* value). *P* values were calculated on the basis of a Student *t* test of the replicate 2^(-ΔCt) values for each gene in the control group and treatment groups. Gene expressions in SCS at 3 and 24 h were considered as the control group, and gene expressions in HOPE 100% at 3 and 24 h as the test group. Differences were considered statistically significant when a *P* value was <0.05 (-log10(*P* value) > 1.301). An exploratory analysis was also conducted showing gene expression at 3 and 24 h in the SCS group, compared with the SCS group at T0. For the second step, the differential expression of endothelial function genes (gene expression at 24 h versus T0) was determined by ANOVA test. Results were expressed as mean ± SEM. Differences were considered statistically significant when a *P* value was <0.05.

RESULTS

First Step: Broad Screening of the Gene Expression Profile

Of the 84 genes evaluated, 6 met the criteria for being considered genes of interest (Figure 2). Qualitative analysis revealed a distinct inverse evolution of their expression over time between the SCS group and the HOPE 100% group in the different samples tested. Five genes were underexpressed in the HOPE 100% preservation group compared with the SCS group. These genes included vascular endothelial growth factor A (*VEGFA*), which is involved in angiogenesis and capillary permeability in response to hypoxia; coagulation factor II receptor-like 1 (*F2RL1*), involved in the coagulation process; Caspase 3 gene, involved in cellular apoptosis; Factor III or thromboplastin (F3), involved in the extrinsic pathway of coagulation activation; and tumor necrosis factor ligand superfamily member 10 (*TNFSF10*), which is involved in cellular apoptosis. One gene, THBD, encoding thrombomodulin with anticoagulant activity and expressed on the endothelial surface, was overexpressed during HOPE 100% preservation compared with SCS. Interestingly, differential gene expression analysis during SCS at 3 and 24 h compared with T0 showed overexpression of a majority of genes involved in the immune response and cell adhesion during SCS (Figure 3).

Second Step: Confirmation Test

The second step evaluated the expression of these 6 genes across all groups and pancreatic samples. Among the 6 genes, the results of the previous step were confirmed for 2 genes: *VEGF* and *THBD*. The results of this second analysis on *F2RL1*, *Caspase 3* gene, *F3*, and *TNFSF10* demonstrated no impact of the preservation mode on the expression level of these 4 genes. Specifically, *VEGF* showed a decrease in expression during preservation in the HOPE 21% and HOPE 100% groups compared with the SCS group (Figure 4A). In contrast, *THBD* showed an increase in expression during preservation in the HOPE 21% and HOPE 100% groups compared with the SCS and HMP groups (Figure 4B).

DISCUSSION

Our study is the first to specifically focus on the modulation of endothelial function and the immune response during the preservation of pancreatic grafts by SCS, HMP, and HOPE. Our study confirms the capacity of hypothermic perfusion preservation modalities to impact gene expression during hypothermic preservation.

The first notable finding of our study is the demonstration that pancreases remain metabolically active during hypothermic preservation. We observed an upregulation of most genes involved in the immune response and platelet adhesion. Hypothermic preservation, therefore, does not stop sterile inflammation processes or prepare for directed immunity. Consequently, this period of hypothermic preservation appears to be particularly suitable for modulating immunity. Contrary to common belief, the normothermic reperfusion phase is not the only phase of interest, and the reduction of sterile inflammation begins during hypothermic preservation. We demonstrated that HOPE at 21% or 100% decreased the expression of the gene coding for *VEGF*. *VEGF* is a growth factor secreted in response to local ischemia and is primarily regulated by the *HIF* system.[12] In the presence of oxygen, the HIF protein is hydroxylated and continuously degraded by the proteasome. Under hypoxic conditions, HIF is stabilized and induces the transcription of genes, particularly *VEGF*. The decrease in *VEGF* expression during preservation is an important signal of decreased parenchymal ischemia. Other

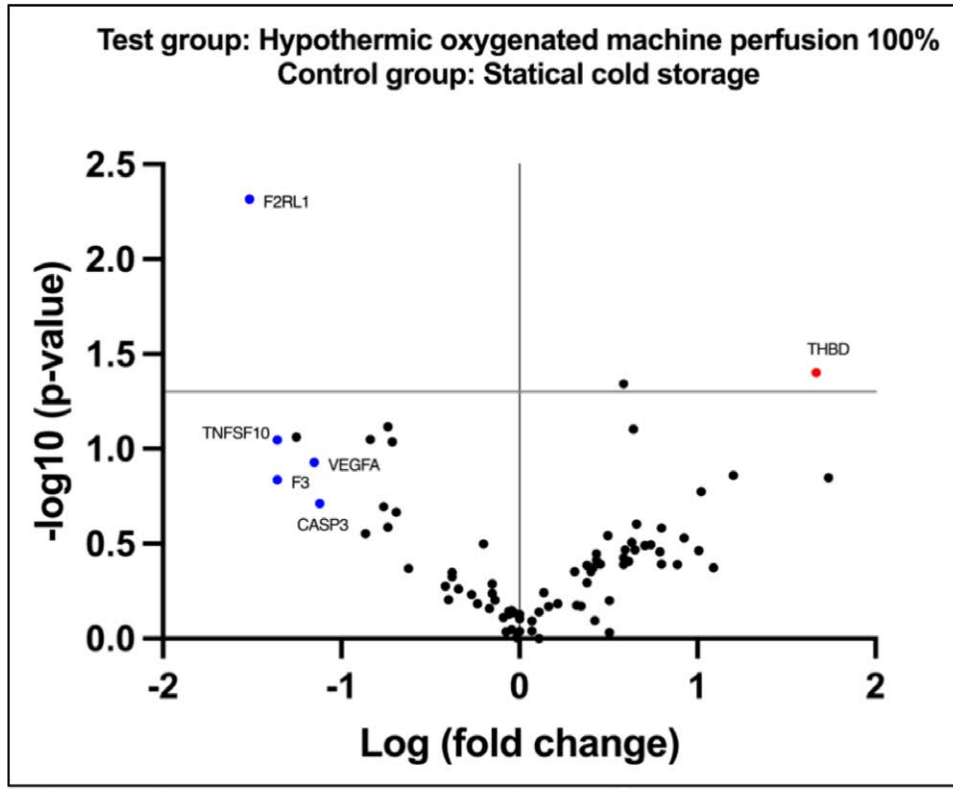

FIGURE 2. Assessment of differential expression of immune response genes and endothelial function. Volcano plot. Test group: hypothermic oxygenated machine perfusion 100% at 3 and 24 h. Control group: static cold storage at 3 and 24 h. *CASP3, Caspase 3*; *F2RL1, coagulation factor II receptor-like 1*; *F3, Factor 3* or *thromboplastin*; *THBD, thrombomodulin*; *TNFSF10, tumor necrosis factor ligand superfamily member 10*; *VEGFA, vascular endothelial growth factor A*.

teams have demonstrated this association between perfusion and decreased *VEGF* expression in different transplantation models. Hernández-Jiménez et al,[13] in a porcine model of lung graft preservation, showed that hypothermic perfusion of transplants, particularly in the case of prolonged ischemia, was associated with a decrease in *VEGF* levels versus SCS. Wszola et al,[14] in a human model of kidney transplantation, demonstrated that kidney grafts preserved on HMP had decreased *HIF* levels compared with SCS for both standard and extended criteria donors.

In our study, HOPE was also associated with increased expression of the gene encoding *THBD*. *THBD* plays a major role in endothelial physiology, with an anticoagulant effect and an anti-inflammatory effect.[15] The expression of *THBD*, as well as endothelial nitric oxide synthase, is regulated by Kruppel-like factor 2.[16] Van thienen et al,[5] in an in vitro model, demonstrated that the expression of Kruppel-like factor 2 by the endothelium was mainly related to the presence of shear stress. Takada et al,[17] also in an in vitro model, demonstrated that endothelial cells exposed to flow showed upregulation of *THBD* levels.[17] Gallinat et al,[18] in a porcine model of kidney transplantation, demonstrated that kidney grafts preserved on HMP showed an increased expression of Kruppel-like factor 2 from the first hour of perfusion. Finally, Burlage et al,[19] in a human preclinical model of liver transplantation with revascularization by normothermic perfusion, found that grafts preserved with HMP presented at reperfusion an overexpression of *THBD*. These data are particularly interesting because they could suggest that HOPE could influence the rate of venous thrombosis of the graft, which to date is not compressible.

Finally, there is a need to prepare pancreatic grafts for transplantation by developing techniques and methods to decrease sterile inflammation and prepare the vascular endothelium for reperfusion. The evaluation of new therapeutics is necessary to decrease the rate of venous thrombosis related to ischemia/reperfusion injury. Some approaches seem promising. The perfusion of certain molecules, such as oxygen carrier (M101), could decrease oxidative stress, necrosis, and cellular stress pathways.[20,21] The choice of perfusion solution associated with polyethylene glycol 35 could reduce the inflammatory response.[22] Finally, the perfusion of anti-inflammatory molecules such as heme oxygenase 1 would reduce the level of certain proinflammatory cytokines and decrease the pancreaticity of the transplant.[23] The demonstration of a specific effect of HOPE would be an important element in favor of its implementation.

Our study had several limitations: first, we used a donation after circulatory death donor model with prolonged preservation time. Although this model represents a minority of pancreatic transplantations, this extreme preservation model facilitates the study of gene expression modulation based on preservation modalities. Nonetheless, we believe this model is applicable to donation after brain death donors with shortened preservation durations. Moreover, we included a limited number of participants per group to evaluate the different perfusion modalities. Additionally, due to technical reasons, we had to focus on 2 key time points for the confirmation analyses. Because the confirmation analyses involved larger sample sizes, we had to limit the study to T0 and 24 h, resulting in a loss of information regarding short- and long-term

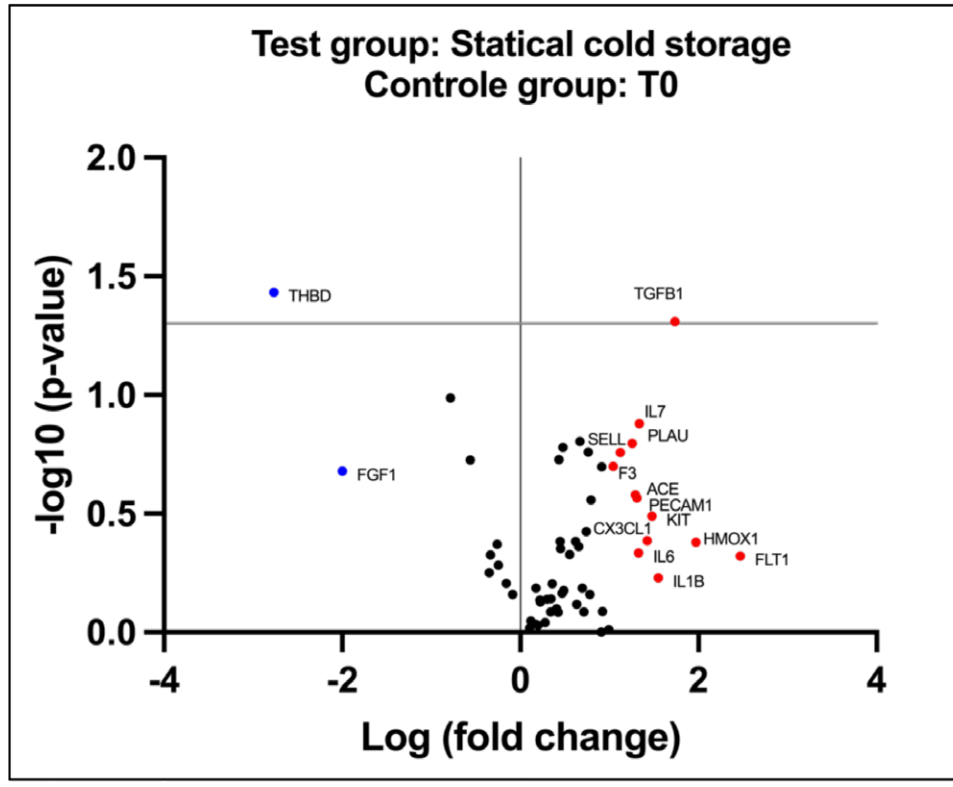

FIGURE 3. Assessment of differential expression of immune response genes and endothelial function. Volcano plot. Test group: static cold storage at 3 and 24 h. Control group: static cold storage at T0. *ACE, angiotensin I converting enzyme*; *CXCL3, chemokine ligand 3*; *heme oxygenase 1*; *F3, Factor 3 or thromboplastin*; *FGF1, fibroblast growth factor 1*; *FLT1, Fms-related receptor tyrosine kinase 1*; *IL-1B, interleukin 1 beta*; *KIT, tyrosine-protein kinase Kit*; *PECAM, platelet endothelial cell adhesion molecule*; *PLAU, plasminogen activator, urokinase*; *SELL, selectin L*; *TGFB1, transforming growth factor beta 1*; *THBD, thrombomodulin*.

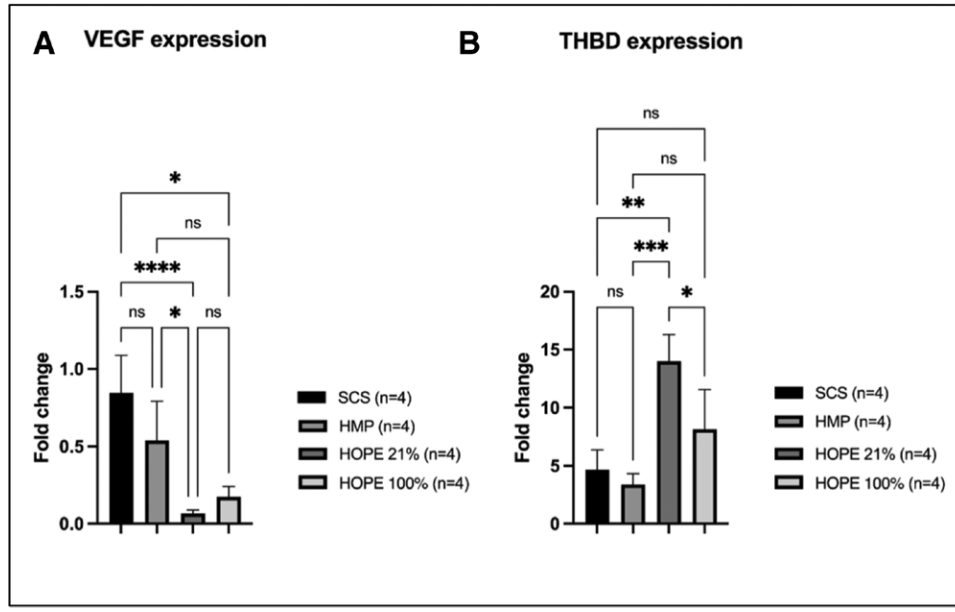

FIGURE 4. Differential expression of *VEGF* and *THBD* genes according to preservation modalities. (Fold change expression between T0 and 24 h). A, Differential expression of *VEGF* gene. B, Differential expression of *THBD* gene. Values expressed as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. HMP, hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; SCS, static cold storage; THBD, thrombomodulin; VEGF, vascular endothelial growth factor.

changes. Finally, we conducted a study based exclusively on the gene expression (RNA) of different genes involved in the immune response and endothelial function. To date, we have not performed phenotypic studies that would allow us to confirm these results and the transition from genetics to phenotype. Studying the rate of complications and thrombosis

based on the preservation modality will be the major criterion for studying pancreatic perfusion modalities in the future.

In conclusion, HMP and HOPE, compared with SCS, are effective preservation modalities to modulate the endothelial function of pancreatic grafts, notably by increasing *THBD* expression and decreasing ischemia and *VEGF* expression.

ACKNOWLEDGMENTS

The authors thank the French National Agency (Agence de la biomedicine) for grant funding, the French Urological Association (Association Française d'Urologie) for grant funding, the Nantes University Hospital for grant funding, the biochemical analysis laboratory of the Nantes University Hospital for logistical support, and the Institut Georges Lopez for grant funding and technical support.

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