The Journal of Physiology

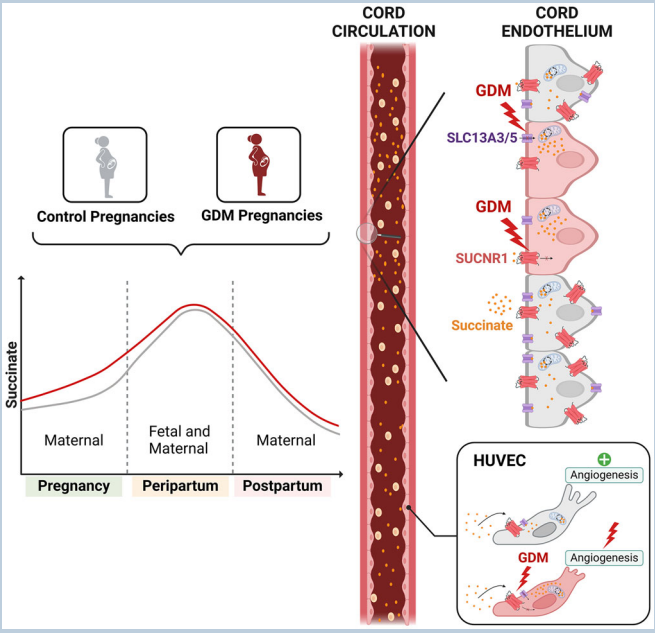
Impaired angiogenesis in gestational diabetes is linked to succinate/SUCNR1 axis dysregulation in late gestation

Sergiy Klid[1,2], Francisco Algaba-Chueca[2,3], Elsa Maymó-Masip[2,3], Mónica Ballesteros[1,2,4], Montse Inglés[1,2,4], Albert Guarque[1,2,4], Nerea Vilanova-Ricart[1,3], Ariadna Prats[3], Azra Kulovic-Sissawo[5], Elisa Weiss[5], Ursula Hiden[5], Joan Vendrell[1,2,3], Sonia Fernández-Veledo[1,2,3] and Ana Megía[1,2,3]

[1]*Department of Medicine and Surgery, Rovira i Virgili University, Tarragona, Spain*
[2]*CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain*
[3]*Department of Endocrinology and Nutrition, Research Unit, University Hospital of Tarragona Joan XXIII-Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain*
[4]*Department of Obstetrics and Gynecology, University Hospital of Tarragona Joan XXIII. Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain*
[5]*Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria*

Handling Editors: Kim Barrett & Luis Sobrevia

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP288010#support-information-section).



Abstract figure legend Gestational Diabetes Mellitus (GDM) impacts on succinate metabolism and angiogenesis in the umbilical cord endothelium. (**Left Panel**) Succinate levels increase in both maternal and fetal circulation at delivery, with higher concentrations observed in GDM pregnancies compared to controls during late gestation. (**Right Panel**) In GDM pregnancies, attenuated uptake succinate transporters (e.g., SLC13A3/5) might compensate excessive intracellular succinate accumulation, while SUCNR1 expression is downregulated in the umbilical cord endothelium. This downregulation disrupts the angiogenic gene profile normally regulated by SUCNR1. (**Lower Inset**) Healthy human umbilical vein endothelial cells (HUVECs) respond to SUCNR1 activation by promoting sprouting and tube formation, enhancing angiogenesis. However, in GDM pregnancies, excessive succinate and reduced SUCNR1 functionality impair these processes, weakening the angiogenic response.

DOI: 10.1113/JP288010

Abstract Recent research has highlighted the significance of succinate and its receptor in gestational diabetes (GDM) pathogenesis. However, a clear interconnection between placenta metabolism, succinate levels, SUCNR1 signalling and pregnancy pathologies remains elusive. Here, we set out to investigate the potential role of succinate on labour and placental mechanisms by combining clinical and functional experimental data at the same time as exploring the specific SUCNR1-mediated effects of succinate on placenta vascularization, addressing its specific agonist actions. According to our data, succinate levels vary throughout pregnancy and postpartum, with a natural increase during the peripartum period. We also show that SUCNR1 activation in the umbilical cord endothelium promotes angiogenesis under normal conditions. However, in GDM, excessive succinate and impaired SUCNR1 function may weaken this angiogenic response. In conclusion, the present study underlines succinate as an emerging signalling molecule in the placenta, regulating labour and placental processes. The reduced sensitivity of the succinate/SUCNR1 pathway in the GDM environment may serve as a protective physiological mechanism or could have a pathogenic effect.

(Received 31 October 2024; accepted after revision 5 March 2025; first published online 31 March 2025)
Corresponding authors S. Fernández-Veledo and A. Megía: Research Unit, University Hospital of Tarragona Joan XXIII, c/ Dr. Mallafré Guasch, 4, 43007 Tarragona. Spain. Email: sonia.fernandez@iispv.cat, amegia.hj23.ics@gencat.cat

Key points

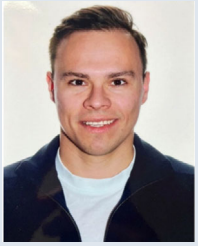
- Succinate levels increase at delivery in maternal and fetal circulation.
- Gestational diabetes (GDM) induces succinate accumulation and SUCNR1 downregulation in umbilical cords.
- GDM compromises angiogenic gene profile modulation by SUCNR1 in umbilical cord endothelium.
- SUCNR1 activation stimulates sprouting and tube-forming capacity of human umbilical vein endothelial cells from healthy, but not GDM pregnancies.

Introduction

Pregnancy is a dynamic and complex physiological process that involves numerous changes in the mother's body to support the growth and development of the fetus (Challis et al., 2009; Kim et al., 2015). The placenta, acting as a complex interface between the mother and fetus, undergoes significant changes during pregnancy, including developing an extensive capillary network for effective maternal–fetal exchange. Although regulating placental angiogenesis through factors responding to changes in oxygen tension and mechanical stimuli has been well explored (Burton et al., 2009; Demir et al., 2004), the potential contribution of placental metabolites to angiogenesis has been largely overlooked.

Gestational diabetes mellitus (GDM), defined as diabetes diagnosed in the second or third trimester of pregnancy that was not overt diabetes before gestation (Elsayed et al., 2023), is one of the most common pregnancy complications. GDM increases the risk of perinatal complications such as pre-eclampsia, fetal macrosomia, preterm delivery, Caesarean section, neonatal hypoglycaemia and hyperbilirubinemia, and poses long-term adverse outcomes for both mother and child (Shou et al., 2019). In pregnancies complicated by GDM, there is evidence suggesting enhanced placental

Sergiy Klid is completing a PhD in Biomedicine at Rovira i Virgili University, Tarragona, Spain. Sergiy's research focuses on understanding the molecular mechanisms underlying pregnancy complications, with a particular emphasis on gestational diabetes mellitus (GDM). His work includes exploring the regulation of angiogenesis in placenta-derived stem cells and its modulation by GDM, investigating the dysregulation of placental lipid metabolism through the ANGPTL3-4-8 axis, as well as examining the implications of disrupted succinate metabolism in GDM for pregnancy outcomes. Sergiy's contributions aim to advance knowledge in placental biology and improve maternal and fetal health.



vascularization, attributed to factors such as fetal hypoxia resulting from increased oxygen demands as a result of hyperglycaemia and hyperinsulinemia (Cvitic et al., 2014; Huynh et al., 2015). The exact molecular mechanisms linking GDM to altered placental angiogenesis remain unclear. Metabolic factors, including placental/fetal metabolites (Husted et al., 2017), may influence angiogenesis by acting as ligands for specific plasma membrane receptors, functioning similarly to hormones and neurotransmitters (Draoui et al., 2017; Du et al., 2021; Jiménez-Valerio & Casanovas, 2017; Liu et al., 2025; Murray & Wilson, 2001; Potente & Carmeliet, 2017).

Succinate is a pleiotropic metabolite that functions dually as both an energetic substrate in the tricarboxylic acid cycle and a signalling molecule that modulates various cellular processes by binding its cognate receptor SUCNR1, a G-protein coupled receptor with higher expression levels than previously anticipated (He et al., 2004). Elevated circulating succinate levels have been described in several inflammation-related pathologies, including obesity and type 2 diabetes, in both human subjects (Astiarraga et al., 2020; Ceperuelo-Mallafre et al., 2019; Serena et al., 2018) and animal models (Fernández-Veledo & Vendrell, 2019; Keiran et al., 2019; Villanueva-Carmona et al., 2023). However, succinate concentrations also transiently increase under certain physiological conditions, such as endurance exercise (Hochachka & Dressendorfer, 1976; Reddy et al., 2020) and the fasted-to-a-fed transition (Astiarraga et al., 2020) in humans. In murine models, cold exposure has been shown to increase succinate concentrations in response to muscle shivering, which subsequently activates thermogenesis (Mills et al., 2018). The succinate/SUCNR1 signalling pathway has been explored in various physiological and pathological contexts in tissue and cell culture models. As an extracellular signalling metabolite, succinate is multifaceted in regulating diverse biological processes. These include the fine-tuning of inflammatory responses (Fernández-Veledo et al., 2021; Huang et al., 2024; Keiran et al., 2019; Krzak et al., 2021; Trauelsen et al., 2021; Winther et al., 2021) and modulation of the circadian clock and leptin expression in adipocytes (Villanueva-Carmona et al., 2023). Moreover, succinate acts as a protective mechanism for damaged hepatocytes in metabolic dysfunction-associated steatosis liver disease (Marsal-Beltran et al., 2023), regulates insulin secretion in pancreatic β cells (Sabadell-Basallote et al., 2024) and contributes to hepatic stellate cell activation (Correa et al., 2007; Li et al., 2016). Additionally, succinate has been implicated in the regulation of the renin–angiotensin system (Peti-Peterdi, 2010; Toma et al., 2008; Vargas et al., 2009), influences haematopoiesis within the bone marrow (Hakak et al., 2009) and inhibits lipolysis in the adipose tissue (An et al., 2021; McCreath et al., 2015). It also plays a role in maintaining ventricular cardiomyocytes (Aguiar et al., 2010) and contributes to skeletal muscle remodelling (Wang et al., 2019). Therefore, although chronic elevations in succinate are closely linked to the progression of metabolic disorders (Fernández-Veledo et al., 2024), the succinate/SUCNR1 axis also orchestrates critical physiological responses, particularly those related to glucose homeostasis (Marsal-Beltran et al., 2023; Sabadell-Basallote et al., 2024; Villanueva-Carmona et al., 2023).

SUCNR1 has been identified as a potential regulator of inflammation-induced pro-labour events in the myometrium (Lim & Lappas, 2020). However, whether circulating succinate undergoes dynamic changes during pregnancy remains unknown. In the context of angiogenesis, succinate triggers this process through SUCNR1 not only during normal retinal development, but also in proliferative ischaemic retinopathy (Sapieha et al., 2008). Additionally, the significance of succinate/SUCNR1 signalling has been elucidated in tumour angiogenesis (Mu et al., 2017). Furthermore, the link between succinate and synovial angiogenesis has been highlighted in rheumatoid arthritis (Li et al., 2018). Recent data confirm the expression of SUCNR1 in placental endothelial cells, providing evidence that succinate, via its receptor, triggers an angiogenic response in these cells (Atallah et al., 2021).

The present study investigates the role of succinate in labour and placental mechanisms, focusing on SUCNR1-mediated effects on placental vascularization in pregnancies complicated by GDM. We combine analyses of succinate levels in maternal and fetal circulation during late gestation, peripartum and postpartum periods with *ex vivo* tissue explants and primary human umbilical vein endothelial cell (HUVEC) cultures. Functional assays and gene expression profiling assess the impact of succinate on angiogenic pathways, uncovering the molecular basis of SUCNR1 axis dysregulation in GDM and its implications for placental function and maternal–fetal outcomes.

Methods

Ethical approval

The present study was performed at the Hospital Universitari de Tarragona Joan XXIII (HUJ23) following the tenets of the latest version of the *Declaration of Helsinki*. The study protocol was approved by the Institut d'Investigació Sanitaria Pere Virgili Research Ethics Board (Ref: 133/2018) and all participants provided written informed consent before inclusion.

Study subjects

The inclusion criteria were women aged 18–45 years, singleton pregnancy, absence of infections and free of

chronic diseases. Exclusion criteria for all subjects were pre-existing type 1 or 2 diabetes, inflammatory or chronic diseases, or use of drugs known to affect carbohydrate metabolism and major neonatal congenital abnormalities.

One hundred pregnant women ($n = 47$ with GDM and $n = 53$ with normal glucose tolerance, acting as controls) and their offspring were included in the study. Blood samples were obtained from maternal ($n = 72$) in the peripartum period and cord blood ($n = 100$) after delivery to determine succinate. In 14 controls and 14 GDM mothers, succinate levels were determined in a maternal blood sample collected in the third trimester of gestation and at 3 months postpartum, whereas placenta and umbilical cord were collected immediately after delivery from mother-offspring pairs of 33 women with GDM and 39 controls.

All mothers were screened for GDM at 24–28 weeks of pregnancy using a two-step approach (Grupo Español de Diabetes y Embarazo (GEDE) & Grupo Español de Diabetes y Embarazo, 2015). Subjects with a 1 h and 50 g glucose challenge test $\geq$140 mg dL^{-1} (screening test) underwent a 3 h and 100 g oral glucose tolerance test, and those with two or more values above the threshold proposed by the National Diabetes Data Group (Diabetes & Group, 1979) were considered to have GDM. Controls had a 1 h and 50 g glucose challenge test $<$140 mg dL^{-1} or all oral glucose tolerance test values below the threshold. Women with GDM were placed on an individualized diet with 40% carbohydrates and instructed to self-monitor blood glucose six times a day (fasting and 1 h post-prandial). Insulin therapy was recommended when fasting glucose was 95 mg dL^{-1} or higher and/or 1 h post-prandial values were above 140 mg dL^{-1}.

The timing of delivery was based primarily on obstetric indications. Gestational age was confirmed by a routine ultrasonographic examination before week 20 of gestation.

Maternal pre-pregnancy weight and height were obtained at the first prenatal visit. Pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) were calculated according to: Pre-pregnancy BMI = pre-pregnancy weight (kg)/(height (m))^2 and GWG = final weight – pre-pregnancy weight. According to BMI, women were classified as normal weight (BMI $<$ 25), overweight (25–29.9) and women with obesity (BMI $\geq$ 30).

Birth weight and body length were obtained and noted after delivery, following fetal sex, type of delivery and gestation age.

Sample collection and processing

Maternal blood was collected at the beginning of the third trimester, at delivery and during postpartum. Samples were drawn in the morning after an 8 h fast, except in cases of vaginal delivery, where maternal blood was collected immediately upon admission. Umbilical cord blood was obtained immediately after delivery in all cases. Serum and plasma were stored at −80°C until analysis. Full-term placentas (37–39 weeks of gestation) were collected after delivery and processed without delay under sterile conditions. Tissue sections from the umbilical cord, the maternal and fetal sides of the placentas, were collected, washed and frozen at −80°C in liquid N_2. For explant secretion data, 1 g of tissue was sliced, washed with phosphate-buffered saline (PBS) containing a 1% antibiotic/antimycotic solution, and incubated for 24 h. Afterward, both the conditioned medium and tissue were collected and stored. To minimize the variability associated with vaginal delivery, only elective placentas obtained after Caesarean section were considered for endothelial cell isolations.

Isolation and culture of human umbilical vein endothelial cells

To isolate human umbilical vein endothelial cells (HUVECs), 25 cm^2 flasks were coated with gelatine (1%; Sigma-Aldrich, St Louis, MO, USA) and left for 30 min at 37°C. Sections of 20–30 cm of umbilical cord were collected and cut with a scalpel on both ends to clearly identify the vein and two arteries. A Vacutainer® cannula (Becton Dickinson, Franklin Lakes, NJ, USA) was inserted at one extremity of the vein and clamped with a surgical clamp and the cord vein was subjected to a first wash with PBS (1X; Sigma-Aldrich) with 1% antibiotic/antimycotic solution (Gibco, Carlsbad, CA, USA) to remove all red blood cells. The washing process was followed by a 10 min digestion in the water bath with collagenase A from *Clostridium histolyticum* (0.2% in PBS; Roche, Basel, Switzerland), having clamped the cord at the other extremity. Afterward, under the hood, the cord was gently squeezed to facilitate cell detachment and cells were collected in a tube containing a complete EGM-2 growth medium (Lonza, Basel, Switzerland) by washing the vein with 1X PBS. The growth medium contained D-glucose (dextrose) (CAS number 50-99-7) at exactly 1.0 g L^{-1} (5.55 mM). After centrifugation at 750 ***g*** for 10 min, the pellet was resuspended in the mentioned medium. Primary cultures of HUVECs at passage 0 were grown to 80%–90% confluence at 37°C, 5% CO_2, and 5% O_2, and non-adherent cells were removed by changing the culture medium 1 day after seeding. After 6–8 days, HUVECs were recovered using TrypLE^{TM} Select Enzyme (1X; Gibco) and amplificated in 75 cm^2 flasks coated with 1% gelatin, changing the medium every 2 days. Cells were treated with the SUCNR1 agonist cis-Epoxysuccinic acid (cESA) (Tokyo Chemical Industry, Tokyo, Japan) (Geubelle et al., 2017) and the SUCNR1 antagonist

NF-56-EJ40 (MedChemExpress, Monmouth Junction, NJ, USA) (Haffke et al., 2019). Independent experiments were conducted from passages 3–6.

Gene expression analysis

Total tissue was processed in liquid N_2, collected and then RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA, USA). HUVEC RNA content was extracted using Trizol® Reagent (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed using an optical density ratio of 260/280 nm. Two micrograms of total RNA were transcribed into cDNA with random primers using a dNTP Mix (100 mM), MultiScribe Reverse Transcriptase (50 U μL^{-1}) and RNase Inhibitors with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Gene expression was evaluated by quantitative real-time PCR on a 7900HT Fast Real-Time PCR System using TaqMan Gene Expression Assays (Applied Biosystems): *ANGPTL4* (Hs01101123_g1), *PTGS2* (Hs00153133_m1), *SLC13A3* (Hs00955744_m1), *SLC13A5* (Hs01586183_m1), *SLC16A1* (Hs01560299_m1), *TNF* (Hs00174128_m1) and *VEGFA* (Hs00900055_m1). Gene expression values were calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$) and normalized to the expression of the housekeeping gene *18S* (Hs03928985_g1).

Matrigel tube formation assay

Analysis of capillary formation was performed using an ECM gel from Engelbreth-Holm-Swarm mouse sarcoma (Sigma-Aldrich). In total, 50 µL of gel matrix solution was applied to each well of a 96-well plate and incubated for 30 min at 37°C. HUVECs (1.2×10^4) were suspended in 150 µL of EGM-2 (Lonza) growth media, plated onto the gel matrix in triplicate and incubated at 37°C. After 5 h of incubation, at least five fields were randomly photographed using an Evos XL Core Imaging System (Invitrogen). Each sample's number of extremities, nodes, junctions and meshes was measured using an Angiogenesis Analyzer for ImageJ (National Institutes of Health, Bethesda, MD, USA), as described previously (Carpentier et al., 2020).

Spheroid sprouting assay

In total, 5×10^6 cells was resuspended in 0.3% methylcellulose (Sigma-Aldrich) working solution, diluted in growth media without serum and seeded in hanging drops. Emerging spheroids were then incubated overnight at 21% O_2 at 37°C, followed by their harvesting using 1 × Hanks' Balanced Salt Solution with 10% fetal bovine serum (Gibco). The suspension was then centrifuged without brake to avoid spheroid sticking. The remaining pellet was overlayed with 1.2% methylcellulose stock solution containing 40% fetal bovine serum and gently mixed with $NaHCO_3$ (15.6 mg mL^{-1}), type 1 collagen (4 mg mL^{-1}) and NaOH (1 M), working constantly on ice. The collagen-spheroid solution was pipetted on a 24-well plate and incubated at 37°C with 21% O_2 for 2 h to allow collagen to polymerize. After that, stimulation media containing diluted compounds for treatment and bVT cocktail (25 µg mL^{-1} vascular endothelial growth factor + 10 ng mL^{-1} b-fibroblast growth factor + 10 ng mL^{-1} tumour necrosis factor α) as a positive control was added and then spheroids were stimulated for a maximum of 16 h. Spheroids were finally fixed with 4% formaldehyde and imaged with an Evos XL Core Imaging System (Invitrogen). The number of sprouts and sprout length were measured using ImageJ (National Institutes of Health).

Laboratory measurements

Third-trimester glucose, cholesterol and triglyceride levels were determined by standard enzymatic methods on the ADVIA 1800 and 2400 autoanalyzer platforms (Siemens AG, Munich, Germany).

Succinate determination

Circulating plasma succinate levels were measured using the EnzyChrom Succinate Assay Kit (BioAssay Systems, Hayward, CA, USA), as described previously (Serena et al., 2018). The assay sensitivity was 12 µM and the intra- and interassay coefficients of variance were <3.5% and 6.9%, respectively.

Statistical analysis

$P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA) or Prism, version 8.0 (GraphPad Software Inc., San Diego, CA, USA). Graphical representations were generated with Prism, version 8. Quantitative data are presented as percentages for categorical variables, mean ± SD for normally distributed continuous variables and median (interquartile range, IQR) for non-normally distributed variables. Data in graphs are shown as the mean ± SD. The normality of the data was tested with the Kolmogorov–Smirnov test. For comparisons of proportions, differences between groups were analysed using the chi-squared test, whereas for comparisons of normally and non-normally distributed quantitative variables, we applied Student's unpaired *t* test or the Mann–Whitney *U* test. A paired *t* test was used to assess differences between two repeated measures.

Table 1. Clinical and metabolic characteristics of the entire cohort

Maternal characteristics	Whole group (n = 100)	Control (n = 53)	GDM (n = 47)	P value
Age (years)	33.4 ± 5.2	33.1 ± 5.5	33.7 ± 4.9	0.556
BMI (kg m^{-2})	26.2 ± 5.4	25.1 ± 5.5	27.3 ± 5.2	0.047
Normal weight n (%)	49 (49)	32 (60.3)	17 (36.2)	0.052
Overweight n (%)	28 (28)	11 (20.8)	17 (36.2)	
Obese n (%)	23 (23)	10 (18.9)	13 (37.6)	
Gestational weight gain (kg)	9.8 ± 5.7	11.2 ± 6.3	8.3 ± 4.5	0.011
Nulliparous, n (%)	49 (49)	26 (49.1)	23 (48.9)	0.990
Glucose (mmol L^{-1})	4.63 ± 0.78	4.45 ± 0.78	4.85 ± 0.74	0.011
Birth characteristics	**Whole group**	**Control**	**GDM**	**P value**
Birth weight (g)	3374 ± 412	3307 ± 386	3449 ± 431	0.085
Gestational age (weeks)	39 (38–40)	39 (38–40)	39 (39–40)	0.572
Caesarean, n (%)	28 (28)	14 (26.4)	14 (29.8)	0.708
Male sex, n (%)	49 (49)	26 (49.1)	23 (48.9)	0.990

Data expressed as the mean ± SD for normal distributed quantitative variables and median (interquartile range) for non-parametric variables. Qualitative variables are expressed as n (%). As required, differences between quantitative variables were assessed using the Student's t test or the Mann–Whitney U test. The chi-squared test assessed differences between qualitative variables. Abbreviation: BMI, body mass index.

As required, one-way ANOVA plus Tukey's multiple comparisons test was used to analyse the differences between more than two groups. Two-way ANOVA was used to assess how succinate concentrations are affected by two factors.

Results

Succinate levels increase at delivery in maternal and fetal circulation

Clinical and analytical data of the total study population are shown in Table 1. Women with GDM had higher BMI, gained less weight during pregnancy, and exhibited higher blood glucose and lower cholesterol concentrations (all $P < 0.05$).

To explore the potential implications of succinate in pregnancy, we measured circulating succinate levels in maternal blood at the beginning of the third trimester, during the peripartum period, 3 months postpartum and in umbilical cord blood immediately after delivery (Fig. 1*A*). Additionally, we measured succinate concentrations at the peripartum period and in cord blood after delivery in 72 mother–child pairs. Remarkably, maternal circulating succinate levels increase dramatically at delivery, with similar concentrations observed in umbilical cord blood. Three months postpartum, maternal succinate levels returned to values similar to those at the beginning of the third trimester ($P < 0.001$). This trend was also observed in pregnancies complicated by GDM ($P < 0.001$).

When comparing succinate concentrations according to GDM, a significant difference was observed only in the third trimester, where women with GDM had significantly higher succinate levels than matched controls. No other differences were detected between the groups at any other time points. The results remained unchanged after adjusting for pregestational BMI (data not shown).

Considering the significant increase in maternal succinate levels during the peripartum period, we hypothesized that labour triggers these succinate fluctuations. We first separated the cohort based on the mode of delivery (Fig. 1*B*). In the control group, 14 women underwent Caesarean section (11 elective) and 39 underwent a vaginal delivery. In the GDM group, 14 women underwent Caesarean section (seven elective), and 33 underwent a vaginal delivery. Notably, vaginal delivery was associated with significantly higher maternal and cord blood succinate levels compared to elective Caesarean section. When analysed by delivery type, no significant differences in succinate levels were observed in maternal or cord blood between the control and GDM groups.

To further confirm the association between succinate levels and labour, women were classified based on their labour status (in labour *vs.* not in labour). Our findings indicate that succinate levels in both maternal and cord blood were significantly higher in women who have entered labour, whether through vaginal delivery or secondary Caesarean, compared to those who underwent elective Caesarean sections without labour onset (Fig. 1*C* and *D*). Only cord blood succinate levels were higher in the spontaneous labour group than in the induced labour

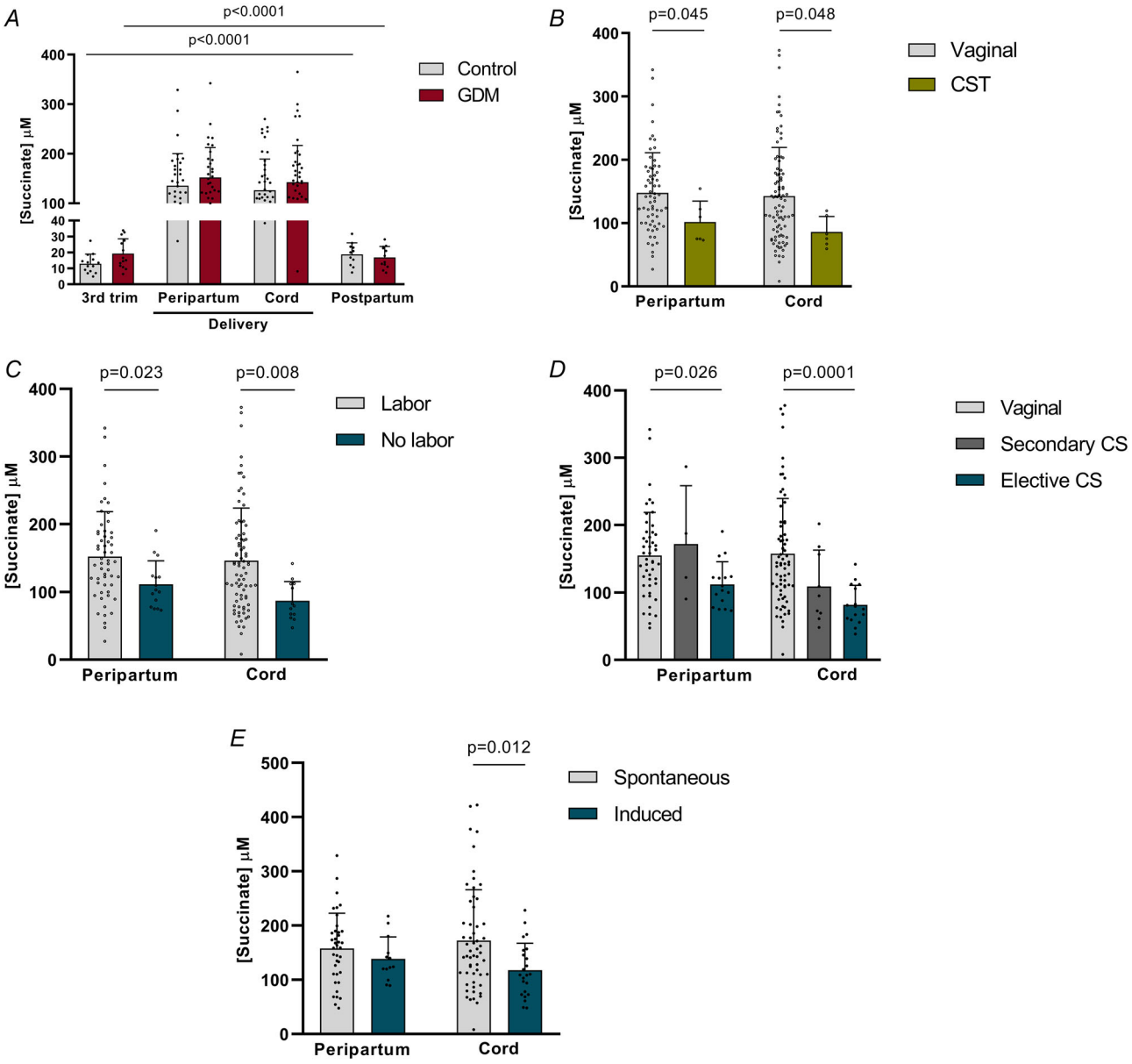


Figure 1. Succinate levels increase in maternal and fetal circulation at delivery and show a distinct dynamic according to delivery type
A, plasma succinate levels in the third trimester ($n = 14 + 14$), peripartum ($n = 36 + 34$), umbilical cord ($n = 47 + 46$) and postpartum ($n = 11 + 12$) of the whole cohort. *B*, plasma succinate levels at the peripartum and umbilical cord, clustering in healthy controls and GDM patients, and categorizing between vaginal and Caesarean deliveries. Maternal peripartum: $n = 27 + 28$ from patients undergoing vaginal delivery; $n = 9 + 6$ from patients undergoing elective Caesarean section. Cord blood succinate levels: $n = 42 + 40$ from patients undergoing vaginal delivery; $n = 8 + 6$ from patients undergoing elective Caesarean section. Plasma succinate levels of the whole cohort are at the peripartum ($n = 36 + 34$) and umbilical cord ($n = 47 + 46$). *C*, comparison of plasma succinate levels according to the presence or absence of labour (maternal peripartum levels: 56 samples from patients undergoing labour and 16 from patients undergoing elective Caesarean section) and cord blood levels: 82 from patients undergoing labour and 18 from patients undergoing elective Caesarean section). *D*, plasma succinate levels according to the type of delivery. Maternal peripartum/cord blood: 51/72 from patients undergoing vaginal delivery, 16/16 from patients undergoing elective Caesarean section and 5/10 from patients undergoing secondary Caesarean section. *E*, plasma succinate concentrations according to the onset of labour. Maternal peripartum: 39 spontaneous onset and 17 inductions; cord blood: 57 spontaneous onset and 25 inductions. Data are presented as the mean ± SD. Differences were assessed by two-way ANOVA with Tukey's multiple comparisons test in (*A*) and Student's *t* test in (*B*) to (*E*). CS, Caesarean section.

group (Fig. 1*E*). No significant differences in succinate levels were observed between the GDM and control groups when analysed based on the presence or onset of labour.

We analysed the groups separately to determine whether GDM influenced succinate levels according to delivery type, labour presence and labour onset. In the GDM group, differences in succinate levels remained statistically significant in maternal and cord blood for delivery type ($P = 0.013$ and $P = 0.003$, respectively) and labour presence ($P = 0.016$ and $P = 0.017$, respectively). By contrast, these differences were only observed in the cord blood in the control group for delivery type and labour presence ($P = 0.013$ and $P = 0.032$, respectively). Finally, considering labour onset, we found that cord blood succinate levels were significantly higher in spontaneous-onset deliveries only in the control group ($P = 0.021$) (see Supporting information, Fig. S1). The statistical analysis of delivery type, considering vaginal delivery, elective Caesarean and non-elective Caesarean, has not been included because some groups had fewer than five cases, making the analysis unfeasible. A similar limitation occurred with labour onset when analysing maternal blood samples, so only the cord blood data are presented.

Overall, these findings suggest that maternal and umbilical cord succinate levels are significantly modulated by labour, highlighting a potential role for succinate in the physiological mechanisms of parturition, which may be altered in the presence of GDM.

Gestational diabetes induces succinate accumulation and SUCNR1 downregulation in umbilical cords

Our data suggest that, at delivery, elevated succinate levels in maternal and fetal circulation expose the placenta and umbilical cord to concentrations sufficient to activate the succinate receptor SUCNR1 (He et al., 2004). During labour, the placenta and umbilical cord can experience hypoxia, which is known to induce tissue release of succinate (Chouchani et al., 2014; Murphy & O'Neill, 2018; Tannahill et al., 2013). This physiological hypoxia can be exacerbated in a diabetic environment because of placental abnormalities and vascular dysfunction, among other factors (Cvitic et al., 2014; Li et al., 2013). We analysed succinate release in the placenta and umbilical cord explants from GDM and control pregnancy. Our findings indicate that umbilical cords from GDM mothers showed significantly higher secretion of succinate, whereas succinate secretion from placental and amniotic membrane tissues remained unchanged (Fig. 2*A*). Correspondingly, succinate levels were significantly higher in umbilical cord tissue lysates from the GDM group than controls (Fig. 2*B*). By contrast, *SUCNR1* and the extracellular uptake succinate transporters *SLC13A3* and *SLC13A5* were downregulated in the umbilical cord of those patients (Fig. 2*C*).

GDM compromises angiogenic gene profile modulation by SUCNR1 in umbilical cord endothelium

Umbilical vein endothelial cells exhibit higher expression of SUCNR1 (Atallah et al., 2021), making them targets for the elevated succinate levels observed during the peripartum period. This elevated extracellular succinate can modulate the production of angiogenic mediators in response to labour stress, a process that might be disrupted in the context of GDM. According to data from umbilical cord explants (Fig. 2*A*), succinate secretion by HUVECs is markedly increased in those isolated from GDM mothers (Fig. 3*A*).

To determine whether SUCRN1 activation impacts the angiogenic expression profile in umbilical cord endothelium, we first treated the HUVECs using the synthetic SUCNR1 agonist cESA, which has a lower EC_{50} compared to succinate (Geubelle et al., 2017), aiming to exclude any intracellular effects of succinate induced by its uptake into cells via dicarboxylic acid transporters (Pajor, 2006, 2014). We saw a pro-angiogenic effect exclusively in the control group. To further confirm the impact of succinate on HUVEC angiogenic profile relies on its extracellular signalling properties, we co-treated the cells with the human-specific SUCNR1 antagonist, NF-56-EJ40. Acute receptor antagonism entirely prevented the cESA-induced pro-angiogenic exacerbation in the control group, with a significant downregulation of *ANGPTL4*, *PTGS2*, *TNF* and *VEGFA* genes, confirming that the pro-angiogenic effect of cESA is dependent on SUCNR1 in a normoglycemic environment. However, SUCNR1 inhibition by NF-56-EJ40 in samples from GDM donors showed an unbiased effect on the target genes (Fig. 3*B*).

SUCNR1 activation stimulates tube-forming capacity and sprouting of HUVECs from healthy but not GDM pregnancies

To validate the role of succinate/SUCNR1 axis in the angiogenic properties of HUVECs and assess how GDM influences this process, we conducted tube-formation (2-D culture that simulates the later stages of angiogenesis) and sprouting assays (a 3-D culture method that mimics the initial stages of angiogenesis) using primary cultures isolated from both normal and GDM pregnancies. An impairment in the ability of HUVECs from GDM pregnancies to form capillary-like structures was detected. Representative fields of healthy HUVECs stimulated with cESA showed a markedly higher number of branching

points and longer tube length than non-treated HUVECs from healthy pregnancies (Fig. 4*A*). The extent of tube formation was significantly greater in cESA-stimulated HUVECs than in non-stimulated ones, as measured by the number of capillary nodes ($P = 0.047$) and junctions ($P = 0.046$). By contrast, the number of extremities ($P = 0.348$) and meshes ($P = 0.176$) remained similar (Fig. 4*B*). GDM-HUVECs did not respond to cESA stimulation, with no significant changes observed in any of the parameters. Similarly, representative spheroids generated from healthy HUVECs showed enhanced sprouting after stimulation with cESA (Fig. 4*C*), as evidenced by a significantly higher number of sprouts ($P = 0.007$) and increased mean ($P = 0.004$) and cumulative sprout length ($P = 0.003$) (Fig. 4*D*). However, spheroids derived from GDM-exposed HUVECs were insensitive to cESA stimulation, with no differences observed in any of the parameters.

Discussion

Pregnancy involves complex alterations in maternal metabolism and placental function, requiring tight regulatory mechanisms to ensure optimal fetal development. In this context, succinate may be a key regulator in this process, with its dual function as both a metabolic substrate and signalling molecule. Interacting with its membrane receptor, SUCNR1, which is expressed in multiple tissues, including the placenta (Atallah et al., 2021), succinate influences important physiological pathways related to glucose metabolism, inflammation and angiogenesis (Fernández-Veledo et al., 2024). These

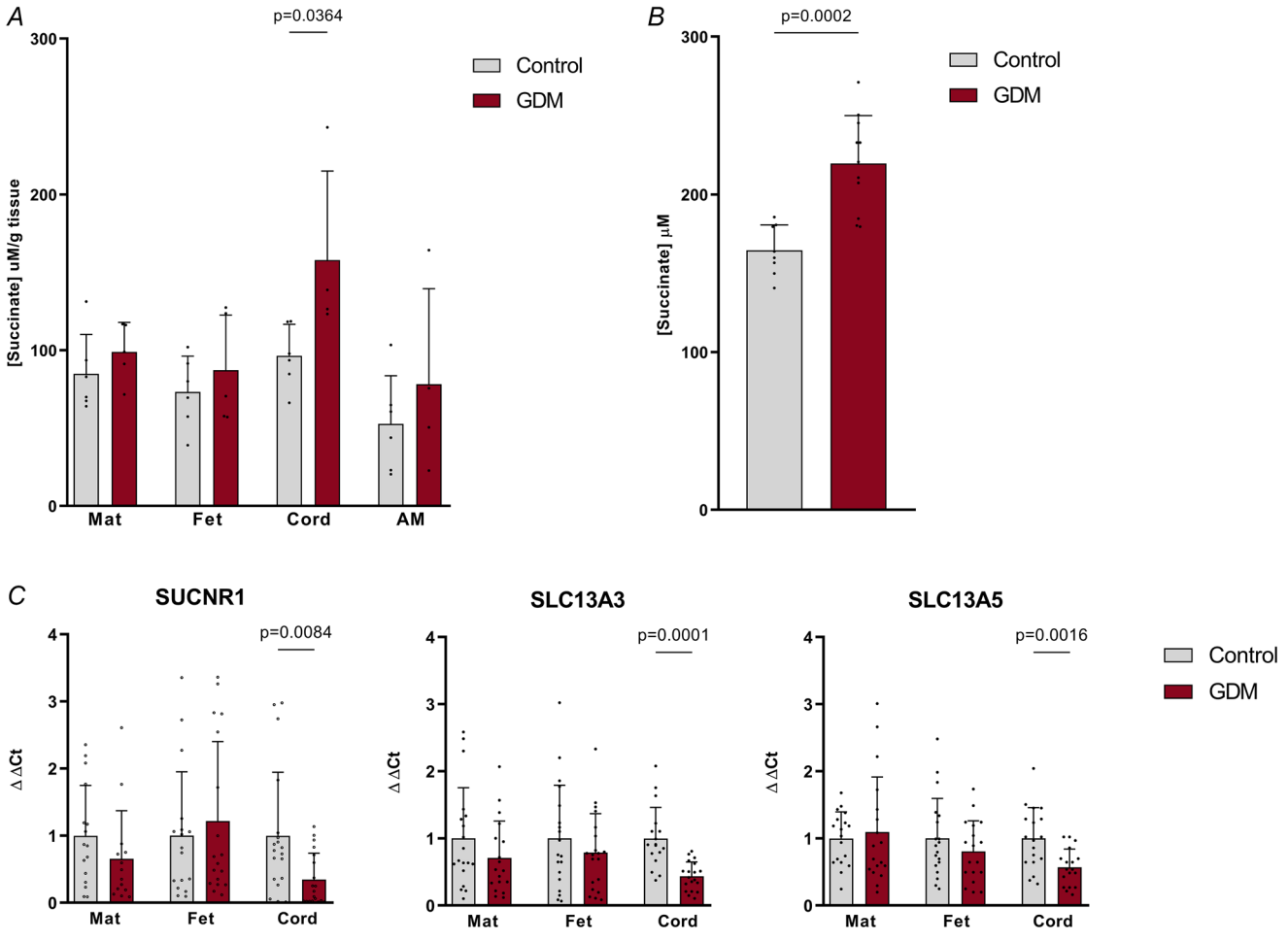


Figure 2. Umbilical cords from GDM isolations release higher amounts of succinate, although with succinate influx attenuated
A, conditioned media from placental, amniotic membrane and umbilical cord tissue explants. *B*, succinate secretion by umbilical cord tissue lysates. Data are presented as the mean ± SD. *C*, succinate receptor and its uptake transporters gene expressions. Data correspond to $\Delta\Delta C_t$ and are shown as the mean ± SD. Differences were assessed by two ANOVAs plus Bonferroni's multiple comparisons test in (*A*) and Student's *t* test in (*B*) to (*C*). AM, amniotic membrane.

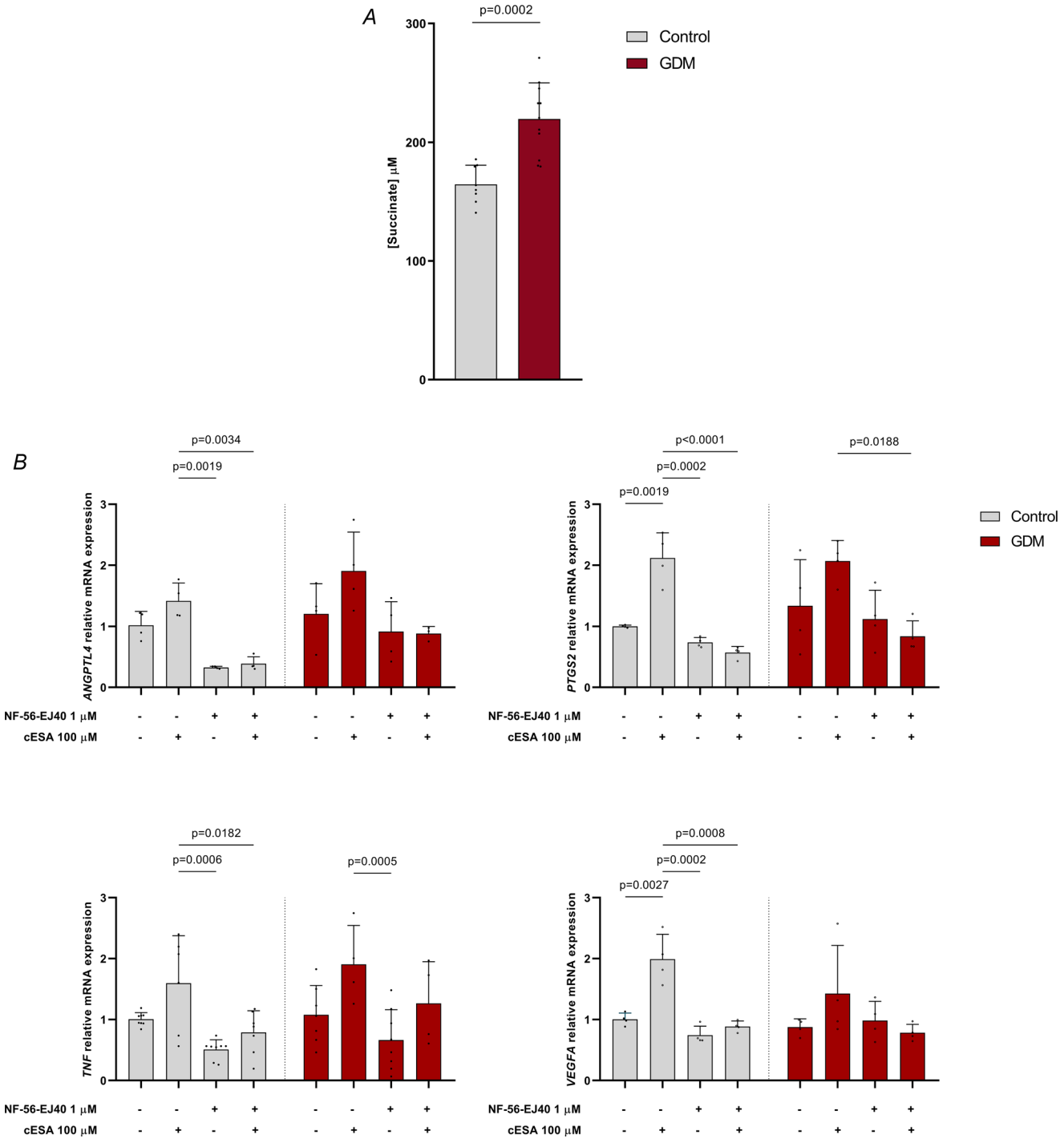


Figure 3. HUVECs from GDM isolations release higher amounts of extracellular succinate, whereas the SUCNR1-modulated angiogenic gene profile becomes compromised
A, HUVEC succinate secretion and intracellular content. Data are presented as the mean ± SD. *B*, angiogenesis-related *ANGPTL4*, *PTGS2* and *TNF* gene expression in HUVEC. Data correspond to $\Delta\Delta C_t$ and are shown as the mean ± SD. Differences were assessed using Student's *t* test in (*A*) and two-way ANOVA with Tukey's test for multiple comparisons in (*B*).

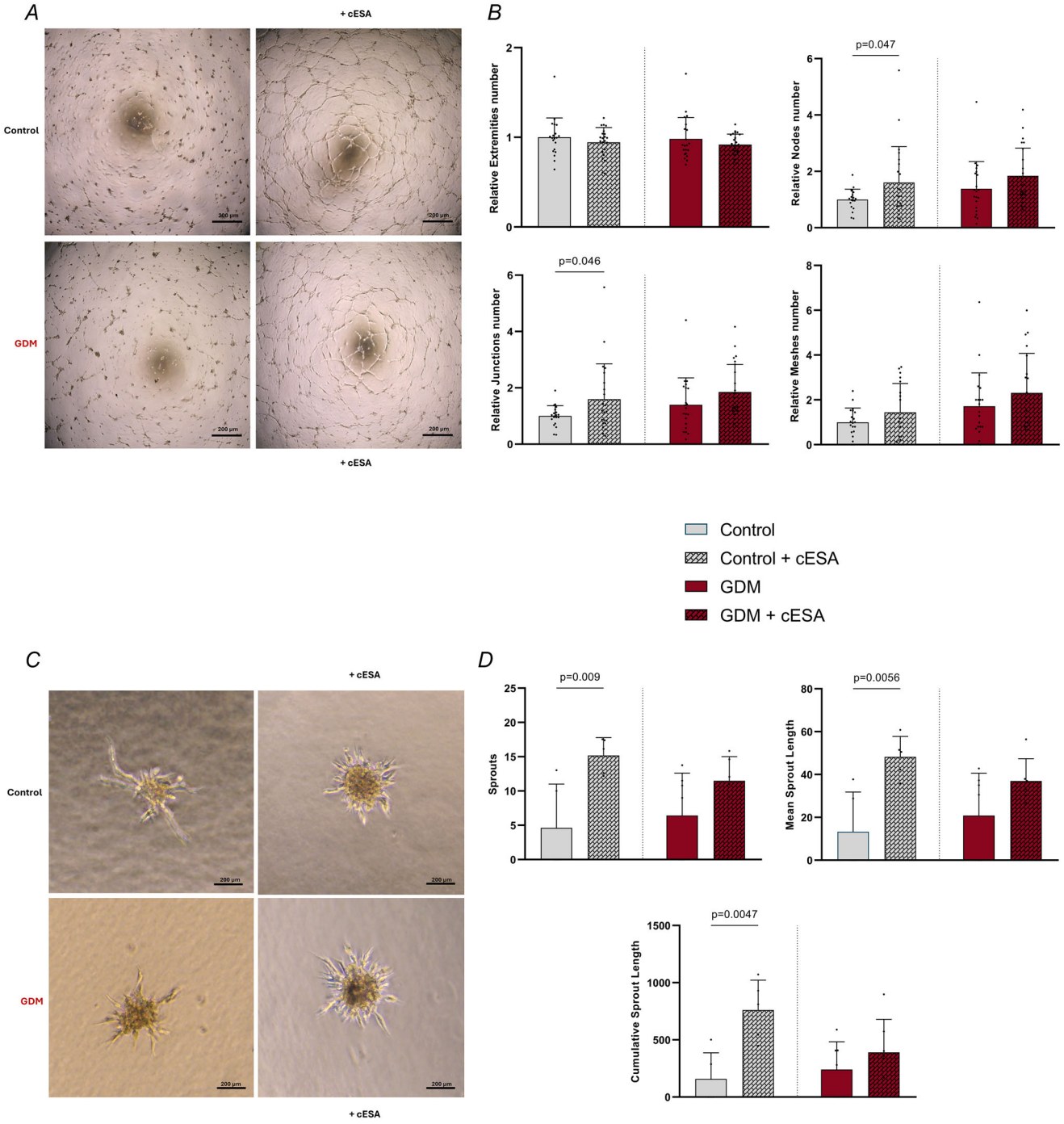


Figure 4. cESA improves network formation and sprouting potential of HUVECs from healthy isolations (controls)
A, visual representation of Matrigel tube formation assay. Control-HUVECs (above) and GDM-HUVECs (below) were stimulated with 100 µM cESA for 5 h. *B*, quantitative data represent differences in the number of extremities, nodes, junctions and meshes between groups (n = 4 HC and 4 GDM). *C*, visual representation of the spheroid sprouting assay. Control-HUVECs (above) and GDM-HUVECs (below) were stimulated with 100 µM cESA for 16 h. Scale bars = 200 µm. *D*, quantitative data represent differences in the number of sprouts, mean sprout length, and cumulative sprout length between groups (n = 4 HC and 4 GDM). Data are normalized to the mean of the controls of each experiment and are shown as the mean ± SD. Differences assessed by Student's t test. Scale bars = 200 µm.

roles suggest that succinate may be a crucial factor in regulating various aspects of pregnancy.

Our data, showing the dynamics of succinate levels during pregnancy and postpartum, its regulation according to GDM, and its physiological increase in the peripartum period, support this idea. We also show how the activation of SUCNR1 promotes angiogenesis in HUVECs from pregnancies with normal glucose tolerance. By contrast, in GDM, excessive succinate release and the compromised function of the SUCNR1 receptor and the succinate transporters may dampen the angiogenic response, positioning succinate as a metabolic sensor in pregnancy.

Succinate levels are elevated in inflammatory and insulin-resistance diseases, such as obesity and type 2 diabetes (Ceperuelo-Mallafre et al., 2019; Serena et al., 2018) and circulating succinate concentrations correlate with BMI, insulin and glucose concentrations (Astiarraga et al., 2020; Ceperuelo-Mallafre et al., 2019; Serena et al., 2018). Also, at the end of the third trimester, before the onset of labour, succinate concentrations are increased in GDM women on insulin treatment as a marker of the severity of the metabolic alteration (Dunn et al., 2012). In our cohort, at the beginning of the third trimester, GDM women exhibited higher succinate concentrations compared to control women. By contrast, no differences were observed in the postpartum period, when the insulin sensitivity was recovered. This agrees with data on type 2 diabetes and other insulin resistance states. By contrast, although obesity has been linked to succinate concentrations outside of pregnancy (Astiarraga et al., 2020; Ceperuelo-Mallafre et al., 2019; Serena et al., 2018), our cohort showed no association between succinate concentrations and pre-pregnancy BMI.

However, we show that the succinate differences observed in the third trimester between GDM and control are lost in the peripartum period, in which there is a marked increase in succinate levels in maternal and fetal blood, reaching concentrations capable of physiologically activating the SUCNR1 receptor and inducing downstream signalling (He et al., 2004). Currently, the molecular signals responsible for the onset of labour are not well established (Ilicic et al., 2020). Although inflammation has been implicated, it is unclear whether it is the main driver of this (Kyathanahalli et al., 2023; Mor et al., 2011) and the potential contribution of succinate or its receptor to labour processes is not well established.

Placental mitochondrial content changes in response to gestational age and labour, probably reflecting shifts in metabolic demands (Holland et al., 2017). Inflammation and hypoxia, among other factors, have been associated with succinate dehydrogenase inhibition, leading to increased succinate accumulation in the mitochondrial matrix. This succinate then diffuses into the cytosol and can be released extracellularly, where it interacts with the SUCNR1 receptor to trigger various signalling pathways (Fernández-Veledo et al., 2021, 2024; Huang et al., 2024). Notably, SUCNR1 expression is higher in the myometrium of women who have undergone labour than those who have not. Furthermore, inhibition of SUCNR1 in myometrial tissues suppresses both the inflammatory response and cellular contractility, suggesting a role of succinate receptor in mediating inflammation and contractile processes during childbirth (Lim & Lappas, 2020).

Additionally, elevated cytosolic succinate levels can stabilize hypoxia-inducible factor 1-alpha (HIF-1α), disrupt ATP synthesis and increase reactive oxygen species production, potentially amplifying angiogenic and inflammatory processes (Atallah et al., 2022; Fernández-Veledo et al., 2024; Huang et al., 2024). Recent studies have reported high HIF-1α mRNA levels in labouring human myometrium (Chaemsaithong et al., 2013; Mittal et al., 2011), suggesting that HIF-1α plays a crucial role in myometrial contractility during labour (Wen et al., 2022). In this context, the differences observed in succinate levels depending on the onset of labour (spontaneous or induced), the type of delivery (vaginal or Caesarean section) and the presence or absence of labour that we observed in our cohort would support the role of succinate as a key element in the birth process.

In pregnancies complicated by diabetes, labour appears to be associated with greater difficulties in cervical ripening during induction (Hawkins et al., 2017) and prolonged active labour phases (Nevander et al., 2023). *In vitro* studies also indicate that the myometrium of women with GDM exhibits reduced contractility compared to non-GDM cases (Al-Qahtani et al., 2012). These findings suggest that labour in women with GDM may be characterized by heightened hypoxia and inflammation, along with increased succinate levels. In the present study, maternal and cord blood succinate levels were comparable between the GDM and control groups when analysed according to mode of delivery, labour and onset of labour. However, when the groups were analysed separately, differences in succinate levels related to mode of delivery and labour were more pronounced in the GDM group. By contrast, differences in cord blood succinate levels based on labour onset were significant only in the control group. Given that GDM placentas exhibit impaired mitochondrial function compared to controls (Abbade et al., 2020) and mitochondrial abnormalities have been documented (Meng et al., 2014), a greater succinate accumulation would be expected under hypoxic-inflammatory conditions. Excess succinate released into the extracellular space could activate SUCNR1, whereas cytosolic accumulation could stabilize HIF-1α, both comprising mechanisms to enhance myometrial contractility. However, *in vitro* studies suggest that hyperglycaemia-associated increases in HIF-1α levels correlate with reduced myometrial cell

contractility (Li et al., 2023). Additionally, it remains unclear whether GDM affects the number and function of SUCNR1 receptors in myometrial cells, as we have observed in HUVECs. Therefore, although succinate may contribute to HIF-1α regulation and SUCNR1 activation in myometrial tissue, the hyperglycaemic environment characteristic of GDM may ultimately contribute to reduced myometrial contractility.

Maintaining a balanced succinate/SUCNR1 axis may be crucial for optimal physiological functioning. As previously stated, in GDM placentas, mitochondrial function appears to be impaired compared to controls (Abbade et al., 2020; Fisher et al., 2021; Muralimanoharan et al., 2016), leading to succinate accumulation within the mitochondrial matrix. This accumulated succinate can diffuse into the cytosol and be released into the extracellular space, interacting with the SUCNR1 receptor to activate angiogenic responses (Atallah et al., 2021). Our tissue explant data revealed that umbilical cords from GDM mothers released significantly higher amounts of succinate, whereas placental and amniotic membrane efflux of succinate remained unchanged. Accordingly, succinate release by umbilical cord lysates was also remarkedly augmented in the GDM group compared to controls. Consistent with our findings, previous studies have documented succinate accumulation in pathological conditions, including elevated levels in synovial joints of subjects with rheumatoid arthritis (Kim et al., 2014) and higher succinate concentrations in pre-eclamptic placentas compared to controls (Dunn et al., 2012). By contrast, we found that *SUCNR1* and the extracellular uptake succinate transporters *SLC13A3* and *SLC13A5* were downregulated in the umbilical cord of those patients. In this context, increased succinate release and reduced cellular uptake may act as a compensatory mechanism to enhance SUCNR1 activation in response to the lower expression of this receptor.

Given that the levels of succinate released from amniotic membrane explants rich in mesenchymal stem cells (Bakhtyar et al., 2018) from women with GDM were similar to those from control women, it is most plausible that the increased succinate release from the umbilical cords of women with GDM originated from endothelial cells. Indeed, SUCNR1 expression has been demonstrated in placental endothelial cells (Atallah et al., 2021), endothelial cells of the afferent arterioles of rabbit and mice kidney (Toma et al., 2008), and human venous and arterial endothelial cells from the umbilical cord (Zhang et al., 2018). Our data show that endothelial cells are exposed to elevated levels of circulating succinate in the peripartum, regardless of GDM. This exposure can activate SUCNR1 signalling, leading to the production of angiogenic mediators during labour.

In HUVECs, angiogenesis has been associated with activating the SUCNR1 receptor (Atallah et al., 2021). Our work validates the succinate/SUCNR1 axis as an amplifying route for angiogenic processes in HUVECs by treating control cells with a SUCRN1-specific agonist, cESA. In line, it has already been confirmed that the pro-angiogenic phenotype induced by succinate in healthy HUVECs was suppressed when knocking down SUCNR1, implying that these responses are mediated through SUCNR1 (Atallah et al., 2021), which is in agreement with our data. However, these effects were not seen in GDM-derived HUVECs, suggesting a dysregulation of the succinate/SUCNR1 axis in GDM. As a novelty in the present study, we observed that HUVECs from women with GDM release more succinate into the environment but appear insensitive to its action. On the one hand, this insensitivity is manifested in the absence of clear positive regulation of the genes involved in angiogenesis after co-treatment with a human-specific SUCNR1 antagonist, NF-56-EJ40. At the same time, this response was observed in the HUVECs of control pregnant women. On the other hand, vascular structures are not generated after stimulation with cESA. It has been proposed that the sensitivity of placental endothelial cells to angiogenic stimuli depends on the need to expand the fetal vasculature to achieve an adequate supply of oxygen to the fetus (Hiden et al., 2009). The absence of response of GDM-HUVECs to the stimulation with cESA suggests that, at least at the end of gestation, this would not be one of the main pathways in GDM expansion and could be related to insufficient generation or inefficient vascular structures. Our data limit us with respect to specifying whether this phenomenon is pathogenic or protective in a maternal environment, where there is a greater influx of nutrients to the placenta. Epigenetic variation, particularly DNA methylation, is recognized as a major mechanism involved in fetal programming, playing a critical role in fetoplacental endothelial dysfunction after exposure to GDM (Cvitic et al., 2018). Epigenetic mechanisms, characterized by a high degree of plasticity and responsiveness to environmental stimuli (Feil & Fraga, 2012), may arise as a hypothetic explanation for why the exposure to GDM could eventually program HUVECs to be insensitive to the succinate/SUCNR1 axis. The pathophysiological implications of succinate/SUCNR1 signalling are evident in conditions involving local stress that impact cellular metabolism, such as hyperglycaemia, ischaemia and hypoxia (Gilissen et al., 2016). Therefore, it is plausible that SUCNR1 functions as a metabolic sensor, adjusting cellular activities in response to succinate levels.

We are aware that this study has some limitations. Considering labour induction and elective Caesarean section as periods in which the mechanisms involved in labour had not been set in motion may be inaccurate because we cannot know how long it would have taken for spontaneous labour to start. However, there is no other

way of classification at present. We also know that gene expression levels do not always relate to protein levels; however, the consistency of the data suggests that they appear to be related in this case.

As a remarked strength, this is the first study to investigate the potential role of succinate on labour and placental mechanisms by combining clinical and functional experimental data. Nevertheless, we focused on exploring the specific SUCNR1-mediated effects of succinate on placenta functionality, addressing its specific agonist actions.

In conclusion, the present study underscores the significant role of succinate as a signalling molecule in the placenta beyond its metabolic functions, regulating labour and placental mechanisms. The insensibility of succinate/SUCRN1 seen in the GDM milieu could represent either a protective physiological mechanism or a pathogenic character. Thus, further investigations into changes in placental metabolism and the establishment of clear causal relationships between tissue metabolism, succinate levels, SUCNR1 signalling and pregnancy could offer clinically valuable insights into pregnancy-related diseases, whereas targeting SUCNR1 in the placenta could present promising new therapeutic opportunities.

References

Abbade, J., Klemetti, M. M., Farrell, A., Ermini, L., Gillmore, T., Sallais, J., Tagliaferro, A., Post, M., & Caniggia, I. (2020). Increased placental mitochondrial fusion in gestational diabetes mellitus: An adaptive mechanism to optimize fetoplacental metabolic homeostasis? *British Medical Journal Open Diabetes Research Care*, **8**(1), e000923.

Aguiar, C. J., Andrade, V. L., Gomes, E. R. M., Alves, M. N. M., Ladeira, M. S., Pinheiro, A. C. N., Gomes, D. A., Almeida, A. P., Goes, A. M., Resende, R. R., Guatimosim, S., & Leite, M. F. (2010). Succinate modulates Ca^{2+} transient and cardiomyocyte viability through PKA-dependent pathway. *Cell Calcium*, **47**(1), 37–46.

Al-Qahtani, S., Heath, A., Quenby, S., Dawood, F., Floyd, R., Burdyga, T., & Wray, S. (2012). Diabetes is associated with impairment of uterine contractility and high caesarean section rate. *Diabetologia*, **55**, 489–498.

An, Y. A., Chen, S., Deng, Y., Wang, Z. V., Funcke, J. B., Shah, M., Shan, B., Gordillo, R., Yoshino, J., Klein, S., Kusminski, C. M., & Scherer, P. E. (2021). The mitochondrial dicarboxylate carrier prevents hepatic lipotoxicity by inhibiting white adipocyte lipolysis. *Journal of Hepatology*, **75**(2), 387–399.

Astiarraga, B., Martínez, L., Ceperuelo-Mallafré, V., Llauradó, G., Terrón-Puig, M., Rodríguez, M. M., Casajoana, A., Pellitero, S., Megía, A., Vilarrasa, N., Vendrell, J., & Fernández-Veledo, S. (2020). Impaired succinate response to a mixed meal in obesity and type 2 diabetes is normalized after metabolic surgery. *Diabetes Care*, **43**(10), 2581–2587.

Atallah, R., Gindlhuber, J., Platzer, W., Bärnthaler, T., Tatzl, E., Toller, W., Strutz, J., Rittchen, S., Luschnig, P., Birner-Gruenberger, R., Wadsack, C., & Heinemann, A. (2021). SUCNR1 is expressed in human placenta and mediates angiogenesis: Significance in gestational diabetes. *International Journal of Molecular Sciences*, **22**(21), 12048.

Atallah, R., Olschewski, A., & Heinemann, A. (2022). Succinate at the crossroad of metabolism and angiogenesis: Roles of SDH, HIF1α and SUCNR1. *Biomedicines*, **10**(12), 3089.

Bakhtyar, N., Jeschke, M. G., Herer, E., Sheikholeslam, M., & Amini-Nik, S. (2018). Exosomes from acellular Wharton's jelly of the human umbilical cord promotes skin wound healing. *Stem Cell Research & Therapy*, **9**(1), 1–14.

Burton, G. J., Charnock-Jones, D. S., & Jauniaux, E. (2009). Regulation of vascular growth and function in the human placenta. *Reproduction (Cambridge, England)*, **138**(6), 895–902.

Carpentier, G., Berndt, S., Ferratge, S., Rasband, W., Cuendet, M., Uzan, G., & Albanese, P. (2020). Angiogenesis analyzer for ImageJ—A comparative morphometric analysis of "end-othelial tube formation assay" and "fibrin bead assay". *Scientific Reports*, **10**(1), 11568.

Ceperuelo-Mallafre, V., Llaurado, G., Keiran, N., Benaiges, E., Astiarraga, B., Martinez, L., Pellitero, S., Gonzalez-Clemente, J. M., Rodriguez, A., Fernandez-Real, J. M., Lecube, A., Megia, A., Vilarrasa, N., Vendrell, J., & Fernandez-Veledo, S. (2019). Preoperative circulating succinate levels as a biomarker for diabetes remission after bariatric surgery. *Diabetes Care*, **42**(10), 1956–1965.

Chaemsaithong, P., Madan, I., Romero, R., Than, N. G., Tarca, A. L., Draghici, S., Bhatti, G., Yeo, L., Mazor, M., Kim, C. J., Hassan, S. S., & Chaiworapongsa, T. (2013). Characterization of the myometrial transcriptome in women with an arrest of dilatation during labor. *Journal of Perinatal Medicine*, **41**(6), 665–681.

Challis, J. R., Lockwood, C. J., Myatt, L., Norman, J. E., Strauss, J. F., & Petraglia, F. (2009). Inflammation and pregnancy. *Reproductive Sciences*, **16**(2), 206–215.

Chouchani, E. T., Pell, V. R., Gaude, E., Aksentijević , D., Sundier, S. Y., Robb, E. L., Logan, A., Nadtochiy, S. M., Ord, E. N. J., Smith, A. C., Eyassu, F., Shirley, R., Hu, C. H., Dare, A. J., James, A. M., Rogatti, S., Hartley, R. C., Eaton, S., Costa, A. S. H., … Murphy, M. P.(2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*, **515**(7527), 431–435.

Correa, P., Kruglov, E. A., Thompson, M., Leite, M. F., Dranoff, J. A., & Nathanson, M. H. (2007). Succinate is a paracrine signal for liver damage. *Journal of Hepatology*, **47**(2), 262–269.

Cvitic, S., Desoye, G., & Hiden, U. (2014). Glucose, insulin, and oxygen interplay in placental hypervascularisation in diabetes mellitus. *BioMed Research International*, **2014**, 1.

Cvitic, S., Novakovic, B., Gordon, L., Ulz, C. M., Mühlberger, M., Diaz-Perez, F. I., Joo, J. E., Svendova, V., Schimek, M. G., Trajanoski, S., Saffery, R., Desoye, G., & Hiden, U. (2018). Human fetoplacental arterial and venous endothelial cells are differentially programmed by gestational diabetes mellitus, resulting in cell-specific barrier function changes. *Diabetologia*, **61**(11), 2398–2411.

Demir, R., Kayisli, U. A., Seval, Y., Celik-Ozenci, C., Korgun, E. T., Demir-Weusten, A. Y., & Huppertz, B. (2004). Sequential expression of VEGF and its receptors in human placental villi during very early pregnancy: Differences between placental vasculogenesis and angiogenesis. *Placenta*, **25**(6), 560–572.

National Diabetes Data Group. (1979). Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*, **28**(12), 1039–1057.

Draoui, N., de Zeeuw, P., & Carmeliet, P. (2017). Angiogenesis revisited from a metabolic perspective: Role and therapeutic implications of endothelial cell metabolism. *Open Biology*, **7**(12), 170219.

Du, W., Ren, L., Hamblin, M. H., & Fan, Y. (2021). Endothelial cell glucose metabolism and angiogenesis. *Biomedicines*, **9**(2), 1–17.

Dunn, W. B., Brown, M., Worton, S. A., Davies, K., Jones, R. L., Kell, D. B., & Heazell, A. E. P. (2012). The metabolome of human placental tissue: Investigation of first trimester tissue and changes related to preeclampsia in late pregnancy. *Metabolomics*, **8**(4), 579–597.

Elsayed, N. A., Aleppo, G., Aroda, V. R., Bannuru, R. R., Brown, F. M., Bruemmer, D., Collins, B. S., Hilliard, M. E., Isaacs, D., Johnson, E. L., Kahan, S., Khunti, K., Leon, J., Lyons, S. K., Perry, M. L., Prahalad, P., Pratley, R. E., Seley, J. J., Stanton, R. C., … the American Diabetes Association. (2023). Classification and diagnosis of diabetes: Standards of care in diabetes—2023. *Diabetes Care*, **46**(Suppl 1), S19–S40.

Feil, R., & Fraga, M. F. (2012). Epigenetics and the environment: Emerging patterns and implications. *Nature Reviews Genetics*, **13**(2), 97–109.

Fernández-Veledo, S., Ceperuelo-Mallafré, V., & Vendrell, J. (2021). Rethinking succinate: An unexpected hormone-like metabolite in energy homeostasis. *Trends in Endocrinology and Metabolism*, **32**(9), 680–692.

Fernández-Veledo, S., Marsal-Beltran, A., & Vendrell, J. (2024). Type 2 diabetes and succinate: Unmasking an age-old molecule. *Diabetologia*, **67**(3), 430–442.

Fernández-Veledo, S., & Vendrell, J. (2019). Gut microbiota-derived succinate: Friend or foe in human metabolic diseases? *Reviews in Endocrine, & Metabolic Disorders*, **20**(4), 439–447.

Fisher, J. J., Vanderpeet, C. L., Bartho, L. A., McKeating, D. R., Cuffe, J. S. M., Holland, O. J., & Perkins, A. V. (2021). Mitochondrial dysfunction in placental trophoblast cells experiencing gestational diabetes mellitus. *The Journal of Physiology*, **599**(4), 1291–1305.

Geubelle, P., Gilissen, J., Dilly, S., Poma, L., Dupuis, N., Laschet, C., Abboud, D., Inoue, A., Jouret, F., Pirotte, B., & Hanson, J. (2017). Identification and pharmacological characterization of succinate receptor agonists. *British Journal of Pharmacology*, **174**(9), 796–808.

Gilissen, J., Jouret, F., Pirotte, B., & Hanson, J. (2016). Insight into SUCNR1 (GPR91) structure and function. *Pharmacology, & Therapeutics*, **159**, 56–65.

Grupo Español de Diabetes y Embarazo (GEDE), & Grupo Español de Diabetes y Embarazo. (2015). Asistencia a la gestante con diabetes. Guía de práctica clínica actualizada en 2014 | Avances en Diabetología. *Avances en Diabetologia*, **31**(2), 45–59.

Haffke, M., Fehlmann, D., Rummel, G., Boivineau, J., Duckely, M., Gommermann, N., Cotesta, S., Sirockin, F., Freuler, F., Littlewood-Evans, A., Kaupmann, K., & Jaakola, V.-P. (2019). Structural basis of species-selective antagonist binding to the succinate receptor. *Nature*, **574**(7779), 581–585.

Hakak, Y., Lehmann-Bruinsma, K., Phillips, S., Le, T., Liaw, C., Connolly, D. T., & Behan, D. P. (2009). The role of the GPR91 ligand succinate in hematopoiesis. *Journal of Leukocyte Biology*, **85**(5), 837–843.

Hawkins, J. S., Stephenson, M., Powers, B., & Wing, D. A. (2017). Diabetes mellitus: An independent predictor of duration of prostaglandin labor induction. *Journal of Perinatology*, **37**(5), 488–491.

He, W., Miao, F. J.-P., Lin, D. C.-H., Schwandner, R. T., Wang, Z., Gao, J., Chen, J.-L., Tian, H., & Ling, L. (2004). Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature*, **429**(6988), 188–193.

Hiden, U., Lang, I., Ghaffari-Tabrizi, N., Gauster, M., Lang, U., & Desoye, G. (2009). Insulin action on the human placental endothelium in normal and diabetic pregnancy. *Current Vascular Pharmacology*, **7**(4), 460–466.

Hochachka, P. W., & Dressendorfer, R. H. (1976). Succinate accumulation in man during exercise. *European Journal of Applied Physiology and Occupational Physiology*, **35**(4), 235–242.

Holland, O. J., Hickey, A. J. R., Alvsaker, A., Moran, S., Hedges, C., Chamley, L. W., & Perkins, A. V. (2017). Changes in mitochondrial respiration in the human placenta over gestation. *Placenta*, **57**, 102–112.

Huang, H., Li, G., He, Y., Chen, J., Yan, J., Zhang, Q., Li, L., & Cai, X. (2024). Cellular succinate metabolism and signaling in inflammation: implications for therapeutic intervention. *Frontiers in Immunology*, **15**, 1–13.

Husted, A. S., Trauelsen, M., Rudenko, O., Hjorth, S. A., & Schwartz, T. W. (2017). GPCR-mediated signaling of metabolites. *Cell Metabolism*, **25**(4), 777–796.

Huynh, J., Dawson, D., Roberts, D., & Bentley-Lewis, R. (2015). A systematic review of placental pathology in maternal diabetes mellitus. *Placenta*, **36**(2), 101–114.

Ilicic, M., Zakar, T., & Paul, J. W. (2020). The regulation of uterine function during parturition: An update and recent advances. *Reproductive Sciences*, **27**(1), 3–28.

Jiménez-Valerio, G., & Casanovas, O. (2017). Angiogenesis and metabolism: Entwined for therapy resistance. *Trends in Cancer*, **3**(1), 10–18.

Keiran, N., Ceperuelo-Mallafré, V., Calvo, E., Hernández-Alvarez, M. I., Ejarque, M., Núñez-Roa, C., Horrillo, D., Maymó-Masip, E., Rodríguez, M. M., Fradera, R., de la Rosa, J. V., Jorba, R., Megia, A., Zorzano, A., Medina-Gómez, G., Serena, C., Castrillo, A., Vendrell, J., & Fernández-Veledo, S. (2019). SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. *Nature Immunology*, **20**(5), 581–592.

Kim, C. J., Romero, R., Chaemsaithong, P., & Kim, J. S. (2015). Chronic inflammation of the placenta: Definition, classification, pathogenesis, and clinical significance. *American Journal of Obstetrics and Gynecology*, **213**(4), S53–S69.

Kim, S., Hwang, J., Xuan, J., Jung, Y. H., Cha, H. S., & Kim, K. H. (2014). Global metabolite profiling of synovial fluid for the specific diagnosis of rheumatoid arthritis from other inflammatory arthritis. *PLoS ONE*, **9**, 1–9.

Krzak, G., Willis, C. M., Smith, J. A., Pluchino, S., & Peruzzotti-Jametti, L. (2021). Succinate receptor 1: An emerging regulator of myeloid cell function in inflammation. *Trends in Immunology*, **42**(1), 45–58.

Kyathanahalli, C., Snedden, M., & Hirsch, E. (2023). Is human labor at term an inflammatory condition?. *Biology of Reproduction*, **108**(1), 23–40.

Li, H. P., Chen, X., & Li, M. Q. (2013). Gestational diabetes induces chronic hypoxia stress and excessive inflammatory response in murine placenta. *International Journal of Clinical and Experimental Pathology*, **6**, 650–659.

Li, T., Fei, J., Yu, H., Wang, X., Bai, J., Chen, F., Li, D., & Yin, Z. (2023). High glucose induced HIF-1α/TREK1 expression and myometrium relaxation during pregnancy. *Frontiers in Endocrinology (Lausanne)*, **14**, 1115619.

Li, Y., Liu, Y., Wang, C., Xia, W. R., Zheng, J. Y., Yang, J., Liu, B., Liu, J. Q., & Liu, L. F. (2018). Succinate induces synovial angiogenesis in rheumatoid arthritis through metabolic remodeling and HIF-1α/VEGF axis. *Free Radical Biology and Medicine*, **126**, 1–14.

Li, Y. H., Choi, D. H., Lee, E. H., Seo, S. R., Lee, S., & Cho, E. H. (2016). Sirtuin 3 (SIRT3) regulates oα-smooth muscle actin (oα-SMA) production through the succinate dehydrogenase-G protein-coupled receptor 91 (GPR91) pathway in hepatic stellate cells. *Journal of Biological Chemistry*, **291**(19), 10277–10292.

Lim, R., & Lappas, M. (2020). Inhibition of GPR91 reduces inflammatory mediators involved in active labor in myometrium. *Mediators of Inflammation*, **2020**, 1.

Liu, Y., Wu, Z., Li, Y., Chen, Y., Zhao, X., Wu, M., & Xia, Y. (2025). Metabolic reprogramming and interventions in angiogenesis. *Journal of Advanced Research*, **70**, 323–338.

Marsal-Beltran, A., Rodríguez-Castellano, A., Astiarraga, B., Calvo, E., Rada, P., Madeira, A., Rodríguez-Peña, M. M., Llauradó, G., Núñez-Roa, C., Gómez-Santos, B., Maymó-Masip, E., Bosch, R., Frutos, M. D., Moreno-Navarrete, J. M., Ramos-Molina, B., Aspichueta, P., Joven, J., Fernández-Real, J. M., Quer, J. C., … Fernández-Veledo, S. (2023). Protective effects of the succinate/SUCNR1 axis on damaged hepatocytes in NAFLD. *Metabolism*, **145**, 155630.

McCreath, K. J., Espada, S., Gálvez, B. G., Benito, M., de Molina, A., Sepúlveda, P., & Cervera, A. M. (2015). Targeted disruption of the SUCNR1 metabolic receptor leads to dichotomous effects on obesity. *Diabetes*, **64**(4), 1154–1167.

Meng, Q., Shao, L., Luo, X., Mu, Y., Xu, W., Gao, C., Gao, L., Liu, J., & Cui, Y. (2014). Ultrastructure of placenta of gravidas with gestional diabetes mellitus. *Fertility and Sterility*, **102**(3), e251.

Mills, E. L., Pierce, K. A., Jedrychowski, M. P., Garrity, R., Winther, S., Vidoni, S., Yoneshiro, T., Spinelli, J. B., Lu, G. Z., Kazak, L., Banks, A. S., Haigis, M. C., Kajimura, S., Murphy, M. P., Gygi, S. P., Clish, C. B., & Chouchani, E. T. (2018). Accumulation of succinate controls activation of adipose tissue thermogenesis. *Nature*, **560**(7716), 102–106.

Mittal, P., Romero, R., Tarca, A. L., Draghici, S., Nhan-Chang, C. L., Chaiworapongsa, T., Hotra, J., Gomez, R., Kusanovic, J. P., Lee, D. C., Kim, C. J., & Hassan, S. S. (2011). A molecular signature of an arrest of descent in human parturition. *American Journal of Obstetrics and Gynecology*, **204**(2), 177.e15–177.e33.

Mor, G., Cardenas, I., Abrahams, V., & Guller, S. (2011). Inflammation and pregnancy: The role of the immune system at the implantation site. *Annals of the New York Academy of Sciences*, **1221**(1), 80–87.

Mu, X., Zhao, T., Xu, C., Shi, W., Geng, B., Shen, J., Zhang, C., Pan, J., Yang, J., Hu, S., Lv, Y., Wen, H., & You, Q. (2017). Oncometabolite succinate promotes angiogenesis by upregulating VEGF expression through GPR91-mediated STAT3 and ERK activation. *Oncotarget*, **8**(8), 13174–13185.

Muralimanoharan, S., Maloyan, A., & Myatt, L. (2016). Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: Role of miR-143. *Clinical Science (London, England: 1979)*, **130**(11), 931–941.

Murphy, M. P., & O'Neill, L. A. J. (2018). Krebs cycle reimagined: The emerging roles of succinate and itaconate as signal transducers. *Cell*, **174**(4), 780–784.

Murray, B., & Wilson, D. J. (2001). A study of metabolites as intermediate effectors in angiogenesis. *Angiogenesis*, **4**(1), 71–77.

Nevander, S., Carlhäll, S., Källén, K., Lilliecreutz, C., & Blomberg, M. (2023). Gestational diabetes mellitus and time in active labor: A population-based cohort study. *Acta Obstetricia Et Gynecologica Scandinavica*, **102**(7), 873–882.

Pajor, A. M. (2006). Molecular properties of the SLC13 family of dicarboxylate and sulfate transporters. *Pflugers Archiv: European Journal of Physiology*, **451**(5), 597–605.

Pajor, A. M. (2014). Sodium-coupled dicarboxylate and citrate transporters from the SLC13 family. *Pflugers Archiv: European journal of physiology*, **466**(1), 119–130.

Peti-Peterdi, J. (2010). High glucose and renin release: The role of succinate and GPR91. *Kidney International*, **78**(12), 1214–1217.

Potente, M., & Carmeliet, P. (2017). The link between angiogenesis and endothelial metabolism. *Annual Review of Physiology*, **79**(1), 43–66.

Reddy, A., Bozi, L. H. M., Yaghi, O. K., Mills, E. L., Xiao, H., Nicholson, H. E., Paschini, M., Paulo, J. A., Garrity, R., Laznik-Bogoslavski, D., Ferreira, J. C. B., Carl, C. S., Sjøberg, K. A., Wojtaszewski, J. F. P., Jeppesen, J. F., Kiens, B., Gygi, S. P., Richter, E. A., Mathis, D., & Chouchani, E. T. (2020). pH-gated succinate secretion regulates muscle remodeling in response to exercise. *Cell*, **183**(1), 62–75.e17.

Sabadell-Basallote, J., Astiarraga, B., Castaño, C., Ejarque, M., Repollés-de-Dalmau, M., Quesada, I., Blanco, J., Núñez-Roa, C., Rodríguez-Peña, M. M., Martínez, L., de Jesus, D. F., Marroquí, L., Bosch, R., Montanya, E., Sureda, F. X., Tura, A., Mari, A., Kulkarni, R. N., Vendrell, J., & Fernández-Veledo, S. (2024). SUCNR1 regulates insulin secretion and glucose elevates the succinate response in people with prediabetes. *Journal of Clinical Investigation*, **145**, 155630.

Sapieha, P., Sirinyan, M., Hamel, D., Zaniolo, K., Joyal, J. S., Cho, J. H., Honoré, J. C., Kermorvant-Duchemin, E., Varma, D. R., Tremblay, S., Leduc, M., Rihakova, L., Hardy, P., Klein, W. H., Mu, X., Mamer, O., Lachapelle, P., di Polo, A., Beauséjour, C., … Chemtob, S. (2008). The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nature Medicine*, **14**(10), 1067–1076.

Serena, C., Ceperuelo-Mallafré, V., Keiran, N., Queipo-Ortuño, M. I., Bernal, R., Gomez-Huelgas, R., Urpi-Sarda, M., Sabater, M., Pérez-Brocal, V., Andrés-Lacueva, C., Moya, A., Tinahones, F. J., Fernández-Real, J. M., Vendrell, J., & Fernández-Veledo, S. (2018). Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *International Society for Microbial Ecology Journal*, **12**(7), 1642–1657.

Shou, C., Wei, Y. M., Wang, C., & Yang, H. X. (2019). Updates in long-term maternal and fetal adverse effects of gestational diabetes mellitus. *Maternal-Fetal Medicine*, **1**(2), 91–94.

Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., Frezza, C., Bernard, N. J., Kelly, B., Foley, N. H., Zheng, L., Gardet, A., Tong, Z., Jany, S. S., Corr, S. C., Haneklaus, M., Caffrey, B. E., Pierce, K., Walmsley, S., … O'Neill, L. A. (2013). Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature*, **496**(7444), 238–242.

Toma, I., Kang, J. J., Sipos, A., Vargas, S., Bansal, E., Hanner, F., Meer, E., & Peti-Peterdi, J. (2008). Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *Journal of Clinical Investigation*, **118**, 2526–2534.

Trauelsen, M., Hiron, T. K., Lin, D., Petersen, J. E., Breton, B., Husted, A. S., Hjorth, S. A., Inoue, A., Frimurer, T. M., Bouvier, M., O'Callaghan, C. A., & Schwartz, T. W. (2021). Extracellular succinate hyperpolarizes M2 macrophages through SUCNR1/GPR91-mediated gq signaling. *Cell reports*, **35**(11), 109246.

Vargas, S. L., Toma, I., Jung, J. K., Meer, E. J., & Peti-Peterdi, J. (2009). Activation of the succinate receptor GPR91 in macula densa cells causes renin release. *Journal of the American Society of Nephrology*, **20**(5), 1002–1011.

Villanueva-Carmona, T., Rodríguez-Peña, M. M., Núñez-Roa, C., Maymó-Masip, E., Repollés-de-Dalmau, M., Badia, J., Keiran, N., Mirasierra, M., Pimenta-Lopes, C., Sabadell-Basallote, J., Bosch, R., Caubet, L., Escolà-Gil, J. C., Fernández-Real, J. M., Vilarrasa, N., Ventura, F., Vallejo, M., … Fernández-Veledo, S. (2023). SUCNR1 signaling in adipocytes controls energy metabolism by modulating circadian clock and leptin expression. *Cell Metabolism*, **35**(4), 601–619.e10.

Wang, T., Xu, Y. Q., Yuan, Y. X., Xu, P. W., Zhang, C., Li, F., Wang, L. N., Yin, C., Zhang, L., Cai, X. C., Zhu, C. J., Xu, J. R., Liang, B. Q., Schaul, S., Xie, P. P., Yue, D., Liao, Z. R., Yu, L. L., Luo, L., Zhou, G., … Jiang, Q. Y. (2019). Succinate induces skeletal muscle fiber remodeling via SUCNR1 signaling. *European Molecular Biology Organization Reports*, **20**(9), 1–16.

Wen, B., Zheng, Z., Wang, L., Qian, X., Wang, X., Chen, Y., Bao, J., Jiang, Y., Ji, K., & Liu, H. (2022). HIF-1α is essential for the augmentation of myometrial contractility during labor. *Biology of Reproduction*, **107**(6), 1540–1550.

Winther, S., Trauelsen, M., & Schwartz, T. W. (2021). Protective succinate-SUCNR1 metabolic stress signaling gone bad. *Cell Metabolism*, **33**(7), 1276–1278.

Zhang, H., Zheng, J., Lin, J., Chen, J., Yu, Z., Chen, C., & Liu, T. (2018). miR-758 mediates oxLDL-dependent vascular endothelial cell damage by suppressing the succinate receptor SUCNR1. *Gene*, **663**, 1–8.

Additional information

Data availability statement

We confirm that the all of the data supporting the findings of the present study are available within the published article.

Competing interests

The authors declare that they have no competing financial interests.

Author contributions

S.K, A.M, J.V and S.F.V were responsible for study conceptualization. S.K, F.A.C, E.M, A.G, M.I, M.B, N.V, E.M, A.P, E.W and A.K.S were responsible for the methodology. S.K, F.A.C and A.M were responsible for the formal analysis. S.K, U.H, F.A.C and E.M were responsible for investigations. A.M, J.V and S.F.V were responsible for resources. S.K was responsible for writing the original draft. S.K, F.A.C, A.M, M.B, J.V and S.F.V were responsible for reviewing and editing the manuscript. A.M, J.V and S.F.V were responsible for supervision. A.M, J.V and S.F.V were responsible for funding. All authors have read and approved the final published version of the manuscript submitted for publication.

Funding

This research was supported by grants RTI2018-093919-B-100 and PID2021-122480OB-I00 (to SFV) from the Spanish Ministry of Science and Innovation (MCIN; MCIN/AEI/10.13039/501100011033) and by the European Regional Development Fund (ERDF), 'ERDF, A way of making Europe.' This research also received funding from 'la Caixa' Foundation (ID 100010434) under grant agreement LCF/PR/HR20/52400013 (to SFV); from the Instituto de Salud Carlos III (PI20/00338 to JV and PI 18/00516 and PI21/01479 to AM), cofinanced by the ERDF; and from CIBER (Consorcio de Investigación Biomédica en Red; CB07708/0012), Instituto de Salud Carlos III. SK was funded by a predoctoral fellowship from AGAUR, Spain (2020FI_B00980). NVR is a recipient of a grant from the Joan Oro for predoctoral research personnel in

training (2024 -FI-100961). SFV and JV acknowledge support from the Agency for Management of University Research Grants of the Generalitat de Catalunya (2021 SGR 01409 and 2021 SGR 00829). SFV acknowledges support from the Miguel Servet Tenure-Track Program (CP10/00438 and CPII16/00008) from the Fondo de Investigación Sanitaria, co-funded by the ERDF.

Acknowledgements

We express particular gratitude to the patients and the Institut d'Investigació Sanitària Pere Virgili BioBank (PT17/0015/0029), integrated into the Spanish National BioBank Ne twork (C.0003609), for their collaboration.

Keywords

angiogenesis, gestational diabetes mellitus, HUVEC, pregnancy, succinate, SUCNR1

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History
Supplementary Figure