The birth of cardiac disease: Mechanisms linking gestational diabetes mellitus and early onset of cardiovascular disease in offspring

Carolina Tocantins1 | Mariana S. Diniz1 | Luís F. Grilo1,2 | Susana P. Pereira1,3

1CNC—Center for Neuroscience and Cell Biology, CIBB—Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
2PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal
3Laboratory of Metabolism and Exercise (LametEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sport, University of Porto, Porto, Portugal

Correspondence
Susana P. Pereira, CNC—Center for Neuroscience and Cell Biology, CIBB—Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal.
Email: pereirasusan@gmail.com

Funding information
Funded by the European Regional Development Fund (ERDF) funds through the Operational Programme for Competitiveness - COMPETE 2020 and national funds by Foundation for Science and Technology under FCT-Post-doctoral Fellowship (SFF, SFRH/BD/116061/2016), FCT-doctoral Fellowship (LFG, SFRH/BD/5539/2020), project grant PTDC/DTP-DES/1082/2014 (POCI-01-0145-FEDER-016657), strategic projects UIDB/04539/2020; UID/04539/2020; UID/DTP/00617/2020 and LA/P/0064/2020; and the H2020 funding No.722619 (FOIE GRAS) and No.734719 (mFOIE GRAS).

Edited by: João Ramalho-Santos, Editor

Abstract
Cardiovascular disease (CVD) is the biggest killer worldwide, composing a major economic burden for health care systems. Obesity and diabetes are dual epidemics on the rise and major risk factors predisposing for CVD. Increased obesity- and diabetes-related incidence is now observed among children, adolescents, and young adults. Gestational diabetes mellitus (GDM) is the most common metabolic pregnancy disorder, and its prevalence is rapidly increasing. During pregnancies complicated by GDM, the offspring are exposed to a compromised intrauterine environment characterized by hyperglycemic periods. Unfavorable in utero conditions at critical periods of fetal cardiac development can produce developmental adaptations that remodel the cardiovascular system in a way that can contribute to adult-onset of heart disease due to the programming during fetal life. Epidemiological studies have reported increased cardiovascular complications among GDM-descendants, highlighting the urgent need to investigate and understand the mechanisms modulated during fetal development of in utero GDM-exposed offspring that predispose an individual to increased CVD during life. In this manuscript, we overview previous studies in this area and gather evidence linking GDM and CVD development in the offspring, providing new insights on novel mechanisms contributing to offspring CVD programming by GDM, from the role of maternal–fetal interactions to their impact on fetal cardiovascular development, how the perpetuation of cardiac programming is maintained in postnatal life, and advance the intergenerational implications contributing to increased CVD premature origin. Understanding the perpetuation of CVD can be the first step to manage and reverse this leading cause of morbidity and mortality.

This article is categorized under:
1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide (Roth et al., 2020; Schnall et al., 2016). In 2019, CVD led to 18.6 million deaths, constituting up to 32% of global deaths. One in five people aged <70 years old will die prematurely due to CVD (Roth et al., 2020). In Europe, CVD accounted for 45% of all deaths in 2017 (Wilkins et al., 2017), and nearly 85 million people were living with CVD in 2015 (Wilkins et al., 2017). CVD-related costs represented 8% of total health care expenditure across the European Union (EU; Wilkins et al., 2017), with direct health care costs reaching 111 billion euros of a total of 210 billion euros per year (Wilkins et al., 2017). Despite the decreasing CVD mortality rates observed in the past years (Schnall et al., 2016), the decline rate is now deaccelerating (Roth et al., 2020) due to the increased incidence of associated risk factors (e.g., hypertension, obesity, and diabetes; Schnall et al., 2016; Yu et al., 2019). For the first time in 50 years, an increase in premature CVD death (<65 years of age) was reported in some EU countries (Timmis et al., 2018), which was noticed even before the COVID-19 outbreak. COVID-19 pandemic dramatically aggravated the situation for more than 520 million people living with CVD (Roth et al., 2020).

CVD belongs to the group of non-communicable diseases (NCDs; Blüher, 2019; WHO, 2018). NCDs contribute to 71% of all deaths, killing 41 million people each year (Blüher, 2019; WHO, 2018). The United Nations set the goal of reducing NCDs-related premature mortality by 30% in the 2030 Agenda for Sustainable Development. Modifiable risk factors contributing to increased CVD risk include high systolic blood pressure, body mass index (BMI), fasting plasma glucose, dietary risks (underconsumption of, e.g., fruits, vegetables and overconsumption of, e.g., processed meat, sugar-sweetened beverages), low physical activity, among others, all of which predispose to metabolic syndrome disorders (George et al., 2010; Roth et al., 2020; Wilkins et al., 2017). Obesity and diabetes are direct risk factors for CVD, constituting global rampant epidemics (Scherer & Hill, 2016). Not only observed in adults, but also extends to the pediatric population, with an estimated 3% overall increase annually for type-1 diabetes mellitus (T1DM) incidence (Atlas, 2019). While T2DM was considered an exclusive condition of adulthood, its prevalence among children and adolescents is increasing in certain countries (Pastore et al., 2020). In Japan, T2DM is more common than T1DM in the pediatric population (Atlas, 2019).

Gestational diabetes mellitus (GDM) is the most common metabolic disorder of pregnancy (Chiefari et al., 2017), with maternal age and obesity being important risk factors for GDM development (Li et al., 2020). The prevalence of overweight and obesity is rising in women of reproductive age (affecting up to 50%), highly contributing to an augmented prevalence of GDM (Rodrigo & Glastras, 2018; Shirley Muller & Nirmala, 2018). GDM is defined as any degree of glucose intolerance developed in late gestation after clearly dismissing pregestational diabetes. The worldwide reported GDM prevalence varies from 1% to 45% of pregnancies (Lawrence et al., 2019). This high prevalence discrepancy among studies results from the lack of consistent diagnosis criteria and study population geographic characteristics (McIntyre et al., 2019). Globally, 13.9% GDM-pregnancies were estimated (Li et al., 2020). Currently, most entities adopt the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study criteria (Metzger, 2010), consisting of a 75 g oral glucose tolerance test between the 24th and 32nd week of gestation. Based on the International Association of Diabetes and Pregnancy Study Groups criteria, a GDM diagnosis is considered when one or more of the following tests have increased values after the OGTT: fasting glucose plasma ≥5.1 mmol/L; 1-h plasma glucose ≥10.0 mmol/L; and 2-h plasma glucose ≥8.5 mmol/L (ADA, 2018a; Egan & Dunne, 2019; Metzger, 2010; Metzger et al., 2008; WHO, 2013).

Since maternal insulin cannot cross the placenta, oppositely to maternal glucose, controlling glucose levels in maternal circulation during GDM is crucial (Westermeyer et al., 2014). In response to the elevated maternal glucose circulating levels that are transferred to the fetus by the placenta, the fetal pancreas overstimulates insulin production, promoting growth and adiposity (Q. Chen et al., 2018). GDM’s treatment is multidisciplinary (Kintiraki & Goulis, 2018).
and aims to reduce blood glucose levels during pregnancy to prevent hyperglycemia-related adverse pregnancy outcomes (e.g., preeclampsia, gestational hypertension) and offspring short-term (e.g., macrosomia, shoulder dystocia, preterm birth; Johns et al., 2018) and long-term complications (e.g., glucose intolerance, obesity, increased risk of NCDs; Dong et al., 2013; Farahvar et al., 2019). Lifestyle changes are the first-line GDM treatment including dietary modifications based on medical nutritional advice and physical exercise with appropriate monitoring (ADA, 2018b; Kintiraki & Goulis, 2018). When this approach fails, pharmacological treatment (insulin and/or oral hypoglycemic agents) is required (McIntyre et al., 2019). It is highly controversial which pharmacological treatment should be administered, especially due to potential offspring-related adverse effects (e.g., neonatal hypoglycemia, macrosomia, admission in the intensive care unit; Bao et al., 2019; Guo et al., 2019; Nachum et al., 2017; Sénat et al., 2018). While efforts are being gathered to establish the best GDM treatment, in the middle time, GDM-treated mothers and offspring still exhibit an increased risk of short- and long-term complications (Farahvar et al., 2019).

The relationship between early-life environmental factors and the development of metabolic disturbances later in life was first pointed by Barker, opening the hypothesis that an adverse intrauterine environment programs the fetus to life-long deleterious consequences (Barker, 2004), namely CVD (Barker, 2000). Studies in this field continue to emerge (Loche et al., 2018; Pereira, Diniz, et al., 2021; Pereira, Tavares, et al., 2021, PMID: 33899910). In utero exposure to GDM is associated with long-term pediatric complications (Abokaf et al., 2018; Gu et al., 2019). Particularly, offspring born to GDM-mothers (OGDM) are at augmented risk of developing obesity, T2DM, and heart disease earlier in life (Chiefari et al., 2017; McIntyre et al., 2019). These observations have been supported by a series of epidemiological studies, summarized in Table 1. Noticeably, being exposed to GDM has been associated with incident diabetes and other disorders of the metabolic syndrome in childhood and youth (Blotsky et al., 2019; Gu et al., 2019), another indication that “diabetes begets diabetes” (Damm et al., 2016). Meanwhile, this cycle continues, predisposing OGDM to develop CVD in adulthood or even childhood (Leybovitz-Haleluya et al., 2018; Yu et al., 2019). More research is needed to clarify and understand the underlying pathological mechanisms linking GDM and increased CVD incidence in OGDM. Herein, we advance a fresh perception on molecular pathways contributing to CVD development in OGDM, covering the latest research on the influence of nitric oxide (NO) metabolism during fetal development and the crucial mitochondrial adaptations that play a critical role in cardiac function after birth. Epigenetics along with recent insights into the vicious cycle of CVD perpetuation potentiate the opportunity to early risk surveillance or even risk modification facilitating the decrease of CVD morbidity and mortality.

2 | KNOW TO TREAT: MECHANISMS BEHIND CARDIOVASCULAR DEVELOPMENTAL PROGRAMMING

2.1 | The impact of GDM on the placenta, the bridge between maternal–fetal interactions

The placenta operates exclusively during pregnancy, having an essential role in fetal nourishing and protection during fetal development, through the mediation of maternal and fetal interactions, with metabolic, endocrine, excretory, and immunologic associated functions (Gauster et al., 2012). Since it has been observed that adverse fetal outcomes are related to a pathological placental and, therefore, intrauterine environment, the placenta is considered “a mirror,” “reflecting” both the maternal and fetal health states (Mehare & Kebede, 2020).

GDM induces a dysregulation in placental morphology and vasculature (Huynh et al., 2015). Increased placental weight and size (Lao et al., 1997; Taricco et al., 2003) occur as an attempt to increase the surface of oxygen exchange and counteract the GDM-associated fetal hypoxia, which is characterized by an unbalance between oxygen demands and supply (Gauster et al., 2012). Other morphologic features such as chorangiosis, marked by an increased number of capillaries in the periphery of chorionic villi, have been registered (Aldahmash et al., 2021; Augustine & Pulikkathodi, 2016; Daskalakis et al., 2008). However, the GDM-induced morphological and vascular alterations (e.g., calcification, decidual vasculopathy, villous edema, among others; Aldahmash et al., 2021) may further exacerbate the hypoxic intrauterine environment (Figueroa et al., 1993).

The hypoxic intrauterine environment associated with GDM-human pregnancies has been evidenced through the evaluation of markers such as erythrocytosis, nucleated red blood cells, and arterial redistribution, or cardiotocographic monitoring (Bhorat et al., 2021; Tarvonen et al., 2021). Prenatal hypoxia can trigger an abnormal increase in reactive
<table>
<thead>
<tr>
<th>Author and year</th>
<th>Country</th>
<th>GDM diagnosis</th>
<th>Exclusion criteria</th>
<th>Observed outcomes in GDM-exposed offspring</th>
<th>Follow-up (years)</th>
<th>Number of GDM/no GDM</th>
<th>Period of GDM-pregnancies (year-year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abokaf et al. (2018)</td>
<td>Israel</td>
<td>According to ACOG two-step approach: OGTT (24–28 weeks), 100 g 3-h glucose tolerance in positive women in OGTT</td>
<td>Perinatal deaths, multiple gestations, mothers with pre-gestational diabetes, lack of prenatal care, congenital malformations, and abnormal karyotype</td>
<td>Higher birth weight, elevated rate of hospitalizations with a diagnosis of endocrine morbidity, higher incidence of diabetes mellitus, overweight, and obesity in children</td>
<td>According to hospitalization or until censored</td>
<td>12,642/21,862</td>
<td>1988–2014</td>
</tr>
<tr>
<td>Blotsky et al. (2019)</td>
<td>Canada</td>
<td>Non-specified (according to hospital)</td>
<td>Father and/or mother with prior diabetes</td>
<td>Two-fold higher incidence of pediatric diabetes before the age of 22</td>
<td>22</td>
<td>36,590/36,590</td>
<td>1990–2007</td>
</tr>
<tr>
<td>Grunnet et al. (2017)</td>
<td>Denmark</td>
<td>ICD-10 classifications O244 and O249 of the Danish National Patient Register and self-diagnosed women</td>
<td>Multiple births, only the first sibling was included</td>
<td>Higher weight, BMI, waist-to-hip ratio, systolic blood pressure, resting heart rate, fasting plasma glucose, insulin, C-peptide, HOMA-IR, and plasma triglycerides. Lower height and decreased HDL levels.</td>
<td>9–16</td>
<td>561/597</td>
<td>1996–2002</td>
</tr>
<tr>
<td>Gu et al. (2019)</td>
<td>China</td>
<td>1-h 50-g glucose screening test and if positive subsequent 2-h OGTT with 75 g glucose load</td>
<td>Non-specified</td>
<td>Increased Z score for BMI-for-age, higher risk of childhood overweight</td>
<td>1–2</td>
<td>1263/704</td>
<td>2005–2009</td>
</tr>
<tr>
<td>Kaseva et al. (2017)</td>
<td>Finland</td>
<td>OGTT after overnight fasting by using a 75 g oral glucose load and abnormal values considered according to national guidelines</td>
<td>Offspring born to mothers with type 1 and type 2 diabetes, subjects with cerebral palsy, mental disability, severe physical disability</td>
<td>Higher fat percentage, fat mass, and waist circumference</td>
<td>20</td>
<td>191/547</td>
<td>1985–1989</td>
</tr>
<tr>
<td>Kearney et al. (2018)</td>
<td>Canada</td>
<td>According to medical records</td>
<td>Children born from a pregnancy complicated by type 1 and type 2 diabetes</td>
<td>Increased waist circumference, total and abdominal adiposity, decreased lean mass and altered glycemic profile</td>
<td>3–12</td>
<td>56/30</td>
<td>2003–2013</td>
</tr>
<tr>
<td>Author and year</td>
<td>Country</td>
<td>GDM diagnosis</td>
<td>Exclusion criteria</td>
<td>Observed outcomes in GDM-exposed offspring</td>
<td>Follow-up (years)</td>
<td>Number of GDM/no GDM</td>
<td>Period of GDM-pregnancies (year-year)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Tam et al. (2017)</td>
<td>China</td>
<td>75 g OGTT between 24 and 32 weeks of gestation</td>
<td>Higher rates of abnormal glucose tolerance, overweight or obesity, BMI, blood pressure, and trend toward reduced β-cell function</td>
<td>7</td>
<td></td>
<td>132/794</td>
<td>2000–2006 (HAPO follow-up study)</td>
</tr>
<tr>
<td>Yu et al. (2019)</td>
<td>Denmark</td>
<td>Non-specified (according to hospital)</td>
<td>Babies with congenital heart disease</td>
<td>Increased rates of early onset CVD from childhood to early adulthood</td>
<td>40</td>
<td>26,272/non-specified</td>
<td>1977–2016</td>
</tr>
</tbody>
</table>

Note: Observed human offspring complications at different ages due to in utero exposure to GDM diagnosed differently between countries. Studies were selected with the aim of (1) covering different ages of the offspring's life, from birth until adulthood; (2) including exclusion criteria that ruled out prior diabetes, type 1 and 2 diabetes mellitus, and others; and (3) denoting the inconsistent diagnosis criteria between different countries worldwide.

Abbreviations: ACOG, American College of Obstetricians and Gynecologists; ICD, International statistical classification of diseases and related health problems.
oxygen and nitrogen species (ROS, RNS) in the placenta (Aljunaidy et al., 2017). On the one hand, a hypoxic environment leads to decreased activity of the mitochondrial electron transport chain (ETC) complexes and activation of NADPH oxidase, thus increasing the production of mitochondrial superoxide anion ($O_2^-$), which in physiological concentrations acts as a signaling molecule, but excessive $O_2^-$ concentrations lead to macromolecules alterations and cell damage (Aljunaidy et al., 2017). At the same time, a low oxygenic tension environment leads to increased levels of cytosolic calcium ($Ca^{2+}$), activating calmodulin, a multifunctional intermediate calcium-binding messenger protein classically described as a regulator of cardiac excitation–contraction coupling, and recently identified as inflammation mediator in CVD (Junho et al., 2020; Morton et al., 2016). Consequently, nitric oxide synthase (NOS) activity is increased, leading to increased production of NO (Morton et al., 2016). Paradoxically, NO reduced bioavailability was described in the human umbilical cord endothelial cells in GDM pregnancies (Di Fulvio et al., 2014; Westermeier et al., 2011). This can be in part explained by the GDM-like oxidative environment, where the production of reactive oxygen species is increased (Di Fulvio et al., 2014; Gauster et al., 2012). The ROS can react with NO, forming peroxynitrite (ONOO$^-$), a highly reactive molecule that can induce nitrosative damage (Morton et al., 2016).

The compromised NO metabolism pathway found in the umbilical vein cord from human pregnancies complicated by GDM (Contreras-Duarte et al., 2021; L. Sobrevia et al., 1998; Vásquez et al., 2004) has been proposed to lead to increased levels of the NO precursor L-Arg transport, increased protein levels and activity of the cationic amino acid transporter (CAT-1), and increased NO synthesis (Contreras-Duarte et al., 2021; L. Sobrevia et al., 1998; Vásquez et al., 2004). Overstimulation of NO metabolism in the fetoplacental unit in GDM-characterized pregnancies is associated with vascular dysfunction (Cabalin et al., 2019; Cornejo et al., 2021; Jensen et al., 2016; Valero et al., 2021), through a possible rise in ONOO$^-$ levels (Morton et al., 2016). This free radical is involved in lipid peroxidation, causing cell damage through cell membrane disruption (Cornejo et al., 2021; Morton et al., 2016). The vascular fetoplacental dysfunction has been reported as being partly caused by the GDM-induced inflammation, as pointed out by increased levels of monocytes in GDM-maternal blood (Angelo et al., 2018), and increased levels of pro-inflammatory cytokines, such as TNF-α, and IL-6 in GDM-maternal serum levels (Cabalin et al., 2019). Nevertheless, the inflammatory response induced by GDM demands further investigation.

Overall, NO comes across as a critical player in the development of GDM-associated complications (Figure 1), firstly, due to NO vasodilation function, and secondly, due to its role in the regulation of mitochondrial function

**FIGURE 1** Gestational diabetes mellitus (GDM) and the impact on the fetoplacental unit, leading to adverse cardiovascular outcomes for the offspring. GDM induces increased pro-inflammatory biomarkers, blood glucose levels and insulin resistance in maternal blood stream. Placental adaptations to GDM include placental increased weight and size, placental hypoxia, marked by increased chorangiosis. An environment characterized by hypoxia could induce placental metabolic adaptations, namely an inhibition of mitochondrial oxidative phosphorylation system (OXPHOS), and increased levels of reactive oxidative and nitrosative species (ROS, RNS). In GDM-human placentas increased levels of cytosolic calcium are observed, with increased activation of calmodulin. In GDM-human umbilical cord veins, nitric oxide (NO) metabolism is impaired. Increased levels and activity of cationic amino acid transporter (CAT-1), l-arginine (NO precursor) transporter led to an increased production of NO. However, the bioavailability of NO has been found to be decreased. These mechanisms could be in the origin of vascular endothelial dysfunction in GDM-human umbilical vein cord. Vascular dysfunction has been associated with several adverse cardiovascular outcomes in the fetus, which lead to an increased predisposition to obesity, diabetes, and early onset of cardiovascular disease (CVD). LDL, low density lipoproteins; TGs, triglycerides
Mitochondria are essential for adenosine triphosphate (ATP) production to fuel placental development (Sferruzzi-Perri, 2021). In fetoplacental endothelium of pregnancies complicated by GDM, increased levels of placental NO lead to the inhibition of mitochondrial ETC complex IV activity and induction of mitochondrial biogenesis (Sobrevia et al., 2020). Indeed, human placenta resultant from GDM pregnancies present decreased activity of the oxidative phosphorylation system (OXPHOS; Muralimanoharan et al., 2016), decreased mitochondrial respiration, and decreased ATP production (Fisher et al., 2021), along with increased fusion events (as evidenced by predominantly elongated mitochondria and altered expression of proteins from the mitochondrial fusion and fission machinery, such as optic atrophy protein 1 [OPA1] and active dynamin-related protein 1 [DRP1]), possibly as a compensatory mechanism (Abbade et al., 2020). GDM-associated alterations in placental morphology, metabolism, and mitochondrial function lead to an impairment in placental development, thus affecting placental buffering ability, placental excretome, and the transport of nutrients required for suitable fetal cardiovascular system development (Cornejo et al., 2021; Fisher et al., 2021; Gauster et al., 2012).

### 2.2 The contribution of NO to GDM-induced vascular dysfunction

The fetal vascular system's main function is to sustain embryonic growth, supporting blood flow across the embryo (Y. M. Jin et al., 2013). This system is composed of a network of tubular structures of different sizes that are irregularly distributed across each tissue (Jensen et al., 2016; Y. M. Jin et al., 2013; Yi Mei Jin et al., 2019). Vascular function is highly dependent on NO signaling. The vasoactive action of NO begins through the activation of the enzyme soluble guanylyl cyclase (sGC), by binding to the heme group of sGC (Ignarro et al., 1999). This enzyme is responsible for activating cyclic guanosine monophosphate (cGMP), which in turn, induces cGMP dependent-PKG, which is responsible for reducing the intracellular levels of calcium, through the action on calcium ion channels, inducing vasodilatation (Yao et al., 2016).

Vascular endothelial dysfunction has been highly associated with the development of hypertension and other types of CVD (de Sá et al., 2017). During pregnancy STZ-induced GDM dams' 120 day-old offspring present increased aortic vasodilation associated with an impaired endothelial function (Porto et al., 2010). In addition, 6- and 12-month-old male rat offspring born to mothers with GDM, induced with STZ during pregnancy, present increased markers of hypertension (de Sá et al., 2017). Interestingly, this could be due to NO reduced bioavailability and release, which was already described in the human umbilical endothelial cells in GDM pregnancies (Villalobos-Labra et al., 2018). Further research is demanded regarding the GDM-offspring NO metabolism and its contribution to CVD early development.

### 2.3 GDM induced fetal cardiac anomalies: The impairment of cardiogenesis

The fetal heart is the first organ to acquire function during fetal development. In humans, the blood flow begins after the eighth week of gestation, the heart continues to grow, having its term in the postnatal stage through hypertrophy (Sharon L. Paige et al., 2015). During postnatal hypertrophy, the heart loses the majority of the regenerative capacity (Abdulla et al., 2004; Muñoz-Chápuli & Pérez-Pomares, 2010).

GDM impairs cardiogenesis (Chaudhari et al., 2008; Dervisoglu et al., 2018), having a deleterious effect. It was estimated, through the available research, that infants born to mothers portraying GDM who needed insulin during the third trimester of pregnancy are around 20 times more likely to develop major anomalies regarding the cardiovascular system (i.e., malformations) than those born to non-GDM mothers, with an absolute risk for infants from diabetic mothers around 9.7% (Becerra et al., 1990; Zhang et al., 2022, PMID: 35104296). Insulin-binding cell receptors control fetal cardiac development and growth, being more abundant in the interventricular septum (Dervisoglu et al., 2018), regulating substrates needed for myocardial growth, therefore playing an essential role in physiological hypertrophy (Dale Abel, 2021). An environment characterized by increased levels of insulin, which is typical in the amniotic fluid of early-stage pregnancies characterized by GDM (O’Neill et al., 2018), might lead to myocardial hypertrophy, which requires glycogen, myocardial protein, and fat synthesis to occur (Chaudhari et al., 2008; Dervisoglu et al., 2018). Fetuses of GDM-mothers present increased cardiac muscle mass, increased intraventricular septal thickness, overall pathological cardiac hypertrophy with decreased cardiac function (Depla et al., 2021; Dervisoglu et al., 2018). The structural and morphological cardiac impairment induced by GDM are accompanied by myocardial dysfunctional (Depla et al., 2021), GDM-fetuses present increased heart rate, and impaired diastolic function (Depla et al., 2021). Although,
in some cases, pathological hypertrophy can be reversed in a post-natal stage. GDM-induced fetal heart structural, morphological, and functional impairment are suggestive that in a postnatal stage these alterations endure and produce long-lasting effects (Figure 1). Indeed, children and adolescents born to GDM-mothers present increased risk factors of CVD (Pathirana et al., 2019). This was also observed in the fetal stage, highlighting the perpetuation of cardiac dysfunction throughout, at least, adolescence (Pathirana et al., 2019). Thus, later in life, GDM could lead to offspring’s cardiac dysfunction that, if not controlled, could culminate in early-onset CVD development.

2.4 Developmental programming of cardiac mitochondrial biology

Metabolic and mitochondrial programming are hallmarks of GDM-related developmental programming in adult offspring (Hebert & Myatt, 2021). Impaired GDM-related maternal metabolism has been associated with compromised mitochondrial function in the placenta and other offspring tissues at the fetal stage (Fisher et al., 2021; Stevanović-Silva et al., 2021). GDM placentas present lower gene and protein expression of mitochondrial fusion markers and mitochondrial biogenesis-related proteins, independently of maternal BMI (Abbade et al., 2020; Kolac et al., 2021), and slower OXPHOS due to reduced FΟ,F1-ATP synthase activity (Luis Sobrevia et al., 2020). Altered placental function in GDM (Section 2.1) is at least partially responsible for developmental programming due to compromising fetoplacental vascular function and metabolic homeostasis (Goto et al., 2021). Wharton’s Jelly stem cells obtained from GDM umbilical cords presented lower expression of mitochondrial regulatory genes and reduced stemness properties compared with healthy pregnancies (Kong et al., 2019). Compromised stemness in fetal stem cells might be related to an impairment in fetal and postnatal tissue regeneration and function.

Usually, offspring from GDM-pregnancies present normal heart function at birth and start to display signals of compromised function in advanced stages of adult life. Fifteen-week-old C57BL/6 mice born to diet-induced GDM mothers show lower ejection fraction, fractional shortening, and increased left ventricle (LV) systolic volume (Cole et al., 2021). In late-gestation STZ GDM-induced Sprague–Dawley rats, a decrease in the systolic ejection fraction is only observed at 12 months old (Louwagie et al., 2020).

Mitochondria play a vital role in cardiac function and metabolism (Chistiakov et al., 2018; Pereira, Tavares et al., 2021). During the prenatal–postnatal transition, heart metabolism shifts from glycolytic-dependent to majorly fatty-acid oxidation- and mitochondrial respiration-dependent (Grilo et al., 2021). Due to the limited cardiac regeneration (Bishop et al., 2021), the developmental programming of heart mitochondrial function during a GDM-pregnancy can affect the cardiometabolic signaling throughout offspring life. Several methodologies can be used for assessing mitochondrial function, providing different parameters with complementary information (for review please read Brand et al., 2013; Gnaiger & MitoEAGLE Task Group, 2019; and Picard & McEwen, 2018).

After birth, mitochondria from GDM-offspring continue to show alterations in several animal models. Neonatal rat cardiomyocytes exposed to prenatal STZ-induced GDM showed increased mitochondrial membrane potential (MMP) and a higher rate of MMP loss (Louwagie et al., 2021). When GDM is STZ-induced at late gestation, neonatal male rat cardiomyocytes show short and wide mitochondria along with fewer mitochondrial fusion events (Larsen et al., 2019). Neonatal rat cardiomyocytes exposed to STZ-induced GDM present decreased basal, maximal and spare oxygen consumption rate (OCR), and palmitate-induced respiration (Mdaki et al., 2016) likely due to the metabolic immature phenotype of the cardiomyocytes. In 15-week-old C57BL/6 mice, GDM-offspring subjected to control diet presented increased basal respiration, states III, and IV and spare capacity for cardiac mitochondrial respiration stimulated through complex-I, but unaffected mitochondrial respiration when supported by complex-II (Cole et al., 2021). Our unpublished data of a high fat high sugar (HFHS) diet-induced GDM Sprague–Dawley rat support the increase of cardiac mitochondrial respiratory control ratio (RCR) at 32-weeks-old when supported with complex-I but not complex-II substrates in male offspring (Pereira, Diniz, et al., 2021; Rodrigues et al., 2019). In Sprague–Dawley rats exposed to late-gestation GDM induced by STZ injection, the mitochondrial basal respiration and proton leak were decreased at 3-weeks but unchanged at 10 weeks, 6 months, and 12 months of age. Nevertheless, lower mitochondrial maximum respiration, respiratory control ratio (RCR, calculated as state 3/state4), respiratory reserve capacity, and palmitate-associated OCR were found in 6-month-old offspring (Louwagie et al., 2020). These results suggest a potential recovery of the immature cardiomyocyte phenotype in offspring cardiac mitochondrial function in early life that becomes prematurely compromised during aging. In another study using Sprague–Dawley rats exposed to high-fat diet and STZ, the mitochondrial OGDHM decreased respiration is concomitant with decreased neonatal rat cardiomyocyte basal, glucose-stimulated and oligomycin-dependent glycolytic capacity (Mdaki et al., 2016). Lower anaerobic glycolysis, confirmed by
the reduced proton production rate (Mdaki et al., 2016), suggested that the hyperglycemic milieu during heart development might have programmed offspring metabolism by, on the one hand, promoting glucose consumption to nucleotide biosynthesis through the pentose phosphate pathway (Nakano et al., 2017) and on the other hand, this metabolic shift can also be promoted by other mediators with maternal, fetal, or placental mitochondrial origin.

Potential mediators related to multiple CVD types, can be behind GDM-cardiac mitochondria programming and maternal–fetal communication, which include both metabolic organs and the maternal endothelium and involve ROS, mitochondrial DNA (mtDNA), pro-inflammatory cytokines, and lipid-derived signals (C. J. McElwain et al., 2020). GDM is often characterized by excessive tissue-specific and circulating ROS levels, usually detrimental to a successful pregnancy and fetal development (Joo et al., 2021). Placental and Wharton’s Jelly stem cells ROS generation is increased in GDM-pregnancy, exposing the fetus to oxidative damage (Hebert & Myatt, 2021; Kong et al., 2019). Fetal cells increased ROS exposure may promote oxidative damage in membrane lipids, impacting mitochondrial membrane permeability and OXPHOS function, deterioration in the mtDNA, due to lower repairing mechanisms and histone-deficiency potentiating mtDNA damage, and may alter other macromolecules structure and function, such as membrane transporters and antioxidant defenses (C. J. McElwain et al., 2020). Mitochondrial oxidative damage induced malfunction of the ETC and an inefficient ATP production, as observed in mice GDM model (Cole et al., 2021), both critical for heart activity. In turn, inefficient mitochondrial respiration promote ROS overgeneration in ETC complex-I and complex-III exacerbating oxidative disturbance in a damage feedback loop characteristic of several CVD types (Peoples et al., 2019). In accordance, higher cardiac lipid peroxidation have been found in neonatal rat cardiomyocytes exposed to STZ-induced GDM (Louwagie et al., 2021).

Accumulation of oxidative damage potentiates an antioxidant response or the activation of quality control mechanisms to maintain cardiac homeostasis. Fifteen-week-old rats exposed to STZ-induced GDM presented no alterations in heart antioxidant enzymes protein expression but increased autophagy-related proteins in male adult offspring (Raji et al., 2021). At 12 months old, GDM-exposed cardiomyocytes from Sprague–Dawley rats showed an elevated mitolysosomes number (Louwagie et al., 2020), suggesting an increased need for mitophagy-related mitochondrial quality control likely due to increased oxidative damage exposure. After the carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)-addition challenge, which results in the loss of the mitochondrial membrane potential forcing the mitochondrial respiratory chain to work its maximum ability, male cardiomyocytes presented a lower capability to...
form new mitolysosomes, potentially due to the already higher basal level or decreased reserve capacity (Louwagie et al., 2020).

This increase in auto/mitophagy suggests a state of cardiac mitochondrial dysfunction, coincident with mitochondrial membrane permeabilization and increased release of mtDNA to the cytosol or extracellular space (Peoples et al., 2019). Circulating mtDNA at 20-weeks of gestation positively correlated with future GDM diagnosis (C. McElwain & McCarthy, 2020). Increased BMI at the second trimester, a clear risk factor to GDM development, was correlated with higher cord blood mtDNA content (Sanchez-Guerra et al., 2019). However, at birth and at 6 months old, lower mtDNA levels were found in the offspring’s heart from late-gestation STZ-induced GDM in Sprague–Dawley rats (Louwagie et al., 2020; Mdaki et al., 2016).

Free mtDNA can trigger various inflammatory and apoptotic pathways once in the cytosol (Bronner & O’Riordan, 2016). Aberrant maternal immune cell adaption is related to GDM low-grade inflammation and poor fetal health outcomes (Kivelä et al., 2021; C. J. McElwain et al., 2021). It has been suggested a potential role of free mtDNA in promoting an immune response and dysregulating toll-like receptors (TLRs)-NLRP3 inflammasome activation (McCarthy et al., 2015; B. Wu et al., 2017; Zhong et al., 2018), which have been described to mediate cardiac disease (Zhou et al., 2018). The inflammasome plays a critical role in the development of atherosclerosis, coronary heart diseases (CHD), heart ischemia–reperfusion (I/R) injury, and NLRP3 inflammasome, thus early activation on these mechanisms due to developmental programming, can be on the offspring CVD genesis. Studies propose that the effective regulation of NLRP3 may help preventing CVD (Tong et al., 2020). Thus, it is likely that GDM stimulates the activation of the mtDNA-induced TLR-NLRP3 pathway, potentially being another “avenue” contributing to the programming of offspring’s future cardiac disease.

The hypothesis that mitochondria play a critical role in offspring disease programming is even more confrontational since most of the offspring's mitochondrial content has origin in the maternal oocyte mitochondria. Oocyte mitochondria have already been shown to present increased mtDNA damage and deficient mitochondrial respiration in diabetic and obese mice (Wang et al., 2009; L. L. Wu et al., 2015). As referred before, altered mitochondrial function and increased ROS production were also observed in GDM umbilical cord Wharton’s Jelly stem cells (Kong et al., 2019).

Neonatal rat cardiomyocytes exposed to STZ-induced GDM present increased apoptosis and mitochondrial membrane potential loss following metabolic stress (Louwagie et al., 2020, 2021). The apoptosis was rescued in male offspring, along with a boost in mitochondrial respiration, by the transference of rat healthy myocardial mitochondria (Louwagie et al., 2021).

These cardiometabolic conditions and mitochondrial alterations do not directly result in CVD but increase offspring susceptibility to disease (Figure 2). A small stimulus/challenge, that in a healthy organism does not directly lead to any pathology, in a compromised development programmed heart can unmask this condition and stimulate cellular signaling pathways that will culminate in early CVD development.

2.5 Epigenetics and CVD: Traits left behind that condition future cardiovascular health

Availability and type of substrate exposure (e.g., circulating nutrient type and concentration, gestational period, placental permeability) during the fetal stage can lead to long-term memory and programming of cellular metabolism through modulation of the epigenetic pattern (Grilo et al., 2021). This epigenetic memory can be a direct result of the cellular environment characteristics, such as substrate availability, or a consequence of the intracellular metabolic milieu (Jiménez-Chillarón et al., 2015; Matilainen et al., 2017). As previously discussed, exposure to GDM during fetal development modulates cardiomyocyte metabolism and cardiac mitochondrial function with metabolic consequences throughout the offspring’s life (Section 2.4). Thus, it is likely that altered metabolite concentrations, which are critical to the development of the epigenetic landscape, most with a mitochondrial origin, are also affected, having consequences in this long-term memory (Agarwal et al., 2018; Cavalli & Heard, 2019).

This mito-nuclear communication works in both directions (Figure 2). Mitochondria generate different metabolites at certain concentrations according to cellular metabolic rate, including acetyl-CoA, S-adenosyl methionine, α-ketoglutarate, or flavin adenine dinucleotide (Quirós et al., 2016). These metabolites are able to control the activity of epigenetic pattern regulators, including histone acetylation (histone acetyltransferases and deacetylases), DNA
methylation (DNA methyltransferases and ten–eleven translocations), and histone methylation (histone methyltransferases, Jumonji C domain demethylases, and lysine-specific demethylases; Grilo et al., 2021). In the opposite direction, the epigenetic-regulated gene expression modulates cellular metabolism and mitochondrial function (Quirós et al., 2016). This results in a positive feedback of the mito-nuclear communication that can start during fetal development and continue throughout offspring aging, highly contributing to disease predisposition (Figure 2).

Limited studies exist regarding the offspring cardiac epigenetic impact of GDM. In children circulating DNA, two genes related to fetal growth and development (MEST and IGF2) were differently methylated in CpG, due to GDM, with a good correlation with macrosomia (Joyce et al., 2021). Also, in the placenta from male offspring of diabetic women, a relation between mitochondria, metabolism and the epigenetic pattern was observed. Decreased PGC-1α protein levels and downstream TFAM correlated well with H3-histone acetylation (H3K27) and PGC-1α promoter b methylation (Jiang et al., 2020). These epigenetic alterations were attributed to lower AMPK activation, being these effects reverted with metformin (4 mg/ml dose) (Jiang et al., 2020).

In cord-blood samples from infants of GDM-mothers, 75 CpG loci were differentially methylated, involving genes related to cellular growth and diabetes pathophysiology (Quilter et al., 2014). GDM induces upregulation of miR-101, reducing enhancer of zester homolog-2 protein expression and consequent histone H3 trimethylation in lysine 27 in

**FIGURE 3** Intergenerational perpetuation of cardiovascular disease (CVD) through maternal gestational diabetes mellitus (GDM). An adverse intrauterine environment during GDM pregnancies (F0) promotes developmental programming of cardiac disease in the offspring (F1), through modulation of epigenetic patterns, mitochondrial adaptations, and impairment of nitric oxide metabolism. Alterations in the germ cells of the F1 and following generation (F2) during GDM pregnancies may be implicated in the observed intergenerational and transgenerational perpetuation of CVD. This results in a vicious disease cycle that consecutively risks the health of future generations.
umbilical cord vein human fetal ECs (Floris et al., 2015). Six-week-old rats born to late-gestation STZ-induced GDM presented enhanced cardiomyocyte global DNA methylation and low Sirt-1 protein expression. Inhibition of Dnmt3A through 5-aza reverted Sirt-1 protein levels, while an antioxidant treatment with N-acetyl-cysteine reversed both DNA hypermethylation and Sirt-1 protein levels (Z. Chen et al., 2019). These data support a clear relation between metabolism and epigenetics with the potential involvement of the redox state in the cardiac tissue. GDM-induced impairment in the mitochondria-epigenetics bridge could imprint cardiac dysfunction in the offspring, contributing to fetal programming of CVD.

3 | **THE INHERITANCE OF CVD AND INTERGENERATIONAL EFFECTS**

The transmission of information between the parents and their offspring is not limited to DNA (Fernandez-Twinn et al., 2015). The environment and lifestyle also play a role in the patterns transmitted across generations, as other molecules are present in the gametes (Fernandez-Twinn et al., 2015; Francisco Perez & Lehner, 2019). The inheritance of patterns can occur, for instance, through the direct influence of maternal exposure to a particular trigger in the developing fetus that will condition offspring physiology and the germline of the following generation, characterized as intergenerational transmission (Breton et al., 2021). Transgenerational transmission is a different term used to depict the inheritance of pattern changes in further generations without the direct impact of a stimulus in the affected organism (Breton et al., 2021; Francisco Perez & Lehner, 2019).

The intergenerational cycle of developmental programming disease has become a problematic public health case of early disease (Breton et al., 2021). On the one hand, maternal (F0) obesity and diabetes predispose the offspring (F1) to metabolic disease development. In turn, the F1 generation was programmed with a heavy predisposition to be obese and/or diabetic, becoming a new generation of obese and diabetic parents, leading to the programming of the next generation (F2) and so on, in a vicious cycle of metabolic disease transmission to future generations (Figure 3). On the other hand, it has been proposed that even if F1 can recover from disease programming, presenting no metabolic complications, the F2 generation may also be programmed. Even without direct exposure, the F2 generation programming can occur by F0 through the F1 germinal line (Figure 3), or altered maternal physiology that would affect offspring development resulting in a metabolic insult during fetal development (Skinner, 2011).

An excellent example of intergenerational disease transmission is observed in the second-generation participants of the Framingham Heart Study. Second-generation offspring present a 3.24-fold increased risk of diabetes having a parent with early-onset diabetes, whereas the risk decreases to 2.19-fold if one or both parents present late-onset diabetes, being diabetes a risk factor for cardiovascular death (1.81-fold), and coronary death (1.75-fold), in the same study (Echouffo-Tcheugui et al., 2020).

Multiple shreds of evidence suggest that transgenerational inheritance can occur both by the maternal and paternal germ cells (Dunn & Bale, 2011; Ponzio et al., 2012; Song et al., 2014), even though the role of maternal programming may persist for more generations (Murrin et al., 2012). Interestingly, maternal obesity-exposed oocyte mitochondria appear to have a critical role in transgenerational programming, by presenting an abnormal morphology (e.g., disarrayed cristae, swelling, and lower matrix electron matrix; Luzzo et al., 2012), impaired beta-oxidation (Boudoures et al., 2016), reduced membrane potential, and affected ATP levels (Reynolds et al., 2015), with the inability to activate mitophagy (Boudoures et al., 2017).

Skeletal muscle of F1, F2, and F3 generations of female C57Bl/6 mice exposed to HFHS diet present mitochondrial dysfunction and an abnormal mitochondrial morphology (Saben et al., 2016). Additionally, F1 and F2 female germ cells contained mitochondrial abnormalities (Saben et al., 2016). Likewise, F1, F2, and F3 generations of HFHS-fed mice were non-obese but presented cardiac mitochondrial abnormalities, such as crystal rarefaction and compromised mitochondrial oxygen consumption, and increased left ventricle mass except for female F3 (Ferey et al., 2019). Specific data for GDM transgenerational disease programming has yet to be collected. However, the results related to maternal obesity strengthen the idea of the intergenerational cycle of disease transmission but also suggest a new possibility of CVD transgenerational programming through three generations, independently of the presence of metabolic disease in the intermediary generations.

4 | **CONCLUSIONS**

An impaired intrauterine environment contributes to life-long repercussions in the offspring. Epidemiological studies support the increased risk of metabolic disease development associated with increased CVD risk in offspring born to
GDM mothers. Moreover, compromised intrauterine conditions during a GDM pregnancy may induce placental changes, adaptations in cardiovascular fetal development, dysregulation of oxidative and nitrosative balance, mitochondrial dysfunction, and lead to epigenetic changes that program the offspring organism to later cardiac disease onset. Furthermore, in utero exposure to GDM contributes to the intergenerational and transgenerational propagation of CVD, perpetuating the disease cycle (Figure 3). Therefore, improvement of research tools and development of reliable animal models targeted to unravel the developmental origins of CVD and prevent the consequent fetal programming induced by a GDM-gestation are vital to guarantee positive health outcomes of future generations and combat the vicious cycle of the deadliest disease worldwide. Spreading the word from the scientific community to society about the implications of maternal habits for offspring health and disease perpetuation becomes prime to raise awareness and help battle disease on a strong side-front, allied to the expanding scientific advances.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest. The funding agencies had no role in the decision to publish the manuscript.

**AUTHOR CONTRIBUTIONS**
Carolina Tocantins: Conceptualization (equal); investigation (equal); resources (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). Mariana S. Diniz: Conceptualization (equal); investigation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). Luis F. Grilo: Conceptualization (equal); investigation (equal); resources (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). Susana P. Pereira: Conceptualization (lead); supervision (lead); writing – review and editing (lead).

**DATA AVAILABILITY STATEMENT**
Data sharing is not applicable to this article as no new data were created or analyzed in this study

**ORCID**
Carolina Tocantins https://orcid.org/0000-0002-3758-0454
Mariana S. Diniz https://orcid.org/0000-0002-2161-9676
Luis F. Grilo https://orcid.org/0000-0002-6278-9241
Susana P. Pereira https://orcid.org/0000-0002-1168-2444

**REFERENCES**


WHO. (2013). *Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy* (pp. 1–63). WHO.


