Role for Tetrahydrobiopterin in the Fetoplacental Endothelial Dysfunction in Maternal Supraphysiological Hypercholesterolemia

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Maternal physiological hypercholesterolemia occurs during pregnancy, ensuring normal fetal development. In some cases, the maternal plasma cholesterol level increases to above this physiological range, leading to maternal supraphysiological hypercholesterolemia (MSPH). This condition results in endothelial dysfunction and atherosclerosis in the fetal and placental vasculature. The fetal and placental endothelial dysfunction is related to alterations in the L-arginine/nitric oxide (NO) pathway and the arginase/urea pathway and results in reduced NO production. The level of tetrahydrobiopterin (BH$_4$), a cofactor for endothelial NO synthase (eNOS), is reduced in nonpregnant women who have hypercholesterolemia, which favors the generation of the superoxide anion rather than NO (from eNOS), causing endothelial dysfunction. However, it is unknown whether MSPH is associated with changes in the level or metabolism of BH$_4$; as a result, eNOS function is not well understood. This review summarizes the available information on the potential link between MSPH and BH$_4$ in causing human fetoplacental vascular endothelial dysfunction, which may be crucial for understanding the deleterious effects of MSPH on fetal growth and development.

1. Introduction

Hypercholesterolemia is considered one of the most important risk factors for the development of cardiovascular disease (Adult Treatment Panel ATP III) [1, 2]. Pregnancy is a physiological process that can involve the development of maternal pathologies, such as preeclampsia, gestational diabetes mellitus (GDM), and metabolic disorders, including maternal pregestational obesity, supraphysiological gestational weight gain (spGWG) [3, 4], and hypercholesterolemia [5, 6]. These alterations may compromise the health of the mother and/or the fetus [5–7]. In normal pregnancies, the mother exhibits a physiological (i.e., normal) increase (30–50%) in the plasma total cholesterol (TCh) level in a process that is referred to as maternal physiological hypercholesterolemia (MPH) [5, 6, 8]. However, in some cases, for mostly unknown
reasons, the TCh level is elevated far beyond the physiological range, which is referred to as maternal supraphysiological hypercholesterolemia (MSPH) [5, 6, 9]. Although studies have shown the potential adverse effects of MSPH on the early development of atherosclerosis [10, 11] and endothelial dysfunction in the fetoplacental vasculature [5, 6], the global prevalence of MSPH remains unknown [12].

Endothelial cells synthesize nitric oxide (NO), a potent vasodilator that is generated by NO synthases (NOS; i.e., L-arginine/NO pathway), following the oxidation of L-arginine in a process that depends on the bioactivity of several cofactors, including tetrahydrobiopterin (BH$_4$) [13–15]. In pregnant women with MSPH, the umbilical vein dilation and endothelial NOS (eNOS) activity are reduced, and arginase (ARG) activity is increased compared with MPH [5, 6]. Remarkably, ARG inhibition results in a partial restoration of human umbilical vein dilation, suggesting that other factors play a role in this phenomenon. In nonpregnant women, hypercholesterolemia reduces the NO bioavailability by mechanisms that include a reduction in BH$_4$ levels [16]. Reduced activity and/or level of BH$_4$ favor(s) the generation of a superoxide anion (O$_2^-$) instead of NO, resulting in endothelial dysfunction [14, 17, 18]. Altogether, this indicates that elevated plasma TCh levels may result in reduced NO synthesis via several mechanisms, leading to endothelial dysfunction. The potential effect of MSPH on BH$_4$ availability and regulation of the biosynthesis of this cofactor, as well as its consequences in the modulation of fetal endothelial function, are unknown [5, 6]. This review summarizes the findings regarding a potential link between MSPH and BH$_4$ and human fetoplacental vascular endothelial dysfunction.

2. Hypercholesterolemia

In nonpregnant women, hypercholesterolemia is mainly related to genetic mutations involving genes that are related to cholesterol traffic, such as lipoprotein receptors and cholesterol transporters, metabolic disorders, and an imbalanced diet [2, 19]. According to the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), this condition is the main risk factor for the development of cardiovascular disease (CVD) [1, 2]. Therefore, the proper management of plasma cholesterol levels can reduce the risk of developing CVD [20]. For this reason, the clinical cut-off point for normal blood TCh in nonpregnant women is rigorously controlled and suggested to be <200 mg/dL [1].

A higher risk of hypercholesterolemia-associated CVD also results from a reduced blood level of high-density lipoprotein cholesterol (HDL, i.e., <40 mg/dL) and/or an increased blood level of low-density lipoprotein cholesterol (LDL, i.e., >100 mg/dL); the latter depends on the global cardiovascular risk, as recently recommended in the Guidelines on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults from the American Heart Association [21]. Although CVD is normally diagnosed in adults, some studies indicate that endothelial dysfunction (i.e., an initial phenomenon in the development of atherosclerosis) and early atherosclerotic lesions (i.e., fatty streaks) begin during intrauterine life, in fetal vessels, as a consequence of increased levels of maternal cholesterol [5, 6, 10]. This indicates the relevance of monitoring the potential adverse effects of maternal cholesterol during pregnancy on the fetal vasculature [22]. This is even more important because the current global prevalence for a high plasma concentration of TCh (>200 mg/dL) in nonpregnant women is ~40% [23]. It is conceivable that a significant number of pregnant women will have increased plasma levels of cholesterol, exposing them to the inherent consequences of this condition as well as the associated fetal vascular alterations [12].

Hypercholesterolemia during Pregnancy. Pregnancy is a physiological condition that is characterized by progressive weeks of gestation-dependent increases (increasing 40–50%) in the maternal blood level of cholesterol and triglycerides [24, 25]. MPH is considered an adaptive response of the mother to satisfy the high cholesterol demand of the growing fetus for membrane and hormone synthesis [26]. Unfortunately, there are no established clinical reference values for the total and lipoprotein cholesterol levels during pregnancy in the global population, including the pregnant Chilean population [5, 6, 8]. A summary of the literature on the TCh, lipoprotein cholesterol, and triglyceride concentrations per trimester of pregnancy for different populations is shown in Table 1. Based on the literature, the estimated mean values for TCh are 179, 226, and 257 mg/dL for the 1st (1–13 weeks of pregnancy), 2nd (14–28 weeks of pregnancy), and 3rd (28–40 weeks of pregnancy) trimesters of pregnancy, respectively. For HDL and LDL, the mean values were 62 and 101, 73 and 131, and 68 and 149 mg/dL for the 1st, 2nd, and 3rd trimesters of pregnancy, respectively. MSPH has been defined by considering an at-term cut-off point for TCh of 280–300 mg/dL [5, 11, 27], and it is associated with vascular alterations at birth [5] and during childhood [28]. Additionally, increased oxidative stress in the maternal and fetal blood and placenta [27] as well as reduced expression of placental LDL receptors [29] was found in pregnancies that had maternal TCh levels that were higher than this cut-off point.

Although lipid trafficking through the placenta is restrictive and children born to mothers with MSPH have normal blood cholesterol levels [5, 31], a positive correlation between the maternal and fetal blood cholesterol in the 2nd and 3rd trimesters of pregnancy has been established [11, 32]. Moreover, increased early atherosclerotic markers, such as fatty streaks and lipid peroxidation, were found in the aortas of human fetuses [11] as well as in 7- to 14-year-old children [12] who were born to mothers with MSPH. Furthermore, endothelial dysfunction in the human umbilical vein from pregnancies with TCh values that were higher than this cut-off point has been proposed to be associated with reduced endothelium dependent vascular relaxation, due to lower eNOS and higher ARG activity [5]. These results provide evidence for the potential effect of MSPH in the placenta, leading to adverse consequences for the fetal vasculature. Interestingly, there is limited information on the prevalence of MSPH in the global population, which is mainly because...
### Table 1: Reported maternal plasma lipid concentration in pregnancy.

| Studied population (number of pregnant women) | Trimester of pregnancy | TCh | HDL | LDL | Tg | Observations                                                                 | Reference |
|----------------------------------------------|------------------------|-----|-----|-----|----|--------------------------------------------------------------------------------|-----------|
| USA (142)                                    | 1                      | 180 | 70  | 110 | 112| Maternal overweight and obesity association with lipid concentration during pregnancy | [37]      |
|                                              | 2                      | 230 | 80  | 137 | 162|                                                                                 |           |
|                                              | 3                      | 260 | 76  | 162 | 212|                                                                                 |           |
| Brazil (288)                                 | 1                      | 186 | 54  | 108 | 97 | Maternal lipid concentration during pregnancy as a risk factor for GDM            | [38]      |
|                                              | 2                      | 228 | 62  | 143 | 150|                                                                                 |           |
|                                              | 3                      | 243 | 62  | 135 | 177|                                                                                 |           |
| Argentina (101)                              | 1                      | 160 | 58  | 78  | 90 | Measurement of maternal plasma lipids during pregnancy                           | [39]      |
|                                              | 2                      | 201 | 62  | 107 | 140|                                                                                 |           |
|                                              | 3                      | 244 | 61  | 144 | 202|                                                                                 |           |
| Chile (265)                                  | 1                      | 178 | 60  | 102 | 108| Maternal lipid concentration association with impaired endothelium dependent dilation of the human umbilical vein | [6]       |
|                                              | 2                      | 232 | 73  | 124 | 179|                                                                                 |           |
|                                              | 3                      | 269 | 75  | 147 | 244|                                                                                 |           |
| Chile (74)                                   | 1                      | —   | —   | —   | — | Cut-off point for TCh in maternal plasma from where fetoplacental vascular dysfunction is seen | [5]       |
|                                              | 2                      | —   | —   | —   | — |                                                                                 |           |
|                                              | 3                      | 238 | 72  | 120 | 232|                                                                                 |           |
| UK (178)                                     | 1                      | 215 | 67  | 124 | 125| Measurement of maternal plasma lipids and apolipoproteins during pregnancy       | [40]      |
|                                              | 2                      | 252 | 81  | 126 | 180|                                                                                 |           |
|                                              | 3                      | 281 | 69  | 159 | 252|                                                                                 |           |
| UK (17)                                      | 1                      | 164 | —   | —   | 77 | Measurement of maternal plasma lipids and markers of oxidative stress in normal and GDM pregnancies | [41]      |
|                                              | 2                      | 212 | —   | —   | 133|                                                                                 |           |
|                                              | 3                      | 261 | —   | —   | 233|                                                                                 |           |
| Ireland (222)                                | 1                      | 197 | 65  | 104 | — | Reference values for maternal lipids during pregnancy                           | [42]      |
|                                              | 2                      | 224 | 75  | 128 | — |                                                                                 |           |
|                                              | 3                      | 278 | 68  | 147 | — |                                                                                 |           |
| Italy (22)                                   | 1                      | 178 | 68  | 97  | 93 | Measurement of maternal plasma lipids during pregnancy                           | [43]      |
|                                              | 2                      | 247 | 73  | 153 | 155|                                                                                 |           |
|                                              | 3                      | 282 | 68  | 168 | 230|                                                                                 |           |
| Sweden (18)                                  | 1                      | 182 | 69  | 104 | 99 | Measurement of maternal plasma lipids during pregnancy                           | [44]      |
|                                              | 2                      | 238 | 79  | 140 | 165|                                                                                 |           |
|                                              | 3                      | 248 | 69  | 153 | 215|                                                                                 |           |
| Spain (45)                                   | 1                      | 166 | —   | —   | 71 | Measurement of maternal plasma LDL oxidation in normal, GDM, and obese pregnancies | [45]      |
|                                              | 2                      | 193 | —   | —   | 106|                                                                                 |           |
|                                              | 3                      | 228 | —   | —   | 150|                                                                                 |           |
| Spain (25)                                   | 1                      | 170 | 68  | 89  | 60 | Maternal lipases activity and hormones concentrations during pregnancy           | [46]      |
|                                              | 2                      | 234 | 82  | 136 | 117|                                                                                 |           |
|                                              | 3                      | 254 | 71  | 153 | 184|                                                                                 |           |
| Serbia (50)                                  | 1                      | 190 | 75  | 97  | 85 | Maternal lipid concentration association with newborn size                        | [47]      |
|                                              | 2                      | 245 | 89  | 126 | 151|                                                                                 |           |
|                                              | 3                      | 267 | 79  | 144 | 219|                                                                                 |           |
| Turkey (801)                                 | 1                      | 166 | 53  | 94  | 93 | Maternal lipid concentrations with fetal growth and development in GDM and preeclampsia | [48]      |
|                                              | 2                      | —   | —   | —   | — |                                                                                 |           |
|                                              | 3                      | 271 | 63  | 155 | 274|                                                                                 |           |
| Israel (3938)                                | 1                      | 175 | 58  | 88  | 100| Association of maternal lipid concentration with preeclampsia and GDM            | [49]      |
|                                              | 2                      | 225 | 63  | 121 | 175|                                                                                 |           |
|                                              | 3                      | 238 | 63  | 137 | 237|                                                                                 |           |
| Nigeria (60)                                 | 1                      | 172 | 41  | 112 | 93 | Atherosclerotic risk in pregnant women                                           | [50]      |
|                                              | 2                      | 203 | 51  | 126 | 128|                                                                                 |           |
|                                              | 3                      | 232 | 63  | 136 | 171|                                                                                 |           |

Women were subjected to lipids determination at 1st trimester (0–14 weeks of gestation), 2nd trimester (14–28 weeks of gestation), or 3rd trimester (28–40 weeks of gestation) of pregnancy. TCh: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Tg: triglycerides. —: not reported; GDM: gestational diabetes mellitus. Values are mean in mg/dL.
the maternal blood cholesterol level is not routinely evaluated during pregnancy. Moreover, in a group of pregnant Chilean women, the prevalence of this maternal condition was ~30% [5, 6]. As a result, a significantly higher number of pregnant women will potentially present with an adverse intrauterine condition that could result in the development of vascular alterations in the growing fetus, such as endothelial dysfunction and early atherosclerosis.

3. Fetoplacental Endothelial Dysfunction in MSPH

The placenta is a physical and metabolic barrier between the fetal and maternal circulations, and it is a crucial organ that supports proper fetal development [33]. Because the placenta and umbilical cord lack autonomic innervation [34], a balance between circulating vasodilators and vasoconstrictors is crucial to maintaining normal fetoplacental function [33, 35]. Endothelial dysfunction is defined as an imbalance between vasodilator and vasoconstrictor molecules that are produced by or acting on endothelial cells [36] and that are critical for normal fetal development.

3.1. L-Arginine/NO Pathway. NO is a gas derived from the metabolism of L-arginine via the enzyme NOS, in a metabolic reaction where there is equimolar generation of L-citrulline and NO (as in the L-arginine/NO pathway) [7]. NOS are a group of enzymes with at least three isoforms that are encoded by different genes in mammals [57], that is, neuronal NOS (nNOS or type 1), inducible NOS (iNOS or type 2), and endothelial NOS (eNOS or type 3). eNOS is the main form that is expressed in endothelial cells [3], and reduced activity of this enzyme may result from lower expression, reduced activation, or increased inactivation [58, 59]. eNOS activity is modulated by different agents, including the level of its cofactor BH$_4$ and posttranslational phosphorylation/dephosphorylation. For example, phosphorylation of serine 1177 (Ser$^{1177}$) via PI3 kinase/Akt is associated with higher activity [60]; however, phosphorylation of threonine 495 (Thr$^{495}$) via protein kinase C (PKC) maintains a low activity of this enzyme [58, 61]. Remarkably, hypercholesterolemia is associated with reduced eNOS expression, an effect that is reversed by restoring the cholesterol levels with BH$_4$ supplementation with the BH$_4$ substrate sepiapterin or by GTPCH1 overexpression in mice [81–83]. As a result, this enzyme likely plays a role in hypercholesterolemia. Incubation with human LDL reduces NOS and GTPCH1 expression in rat vascular smooth muscle cells [84, 85]. Additionally, a reduced level of GTPCH1 due to hyperglycemia in human aortic endothelial cells decreases the BH$_4$ level and NO synthesis, which is reversed by GTPCH1 overexpression [86]. Interestingly, and in corroboration with these findings, there are results showing similar changes in HUVECs that L-arginine, leading to the synthesis of L-ornithine and urea [68]. Interestingly, hypercholesterolemia is associated with increased ARG activity in animal models [69, 70] and humans [71, 72]. The activity of ARGs is also increased in HUVECs from pregnancies with MPH compared with MP pregnancies [5]. Because the pharmacological blockade of ARG with S-(2-boronoethyl)-L-cysteine (BEC) partially reverses the reduced eNOS activity observed in HUVECs in MPH pregnancies, ARGs are likely involved in modulating eNOS activity in this cell type [4].

4. BH$_4$ Metabolism in MSPH

4.1. BH$_4$. BH$_4$ is a cofactor required for NOS activity because this molecule stabilizes the enzyme as an active dimer, allowing for optimal oxidation of L-arginine into NO [13, 15]. A reduction in the BH$_4$ level leads to reduced eNOS activation, which is likely due to the uncoupling that results in the generation of superoxide anion ($\text{O}_2^-$) rather than NO, promoting vascular oxidative stress and endothelial dysfunction [14]. In the endothelium, BH$_4$ is synthesized by at least two metabolic pathways: de novo biosynthesis from guanosine triphosphate (GTP) and the salvage pathway from sepiapterin to BH$_4$ and BH$_2$ [14] (Figure 1(a)). De novo biosynthesis involves the sequential action of GTP cyclohydrolase I (GTPCH1), 6-piruvil tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). The GTPCH1 step is the limiting step of the pathway, and it is highly regulated at the transcriptional, translational, and posttranslational levels [73]. For the salvage pathway, the reduction of BH$_2$ to BH$_4$ is the limiting step and requires the enzyme dihydrofolate reductase (DHFR) [73]. The BH$_4$ level could be reduced by decreased synthesis and by the oxidation of BH$_2$ to BH$_3$ via oxygen-derived reactive species and peroxynitrite (ONOO$^-$), resulting in eNOS uncoupling (Figure 1(b)) [13, 18, 74]. The latter is a phenomenon that occurs in a variety of clinical conditions that are associated with vascular disease, including diabetes mellitus, hypertension, atherosclerosis [75–77], and hyperglycemia [18].

4.2. BH$_4$ Metabolism in Hypercholesterolemia. Patients with hypercholesterolemia have low NO availability [78, 79] as well as a lower BH$_4$ level (Table 2). Interestingly, oral or local supplementation with BH$_4$ restores the impaired NO-dependent vasodilation in subjects with hypercholesterolemia [13, 52, 55, 80]. The association between human hypercholesterolemia and a reduced level of GTPCH1 has not yet been addressed [14, 18]. However, reduced eNOS activity is reversed by supplementation with the BH$_4$ substrate sepiapterin or by GTPCH1 overexpression in mice [81–83]. As a result, this enzyme likely plays a role in hypercholesterolemia. Incubation with human LDL reduces NOS and GTPCH1 expression in rat vascular smooth muscle cells [84, 85]. Additionally, a reduced level of GTPCH1 due to hyperglycemia in human aortic endothelial cells decreases the BH$_4$ level and NO synthesis, which is reversed by GTPCH1 overexpression [86]. Interestingly, and in corroboration with these findings, there are results showing similar changes in HUVECs that
Figure 1: Tetrahydrobiopterin metabolism and endothelial nitric oxide synthase uncoupling. (a) The first step in the de novo synthesis of tetrahydrobiopterin (BH₄) is the rate limiting reaction involving the enzyme GTP cyclohydrolase 1 (GTPCH1), whose substrate is GTP. An alternative salvage pathway for BH₄ synthesis is the reduction of 7,8-dihydrobiopterin (BH₂) to BH₄ by the enzyme dihydrofolate reductase (DHFR). BH₂ is generated from sepiapterin by the sepiapterin reductase enzyme (SR). Oxidative stress may be an environmental condition that promotes the oxidation of BH₄ to BH₂, decreasing the bioavailability of BH₄. (b) Under physiological conditions, nitric oxide synthases (NOS, coupled NOS) generate nitric oxide, following the metabolism of L-arginine into L-citrulline in the presence of BH₄. However, uncoupling NOS (uncoupled eNOS) with these enzymes may result in the generation of a superoxide anion (O₂⁻). This phenomenon results from a deficiency in BH₄ and an increased BH₂ bioavailability (from data in [6, 17, 30]).

were subjected to the pharmacological induction of GTPCH1 expression [87].

The potential effect of MSPH on BH₄ availability in the regulation of the synthesis of this cofactor and its effect on the modulation of fetal endothelial function are unknown [5, 6]. Because NO synthesis is reduced in the fetal endothelium from pregnancies with MSPH via mechanisms involving increased ARG but reduced eNOS activity, it is hypothesized that this maternal condition could also result in reduced BH₄ bioavailability for NO synthesis. As a result, these potential mechanisms could explain the endothelial dysfunction and reduced vascular relaxation observed in MSPH. Preliminary results show that the BH₄ level is reduced in HUVECs from MSPH [88] (Leiva A., Sobrevia L., unpublished). However, it is unknown whether BH₄ metabolism is altered in human fetoplacental endothelial cells in pregnancies with MSPH or whether restoration of the BH₄ level improves the endothelial dysfunction in MSPH [4–6, 33].

5. Concluding Remarks
The prevalence of MSPH in the global population has not been evaluated, although it is estimated as approximately 30% [5, 6]. MSPH is a factor that favors the development of vascular changes in the growing fetus and, eventually, in children [12]. These vascular disorders include endothelial
Table 2: Effect of hypercholesterolemia on tetrahydrobiopterin availability and endothelial function.

| Study model           | Tissue or cell type                  | Experimental condition | BH₄ level  | Parameter                     | Effect          | Reference |
|-----------------------|--------------------------------------|------------------------|------------|-------------------------------|-----------------|-----------|
| Hypercholesterolemia  | Human brachial artery                 | Basal                  | Reduced    | Endothelium dependent vasodilation | Reduced         | [13]      |
|                       |                                       | BH₄ infusion           | Increased  | Endothelium dependent vasodilation | Increased       |           |
| Hypercholesterolemia  | Human coronary artery                 | Basal                  | Reduced    | Coronary artery diameter and flow | Reduced         | [51]      |
|                       |                                       | BH₄ infusion           | Increased  | Coronary artery diameter and flow | Increased       |           |
| Hypercholesterolemia  | Human brachial artery                 | Basal                  | Reduced    | Endothelium dependent vasodilation | Reduced         | [52]      |
|                       |                                       | BH₄ supplementation    | Increased  | Endothelium dependent vasodilation | Increased       |           |
| Hypercholesterolemia  | Human coronary microcirculation       | Basal                  | nr         | Myocardial blood flow          | Reduced         | [53]      |
|                       |                                       | BH₄ infusion           | nr         | Myocardial blood flow          | Increased       |           |
| Hypercholesterolemia  | Human skin                            | Basal                  | nr         | Endothelium dependent vasodilation | Reduced         | [54]      |
|                       |                                       | R-BH₄ infusion         | nr         | Endothelium dependent vasodilation | Increased       |           |
|                       |                                       | S-BH₄ infusion         | nr         | Endothelium dependent vasodilation | Reduced         |           |
| Hypercholesterolemia  | Human skin                            | Basal                  | nr         | Endothelium dependent vasodilation | Reduced         | [55]      |
|                       | Incubation with oxLDL                | Reduced NO generation  | Reduced    |                              |                 | [56]      |
|                       | Incubation with oxLDL + sepiapterin  | Increased NO generation| Increased  | Superoxide generation         | Increased       |           |
| Cell culture          | Human aortic endothelial cells        | Incubation with LDL    | Reduced    | NO generation                 | Reduced         | [52]      |
|                       |                                       | Incubation with LDL + BH₄ | Reduced    | NO generation                 | Reduced         |           |
|                       |                                       |                         | nr         | NO generation                 | Increased       |           |

Basal corresponds to no treatment. BH₄: tetrahydrobiopterin; R-BH₄: R-tetrahydrobiopterin (NO synthase cofactor and antioxidant); S-BH₄: stereoisomer of BH₄ (antioxidant); oxLDL: oxidized low-density lipoprotein; LDL: low-density lipoprotein; NO: nitric oxide; nr: not reported.

dysfunction in the fetus and placenta, disrupting the equilibration between the ARG/urea and L-arginine/NO signaling pathways. However, it is unknown whether these alterations correlate with the degree of MSPH in pregnancy or the alterations in BH₄ metabolism and eNOS function (Figure 2). Drugs that control the TCh plasma level in adult, nonpregnant subjects are not used during pregnancy. This condition limits our present knowledge regarding the correlation between the mother’s and fetus’s TCh level and the vascular function of the fetus during pregnancy. However, based on the available evidence from subjects with hypercholesterolemia, we propose that restoration of the BH₄ level will improve the fetoplacental endothelial function in humans. Therefore, it is essential to focus future studies on exploring the dynamics of the BH₄ metabolism in MSPH pregnancies and the possible contribution that restoring this cofactor could have on this maternal condition and vascular function.

Conflict of Interests
There is no conflict of interests.

Authors’ Contribution
Andrea Leiva and Luis Sobrevia designed research study; Andrea Leiva, Bárbara Fuenzalida, and Carlos Salomón collected clinical data; Andrea Leiva, Bárbara Fuenzalida, Francisco Westermeier, Fernando Toledo, Jaime Gutiérrez, Carlos Sanhueza, and Fabián Pardo collected and analyzed literature.
Figure 2: Effect of maternal supraphysiological hypercholesterolemia on the endothelial L-arginine/NO signaling pathway. In umbilical vein endothelial cells from pregnancies complicated by maternal physiological hypercholesterolemia, the amino acid L-arginine is taken up by the human cationic amino acid transporter 1 (hCAT-1) and metabolized by endothelial nitric oxide synthase (eNOS) and, to a lesser extent, arginases. This phenomenon occurs in the presence of tetrahydrobiopterin (BH₄), resulting in NO generation. BH₄ is generated by the enzyme GTP cyclohydrolase 1 (GTPCH1), which is coded by the gch1 gene and whose substrate is GTP. In cells from pregnancies where the pregnant women had maternal supraphysiological hypercholesterolemia, hCAT-1-mediated L-arginine transport is increased (⇑), increasing the availability of this amino acid for eNOS and arginases. In this pathological condition, L-arginine is mainly used by arginases, limiting the formation of NO via eNOS. In addition, eNOS has reduced (⇓) activity because of the lower phosphorylation of Ser₁₁⁷⁷ and the bioavailability of BH₄. The reduction in the BH₄ concentration results from a reduced expression of gch1, leading to eNOS uncoupling and the generation of a superoxide anion (O₂⁻). The O₂⁻ reacts with NO to form peroxynitrite (ONOO⁻; from data in [5–7, 14]).

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