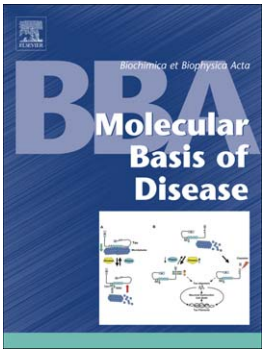

Tetrahydrobiopterin role in human umbilical vein endothelial dysfunction in maternal supraphysiological hypercholesterolemia

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Tetrahydrobiopterin Role in Human Umbilical Vein Endothelial Dysfunction in Maternal Supraphysiological Hypercholesterolemia

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Short title: BH_4 improves fetal endothelial function in MSPH

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Abstract

Maternal physiological hypercholesterolemia (MPH) allows a proper foetal development; however, maternal supraphysiological hypercholesterolemia (MSPH) associates with foetal endothelial dysfunction and early development of atherosclerosis. MSPH courses with reduced endothelium–dependent dilation of the human umbilical vein due to reduced endothelial nitric oxide synthase activity compared with MPH. Whether MSPH modifies the availability of the nitric oxide synthase cofactor tetrahydrobiopterin is unknown. We investigated whether MSPH–associated lower umbilical vein vascular reactivity results from reduced bioavailability of tetrahydrobiopterin. Total cholesterol <7.2 mmol/L was considered as maternal physiological hypercholesterolemia (n = 72 women) and ≥ 7.2 mmol/L as MSPH (n = 35 women). Umbilical veins rings were used for vascular reactivity assays (wire myography), and primary cultures of human umbilical vein endothelial cells (HUVECs) to measure nitric oxide synthase, GTP cyclohydrolase 1, and dihydrofolate reductase expression and activity, as well as tetrahydrobiopterin content. MSPH reduced the umbilical vein rings relaxation caused by calcitonine gene-related peptide, a phenomenon partially improved by incubation with sepiapterin. HUVECs from MSPH showed lower nitric oxide synthase activity (L-citrulline synthesis from L-arginine) without changes in its protein abundance, as well as reduced tetrahydrobiopterin level compared with MPH, a phenomenon reversed by incubation with sepiapterin. Expression and activity of GTP cyclohydrolase 1 was lower in MSPH, without changes in dihydrofolate reductase expression. MSPH is a pathophysiological condition reducing human umbilical vein reactivity due to lower bioavailability of tetrahydrobiopterin leading to lower NOS activity in the human umbilical vein endothelium.

Keywords: tetrahydrobiopterin; fetal; endothelium; cholesterol; pregnancy

Abbreviations

MPH	maternal physiological hypercholesterolemia
MSPH	maternal supraphysiological hypercholesterolemia
GTP	guanosine triphosphate
GTPCH1	GTP cyclohydrolase 1
DHFR	dihydrofolate reductase
BH_2	dihydrobiopterine
BH_4	tetrahydrobiopterine
HUVECs	human umbilical vein endothelial cells
TCh	total cholesterol
LDL	low density lipoprotein
vLDL	very-low density lipoprotein
HDL	high-density lipoprotein
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
CGRP	calcitonine gene related peptide
L-NAME	N^G-nitro-L-arginine methyl ester

1. Introduction

Human pregnancy courses with increased maternal content of total cholesterol (TCh), i.e., maternal physiological hypercholesterolemia in pregnancy (MPH), to satisfy the demand of lipids by the growing foetus [1]. However, some pregnant women show an excessive increase in TCh content over the MPH, i.e., maternal supraphysiological hypercholesterolemia in pregnancy (MSPH) [2-5]. MSPH associates with foetal endothelial dysfunction [4-6], early development of atherosclerosis [7,8], and cardiovascular disease [8-10]. MSPH associates with reduced synthesis of nitric oxide (NO) from human umbilical vein endothelial cells (HUVECs) and NO-dependent dilation of human umbilical vein rings compared with MPH [4], with a *cut-off* point >7.3 mmol/L maternal TCh at term [4,5]. Additionally, HUVECs from MSPH show reduced endothelial NO synthase (eNOS) activity, without altering its protein abundance, compared with MPH [4-5]. In non-pregnant women, hypercholesterolemia leads to lower endothelium–derived NO by a mechanism involving reduced level of the NOS cofactor tetrahydrobiopterin (BH_4) [11-18]. Thus, it is likely that BH_4 is involved in the MSPH–associated lower eNOS activity in the fetoplacental vasculature.

BH_4 is generated by *the novo* from guanosine triphosphate (GTP), where GTP cyclohydrolase 1 (GTPCH1) activity is the limiting step [19], and through a salvage pathway from sepiapterin [20], where reduction of dihydrobiopterin (BH_2) to BH_4 is the limiting step requiring dihydrofolate reductase (DHFR) activity [19]. It is reported that high low-density lipoprotein (LDL) content associates with reduced NOS and GTPCH1 expression in rat vascular smooth muscle cells [21,22], suggesting that GTPCH1 may play a role in hypercholesterolemia. We hypothesize that BH_4 level, and DHFR or GTPCH1 expression and activity will be altered in human fetoplacental endothelium from MSPH. The results show that

reduced endothelium-dependent dilation of vein rings from MSPH is improved by incubation with sepiapterin. The cellular mechanisms involved in this phenomenon include reduced content of BH_4 and GTPCH1 expression and activity without changes in DHFR expression in HUVECs from this pathological condition.

2. Material and Methods

Full version of Material and Methods are available in the Supplementary Material.

2.1 Study groups

Human placentas with their umbilical cords were collected from 107 full-term pregnancies (Table 1). The investigation conforms to the principles outlined in the Declaration of Helsinki. Ethics Committee approval from Faculty of Medicine of the Pontificia Universidad Católica de Chile and patient informed consents were obtained. Blood cholesterol level before pregnancy was obtained for women enrolled in the study. All pregnant women were screened for total blood cholesterol (TCh), high-density lipoprotein (HDL), low-density lipoprotein (LDL) or very-low density lipoprotein (vLDL) cholesterol and triglycerides in whole brachial venous blood taken during the first trimester, second trimester and at the term of pregnancy (third trimester). At birth, whole umbilical blood was collected and assayed for TCh, HDL-cholesterol and triglycerides via enzymatic-colorimetric assays, from where LDL- and vLDL-cholesterol were estimated [4,5].

2.2 Blood cholesterol and triglyceride measurement

TCh, HDL-, LDL-, or vLDL-cholesterol and triglycerides (Tg) was determined in maternal whole brachial venous blood taken at term of pregnancy as described [4,5]. Women with <7.2 mmol/L TCh were considered as MPH and with ≥7.2 mmol/L TCh corresponded to MSPH in this study, based in the *cut-off* point for MSPH, a value from when human fetoplacental endothelial and vascular dysfunction is seen, as previously reported [4,5].

2.3 Human umbilical vein reactivity

Isometric force was measured in a myograph in umbilical vein rings in response to calcitonine gene related peptide (CGRP, 0.1 – 1000 nmol/L, 5 minutes) in 32.5 mmol/L KCl preconstricted vessels, in the absence or presence of 100 µmol/L N^G-nitro-L-arginine methyl ester (L-NAME, NOS inhibitor, 30 minutes), 100 µmol/L sepiapterin (1 hour) or 5 mmol/L 2,4-diamino-6-hydroxypyrimidine (DAHP, 1 hour, GTPCH1 inhibitor) (Sigma-Aldrich, St Louis, MO, USA), as reported [4,5,23].

2.4 Cell culture

HUVECs were isolated by collagenase digestion from umbilical cords at birth from pregnancies with MPH or MSPH and cultured as described [4,5]. Experiments were performed in the absence or presence of L-NAME (100 µmol/L, 30 minutes), sepiapterin (100 µmol/L, 24 hours) and/or DAHP (5 mmol/L, 12 hours) [24,25].

2.5 Reverse transcription and quantitative RT-PCR

Total RNA was isolated using the Qiagen RNAeasy kit (Qiagen, Crawley, UK). RNA quality and integrity were insured by gel visualization and spectrophotometric analysis

($OD_{260/280}$), quantified at 260 nm. Aliquots (1 μg) of total RNA were reversed transcribed into cDNA as described [26]. Quantitative RT-PCR was performed using a Step One real time PCR system (Applied Biosystem, CA, USA) as described [26]. Oligonucleotide primers for *GCH1*, *DHFR* and *28S* were used.

2.6 Western blotting

Total protein was probed with primary polyclonal rabbit *anti*-total eNOS (1:200 dilution, 2 hours, room temperature) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), monoclonal mouse *anti*-eNOS phosphorylated at Serine^{1177} (*P*~Ser^{1177}-eNOS, 1:1000 dilution, 1 hour, room temperature) or *anti*-eNOS phosphorylated at Threonine^{495} (*P*~Thr^{495}-eNOS, 1:1000 dilution, 1 hour, room temperature) (BD Transduction Laboratories, San Jose, CA, USA), monoclonal rat *anti*-GTPCH1 (1:1000 dilution, 8 hours, 4°C) and mouse *anti*-ß-actin (1:5000, 1 hour, room temperature) (Sigma-Aldrich) antibodies [4].

2.7 eNOS monomer/dimer determination

eNOS dimers and monomers were determined following a modification of a previously described method [27]. Confluent HUVECs from MPH and MSPH pregnancies were incubated with sepiapterin (100 μmol/L, 24 hours) or L-NAME (100 μmol/L, 30 minutes) and harvested in lysis buffer (20 mmol/L Tris/HCl (pH 7.4), 150 mmol/L NaCl, 2 mmol/L EGTA, 2 mmol/L EDTA, 0.5% Nonidet P-40, 1% Triton X100). Total protein were mixed with Laemmli buffer (0.32 mol/L Tris/HCl (pH 6.8), 0.5 mol/L glycine, 10% sodium dodecylsulphate, 50% glycerol, 0.03% bromophenol blue) and separated in a 6% reducing polyacrylamide gel (2.5% 2-mercaptoethanol). Proteins were probed for total eNOS and ß-actin as above.

2.8 NOS activity

NOS activity was assayed by determination of intracellular content of L-citrulline was determined by high performance liquid chromatography (HPLC) in confluent HUVECs in the absence or presence of 100 µmol/L N^G-nitro-L-arginine methyl ester (L-NAME, inhibitor of NOS activity), as reported [26].

2.9 Intracellular reactive oxygen species (ROS) determination

Intracellular ROS levels were determined using the fluorescent dye 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) (Molecular Probes, Leiden, The Netherlands) as previously described [28]. Cells were exposed (30 minutes, 37°C) to 10 µmol/L of CM-H_2DCFDA and fluorescence ($\lambda_{exc}/\lambda_{em}$: 495/520 nm) was determined in an Infinite M200 Pro (Tecan, Untersbergstr, Austria) microplate reader.

2.10 Tetrahydrobiopterin (BH_4) determination

The level of BH_4 was determined by an acid-base oxidation method followed by fluorometric detection by HPLC as described [28].

2.11 GTPCH1 activity

GTPCH1 activity was determined as iodine oxidation of neopterin synthesized from GTP by HPLC activity following a modification of the method described by Werner-Felmayer & Gross [29]. Basal activity of the enzyme was quantified in the absence of exogenous GTP, and enzyme activity was induced by addition of 10 µL of 10 mmol/L GTP.

2.12 Statistical analysis

Values for lipids are given as mean ± S.D. For assays *in vitro* the values are mean ± S.E.M., where *n* indicates the number of different cell cultures (3-4 replicates). Comparison between groups was performed by parametric or non-parametric tests (see Supplemental Material and Methods). $P<0.05$ was considered statistically significant.

3. Results

3.1 Study groups

All pregnant women coursed with a normal pregnancy and showed comparable age, height, weight, basal glycaemia and OGTT (Table 1). Maternal weight and body mass index increased as pregnancy progressed in MPH and MSPH. Systolic and diastolic blood pressure at 3rd trimester were higher than at 1st trimester in both groups of pregnant women. None of the maternal or newborn variables were significantly altered when compared between MPH and MSPH.

3.2 Maternal plasma lipids

The maternal blood levels for TCh and LDL in the MSPH group were higher to in the MPH group at term of pregnancy (Table 2). However, HDL, vLDL and Tg levels were similar between both groups.

3.3 Umbilical vein reactivity

In the absence of L-NAME, CGRP caused a maximal dilation (D_{max}) of umbilical vein rings from MPH (D_{max} = 24 ± 3%) (Figure 1A) that was higher than in vessels from MSPH

(D_{max} = 1.5 ± 0.5%) (Figure 1B). The CGRP D_{max} in MPH was unaltered by sepiapterin; however, this molecule increased CGRP D_{max} (10 ± 1%) in vein rings from MSPH. DAHP caused a reduction in the CGRP D_{max} (15 ± 1%) in MPH, but did not alter this value in vein rings from MSPH. CGRP EC_{50} value was higher in MSPH compared with MPH, an effect reversed by sepiapterin and partially reduced by DAHP (Table 3).

L-NAME blocked CGRP–dilation in vein rings from MPH and MSPH, and this effect was unaltered by sepiapterin or DAHP. All the CGRP EC_{50} values in the presence of L-NAME were higher than in the absence of this inhibitor in MPH (Table 3). The CGRP EC_{50} in the presence DAHP was unaltered, but it was reduced in the presence of sepiapterin in MPH (Table 3). SNP caused a comparable dilation in veins from MPH and MSPH in the absence or presence of sepiapterin or DAHP (Figure 1C).

3.4 eNOS expression and NOS activity

L-Citrulline content in HUVECs from MSPH was lower than in cells from MPH, and was reduced by L-NAME to similar values in cells from both conditions (Figure 2A). Sepiapterin did not alter L-citrulline content in MPH, but caused an increase in MSPH reaching values that were higher (3.1 ± 0.4 fold) than those in MPH. L-NAME reduced L-citrulline content in the presence of sepiapterin. L-NAME–inhibited L-citrulline content (i.e., NOS activity) was lower in MSPH compared with MPH (Figure 2B). Incubation of cells with sepiapterin caused an increase in NOS activity in MSPH, but not in MPH.

Total eNOS protein abundance was unaltered in MSPH in the absence or presence of L-NAME or sepiapterin (Figure 3A). However, *P*~Thr^{495}-eNOS (Figure 3B) and *P*~Ser^{1177}-eNOS (Figure 3C) were lower in MSPH, and unaltered by L-NAME or sepiapterin.

3.5 Intracellular ROS

Intracellular level of ROS was higher in HUVECs from MSPH compared with MPH (Figure 4A). Incubation of cells with sepiapterin did not alter ROS level in cells from MSPH or MPH, a phenomenon that was similar in the absence or presence of L-NAME (Figure 4B).

3.6 eNOS monomer and dimer

Cells from MSPH show similar levels of monomeric (Figure 5A,B) and dimeric (Figure 5A,C) eNOS. Incubation of cells with sepiapterin caused a significant reduction in eNOS monomer, but an increase in eNOS dimer proteins, leading to a lower eNOS monomer/dimer ratio (Figure 5D). Incubation of cells with L-NAME caused a minor pronounced effect on eNOS monomer/dimer ratio.

3.7 BH_4 content

Total biopterins (i.e., BH_4 + BH_2 + biopterins) and BH_4 content were lower in MSPH compared with MPH in the absence of sepiapterin (Figure 6A). Out of total biopterins, the BH_4 content in cells from MSPH was lower than in cells from MPH. Incubation of cells with sepiapterin caused an increase in total biopterins and BH_4 content reaching similar values in cells from both conditions. The absolute increase in total biopterins and BH_4 contents caused by sepiapterin were similar for MPH or MSPH; however, this molecule caused a higher fold of increase in cells from MSPH (3.1 ± 0.5 fold) compared with MPH (1.8 ± 0.4 fold).

3.8 DHFR and GTPCH1 expression and activity

The *DHFR* mRNA expression was unaltered in cells from MSPH compared with MPH (Figure 6B); however, *GCH1* mRNA expression (Figure 6C) and GTPCH1 protein abundance (Figure 6D) were lower in cells from MSPH. Generation of neopterin was also lower in MSPH compared with MPH (Figure 6E). Neopterin content was increased by GTP in cells from MPH, an effect blocked by DAHP. However, GTP did not alter neopterin content in the absence or presence of DAHP in cells from MSPH. DAHP reduced neopterin content in cells from MPH, but not from MSPH.

4. Discussion

This study shows that MSPH–associated reduction in the dilation of the human umbilical vein rings [4,5] is partially recovered by sepiapterin, a substrate for BH_4 biosynthesis, via a mechanism involving increased NOS activity. Additionally, MSPH associates with reduced expression and activity of GTPCH1 in HUVECs. Thus, an increase in the maternal plasma level of TCh in MSPH results in reduced *GCH1* expression and GTPCH1 protein abundance and activity in the human fetoplacental vasculature. This study also confirms the previously proposed *cut-off* point for MSPH–associated reduction in the dilation of human umbilical vein rings (>7.2 – 7.5 mmol/L TCh at term) [4,5] and extended this observation to show reduced BH_4 content in HUVECs from MSPH in women with TCh over this *cut-off* point.

MSPH associates with reduced umbilical vein dilation in response to the endothelium–dependent vasodilator CGRP [30] with EC_{50} values that were ~18 fold higher in MSPH compared with vein rings from MPH, confirming previous observations in this umbilical vessel [4,5]. Since dilation caused by sodium nitroprusside (SNP), a spontaneous NO donor, was similar in MSPH and MPH, the umbilical vein endothelium rather than the vascular smooth

muscle is likely altered in MSPH. A major findings in this study is that reduced dilation of vein rings in response to CGRP in MSPH was reversed by sepiapterin suggesting that a reduced BH_4 content in human umbilical vein endothelium could limit CGRP–mediated dilation in these vessels. This phenomenon seems to occur only in vein rings from MSPH since sepiapterin increased the sensitivity to CGRP in these vessels (EC_{50} in the absence *vs* presence of sepiapterin (MSPH-$EC_{50}^{-Sep/+Sep}$) ~920), but not in vein rings from MPH (MPH-$EC_{50}^{-Sep/+Sep}$ ~1). Thus, this data strongly suggest that a BH_4 deficiency could result in lower umbilical vein endothelium–dependent reactivity in pregnancies where the mother courses with MSPH.

Reversal of the MSPH–associated lower umbilical vein dilation by sepiapterin could result from modulation of NOS activity or by an antioxidant effect of BH_4. The latter is supported by findings showing that BH_4 acts as scavenger for reactive oxygen species (ROS) increasing NO bioavailability [31,32]. Interestingly, ROS formation was higher in MSPH, an effect unaltered by sepiapterin. Since sepiapterin caused only a partial restoration of the MSPH–reduced umbilical vein dilation it is suggested that reduced dilation may results from a dual effect including lower BH_4 bioavailability for NOS activity and increased ROS generation in this vascular tissue. Furthermore, sepiapterin–dependent restoration of the dilation of vein rings in response to CGRP associates with higher eNOS activity in HUVECs from MSPH. Accordingly, MSPH–reduced BH_4 content in HUVECs was restored by sepiapterin to levels detected in cells from MPH. Additional assays show that incubation of cells with sepiapterin did not alter the MSPH–associated lower level of activatory (Serine^{1177}) or inhibitory (Thr^{495}) phosphorylation states of eNOS, or the total protein abundance of this enzyme. Thus, the BH_4 bioavailability rather than eNOS protein abundance or phosphorylation states at these specific residues are likely accounting for the umbilical vein reactivity restoration by sepiapterin.

Additionally, sepiapterin increased the formation of eNOS dimers (i.e., coupled eNOS) [11], an effect that was similar in cells from MPH or MSPH. Thus, sepiapterin restores the human umbilical vein endothelial dysfunction detected in MSPH, a phenomenon that involves higher eNOS activity, likely not be due to increased eNOS coupling in MSPH, agreeing with reports in coronary arterioles from patients with atherosclerosis [33]. Interestingly, MSPH associates with reduced content of total biopterins in HUVECs, which could result from a lower BH_4 content (reduction in total biopterins content / reduction in BH_4 content ~1.2). Thus, a lower *de novo* synthesis of biopterins (from GTP to BH_4) in MSPH is likely. These results differ from those reported in diabetes mellitus, where the level of BH_4 was reduced without changes in the total level of biopterins, which results from BH_4 loss by oxidative conversion of BH_4 to BH_2 rather than a change in biosynthesis of biopterins [31,32]. Since MSPH associates with reduced activity of GTPCH1, and because this reduction (~83%) was higher than the reduction seen for its protein abundance (~57%), a lower GTPCH1–dependent *de novo* synthesis of biopterins in HUVECs from MSPH is feasible. This is supported by findings showing that atherosclerosis–associated reduction in endothelial BH_4 level result from inhibition of GTPCH1 activity or from oxidative degradation of BH_4 by peroxynitrite rather than by regulation of the GTPCH1 expression [34].

Although the synthesis of total biopterins and BH_4 is reduced in MSPH, BH_4 reduction in relation to total biopterins was lower in MSPH ($MSPH^{BH4/total\ biopterins}$ ~52%) compared with MPH ($MPH^{BH4/total\ biopterins}$ ~85%). Thus, a larger increase in BH_2 compared with BH_4 content in MSPH is likely. It is reported that dihydrofolate reductase (DHFR) regenerates BH_4 from BH_2 and that BH_4/BH_2 ratio is reduced when the total biopterins availability is reduced [35]. Our results show that DHFR expression was unaltered in MSPH, suggesting that BH_4 generation

from BH_2 is likely to be normal in MSPH. Alternatively, a reduced BH_4/total biopterins ratio in MSPH could results from higher oxidation of BH_4 to form BH_2 as reported in hyperglycaemia where a reduced BH_4 level instead of increased BH_2 level is seen under oxidative stress [28,31]. Since pregnant women coursing with TCh >7.2 mmol/L show increased oxidative stress in the maternal and fetal blood, and in homogenized placenta [36,37], an oxidative state in MSPH could also contribute to the altered proportion of BH_4 in relation to total biopterins.

In summary MSPH is a maternal condition that leads to endothelial dysfunction of the fetoplacental vasculature by a mechanism involving reduced synthesis of BH_4. We have identified a new mechanism involved in the fetoplacental vascular alterations described for MSPH. Because this study has been performed in primary cultures of HUVECs and vein rings *in vitro*, the possibility that these alterations reflect alterations in the foetal vasculature should be considered with caution. However, we propose the possibility of BH_4 as a new therapeutic target to prevent the initiation and/or progression of MSPH–associated foetal cardiovascular disease. This is supported by findings showing that restoration of BH_4 content restores the endothelial dysfunction seen in pathological conditions coursing with a reduced level of this molecule including patients coursing with hypercholesterolemia [12,14,15,38,39]. Therefore, the findings described in this study could be considered in therapeutic protocols for prevention of fetoplacental vascular dysfunction in human pregnancies with MSPH aiming to reduce the potential consequences in the health of the newborn and in its adulthood.

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Disclosures

None.

References

1. Barrett HL, Dekker NM, McIntyre HD, Callaway LK. Normalizing metabolism in diabetic pregnancy: is it time to target lipids? Diabetes Care. 2014;37:1484–1493. doi:10.2337/dc13-1934.
2. Montes A, Walden CE, Knopp RH, Cheung M, Chapman MB, Albers JJ. Physiologic and supraphysiologic increases in lipoprotein lipids and apoproteins in late pregnancy and postpartum. Possible markers for the diagnosis of "prelipemia". Arteriosclerosis. 1984;4:407–417. doi: 10.1161/01.ATV.4.4.407.
3. Basaran A. Pregnancy-induced hyperlipoproteinemia: Review of the literature. Reprod Sci. 2009;16:431–437. doi:10.1177/1933719108330569.
4. Leiva A, de Medina CD, Salsoso R, Sáez T, San Martín S, Abarzúa F, Farías M, Guzmán-Gutiérrez E, Pardo F, Sobrevia L. Maternal hypercholesterolemia in pregnancy associates with umbilical vein endothelial dysfunction: role of endothelial nitric oxide synthase and arginase II. Arterioscler Thromb Vasc Biol. 2013;33:2444–2453. doi:10.1161/ATVBAHA.113.301987.
5. Leiva A, Salsoso R, Sáez T, Sanhueza C, Pardo P, Sobrevia L. Cross-sectional and longitudinal lipid determination studies in pregnant women reveal an association between increased maternal LDL cholesterol concentrations and reduced human umbilical vein relaxation. Placenta. 2015;36:895–902. doi:10.1016/j.placenta.2015.05.012.
6. Zhang H, Temel RE, Martel C. Cholesterol and lipoprotein metabolism, Early Career Committee contribution. Arterioscler Thromb Vasc Biol. 2014;34:1791–1794. doi:10.1161/ATVBAHA.114.304267.

7. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low-density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest. 1997;100:2680–2690. doi:10.1172/JCI119813.

8. Napoli C, Glass CK, Witztum JL, Deutsch R, D'Armiento FP, Palinski W. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of early lesions in children (FELIC) study. Lancet. 1999;354:1234–1241. doi:10.1016/S0140-6736(99)02131-5.

9. Pardo F, Arroyo P, Salomón C, Westermeier F, Salsoso R, Sáez T, Guzmán-Gutiérrez E, Leiva A, Sobrevia L. Role of equilibrative adenosine transporters and adenosine receptors as modulators of the human placental endothelium in gestational diabetes mellitus. Placenta. 2013;34:1121–1127. doi:10.1016/j.placenta.2013.09.007.

10. Palinski W. Effect of maternal cardiovascular conditions and risk factors on offspring cardiovascular disease. Circulation. 2014;129:2066–2077. doi:10.1161/CIRCULATIONAHA.113.001805.

11. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Lüscher T, Rabelink T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. J Clin Invest. 1997;99:41–46. doi:10.1172/JCI119131.

12. Cosentino F, Hürlimann D, Delli Gatti C, Chenevard R, Blau N, Alp NJ, Channon KM, Eto M, Lerch P, Enseleit F, Ruschitzka F, Volpe M, Lüscher TF, Noll G. Chronic treatment with tetrahydrobiopterin reverses endothelial dysfunction and oxidative stress

in hypercholesterolaemia. Heart. 2008;94:487–492. doi:10.1161/ATVBAHA.107.156059.

13. Aoki C, Nakano A, Tanaka S, Yanagi K, Jojima T, Kasai K, Takekawa H, Hirata K, Hattori Y. Fluvastatin upregulates endothelial nitric oxide synthase activity via enhancement of its phosphorylation and expression and via an increase in tetrahydrobiopterin in vascular endothelial cells. Int J Cardiol. 2012;156:55–61. doi:10.1016/j.ijcard.2010.10.029.
14. Fukuda Y, Teragawa H, Matsuda K, Yamagata T, Matsuura H, Chayama K. Tetrahydrobiopterin restores endothelial function of coronary arteries in patients with hypercholesterolaemia. Heart. 2002;87:264–269. doi:10.1136/heart.87.3.264.
15. Wyss CA, Koepfli P, Namdar M, Siegrist PT, Luscher TF, Camici PG, Kaufmann PA. Tetrahydrobiopterin restores impaired coronary microvascular dysfunction in hypercholesterolaemia. Eur J Nucl Med Mol Imaging. 2005;32:84–91. doi:10.1007/s00259-004-1621-y.
16. Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. Curr Opin Pharmacol. 2013;13:161–167. doi:10.1016/j.coph.2013.01.006.
17. Alexander LM, Kutz JL, Kenney WL. Tetrahydrobiopterin increases NO-dependent vasodilation in hypercholesterolemic human skin through eNOS-coupling mechanisms. Am J Physiol. 2013;304:R164–R169. doi:10.1152/ajpregu.00448.2012.
18. Ma S, Ma CC. Recent developments in the effects of nitric oxide-donating statins on cardiovascular disease through regulation of tetrahydrobiopterin and nitric oxide. Vascul Pharmacol. 2014;63:63–70. doi:10.1016/j.vph.2014.08.001.

19. Thony B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J. 2000;347:1–16. doi:10.1042/bj3470001.

20. Crabtree MJ, Channon KM. Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. Nitric Oxide. 2011;25:81–88. doi:10.1016/j.niox.2011.04.004.

21. Dulak J, Polus M, Guevara I, Polus A, Hartwich J, Dembińska-Kieć A. Regulation of inducible nitric oxide synthase (iNOS) and GTP cyclohydrolase I (GTP-CH I) gene expression by ox-LDL in rat vascular smooth muscle cells. J Physiol Pharmacol. 1997;48:689–697.

22. Dulak J, Polus M, Guevara I, Hartwich J, Wybrańska I, Krzesz R, Dembińska-Kieć A. Oxidized low density lipoprotein inhibits inducible nitric oxide synthase, GTP cyclohydrolase I and transforming growth factor beta gene expression in rat macrophages. J Physiol Pharmacol. 1999;50:429–441.

23. Dubois M, Delannoy E, Duluc L, Closs E, Li H, Toussaint C, Gadeau AP, Gödecke A, Freund-Michel V, Courtois A, Marthan R, Savineau JP, Muller B. Biopterin metabolism and eNOS expression during hypoxic pulmonary hypertension in mice. PloS One. 2013;8:e82594. doi:10.1371/journal.pone.0082594.

24. Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Schmidt K, Weiss G, Wachter H. Pteridine biosynthesis in human endothelial cells. Impact on nitric oxide-mediated formation of cyclic GMP. J Biol Chem. 1993;268:1842–1846.

25. Ikemoto K, Matsumoto T, Ohtsuki M, Itoh M, Tada S, Udagawa Y, Sumi-Ichinose C, Nomura T. 2,4-Diamino-6 hydroxypyrimidine (DAHP) suppresses cytokine-induce VCAM-1 expression on the cell surface of human umbilical vein endothelial cells in a

BH4-independet manner. Biochim Biophys Acta. 2008;1780:960–965. doi:10.1016/j.bbagen.2008.03.012.

26. Pardo F, Silva L, Sáez T, Salsoso R, Gutiérrez J, Sanhueza C, Leiva A, Sobrevia L. Human supraphysiological gestational weight gain and fetoplacental vascular dysfunction. Int J Obes (Lond). 2015;39:1264–1273. doi:10.1038/ijo.2015.57.

27. Zou M, Shi Ch, Cohen R. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest. 2002;109:817–826. doi:10.1172/JCI200214442.

28. González M, Rojas S, Avila P, et al. Insulin reverses d-glucose-increased nitric oxide and reactive oxygen species generation in human umbilical vein endothelial cells. PLoS One. 2015;10:e0122398. doi:10.1371/journal.pone.0122398.

29. Werner-Felmayer G, Gross SS. Analysis of tetrahydrobiopterin and its role in nitric oxide synthesis. In: Feelish M, Stamler JS, eds. Methods in Nitric Oxide Research. New York, John Wiley & Sons, Inc.; 1996;271–299. ISBN: 0471955248.

30. Pernow J. Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP and VIP) peptides on pig splenic and human skeletal muscle arteries: Involvement of the endothelium. Br J Pharmacol. 1989;97:983–989. doi:10.1111/j.14765381.1989.tb12040.x.

31. Alp NJ, Mussa S, Khoo J, Cai S, Guzik T, Jefferson A, Goh N, Rockett KA, Channon KM. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. J Clin Invest. 2003;112:725–735. doi:10.1172/JCI200317786.

32. Channon KM. Tetrahydrobiopterin: a vascular redox target to improve endothelial function. Curr Vasc Pharmacol. 2012;10:705–708. doi:10.2174/157016112803520819.

33. Tiefenbacher CP, Bleeke T, Vahl C, Amann K, Vogt A, Kübler W. Endothelial dysfunction of coronary resistance arteries is improved by tetrahydrobiopterin in atherosclerosis. Circulation. 2000;102:2172–2179. doi:10.1161/01.CIR.102.18.2172.

34. Alp NJ, Channon K. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. Arterioscler Thromb Vasc Biol. 2004;24:413-420. doi:10.1161/01.ATV.0000110785.96039.f6.

35. Crabtree MJ, Hale AB, Channon KM. Dihydrofolate reductase protects endothelial nitric oxide synthase from uncoupling in tetrahydrobiopterin deficiency. Free Radic Biol Med. 2011;50:1639–1646. doi:10.1016/j.freeradbiomed.2011.03.010.

36. Liguori A, D'Armiento FP, Palagiano A, Balestrieri ML, Williams-Ignarro S, de Nigris F, Lerman LO, D'Amora M, Rienzo M, Fiorito C, Ignarro LJ, Palinski W, Napoli C. Effect of gestational hypercholesterolaemia on omental vasoreactivity, placental enzyme activity and transplacental passage of normal and oxidised fatty acids. Br J Obstet Gynecol. 2007;114:1547–1556. doi:10.1111/j.1471-0528.2007.01510.x.

37. Marseille-Tremblay C, Ethier-Chiasson M, Forest JC, Giguere Y, Masse A, Mounier C, Lafond J. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. Mol Reprod Dev. 2008;75:1054–1062. doi:10.1002/mrd.20842.

38. Holowatz LA, Kenney WL. Acute localized administration of tetrahydrobiopterin and chronic systemic atorvastatin treatment restore cutaneous microvascular function in

hypercholesterolaemic humans. J Physiol. 2011;589:4787–4797. doi:10.1113/jphysiol.2011.212100.

39. Leiva A, Fuenzalida B, Westermeier F, Toledo F, Salomon C, Gutiérrez J, Sanhueza C, Pardo F, Sobrevia L. Role for tetrahydrobiopterin in the fetoplacental endothelial dysfunction in maternal supraphysiological hypercholesterolemia. Oxid Med Cell Longev. 2015;2015:5346327. doi: http://dx.doi.org/10.1155/2016/5346327.

Figure 1. Human umbilical vein rings reactivity in MPH and MSPH. Dilation in response to calcitonine gene-related peptide (CGRP, 5 minutes) in human umbilical vein rings at birth from pregnancies where the mother showed (**A**) maternal physiological hypercholesterolemia (MPH, total plasma cholesterol 6.4 mmol/L (range 4.0-7.2 mmol/L)) or (**B**) maternal supraphysiological hypercholesterolemia (MSPH, total plasma cholesterol 8.4 mmol/L (range 7.6-10.8 mmol/L)). The relative responses were expressed as percentage of the fraction of the initial vessel response to KCl (see Methods). Vein rings were preincubated in the absence (Control, white symbols) or in the presence (grey symbols) of 100 μmol/L N^G- nitro-L-arginine methylester (L-NAME, 30 minutes), 100 μmol/L sepiapterin (1 hour), 5 mmol/L 2,4-diamino-6-hydroxypyrimidine (DAHP, 1 hour). **C**. Dilation in response to 10 μmol/L sodium nitroprusside (5 minutes) in umbilical vein rings from MPH or MSPH. In A, *P<0.05 vs Control in the absence of L-NAME. All values in the presence of L-NAME (except -11 log mol/L CGRP) are significantly different (P<0.05) from values in the absence of L-NAME. In B, Values in the presence of sepiapterin in absence of L-NAME (except -11 log mol/L CGRP) are significantly different (P<0.05) from all other values. Values are mean ± S.E.M. (n = 12–18).

Figure 2. Tetrahydrobiopterin involvement on nitric oxide synthase activity. A. L-Citrulline content in primary cultures of human umbilical vein endothelial cells (HUVECs) from pregnancies where the mother showed maternal physiological hypercholesterolemia (MPH) or maternal supraphysiological hypercholesterolemia (MSPH) in the absence (–) or presence (+) of 100 μmol/L N^G-nitro-L-arginine methylester (L-NAME, 30 minutes) and/or 100 μmol/L sepiapterin (24 hours) (see Methods). **B**. NOS activity estimated as the fraction of L-citrulline formation inhibited by L-NAME in the absence (–) or presence (+) of sepiapterin as in A. In A, **P*<0.05 vs corresponding values in MPH, †*P*<0.05 vs all other corresponding values in MPH or MPSH, ‡*P*<0.05 vs value in the absence of L-NAME and sepiapterin in MSPH. In B, **P*<0.05 vs corresponding values in MPH, †*P*<0.05 vs corresponding value in MPSH in the absence of sepiapterin. Values are mean ± S.E.M. (*n* = 6).

Figure 3. Tetrahydrobiopterin involvement on nitric oxide synthase expression. A. Western blots (representative blot of other 8 different umbilical veins from different patients) for total endothelial nitric oxide synthase (Total eNOS), or phosphorylated eNOS at Serine^{1177} (*P*~Ser^{1177}-eNOS) or Threonine^{495} (*P*~Thr^{495}-eNOS) in primary cultures of human umbilical vein endothelial cells (HUVECs) (β-actin was internal control) from pregnancies where the mother showed maternal physiological hypercholesterolemia (MPH) or maternal supraphysiological hypercholesterolemia (MSPH). Experiments were performed in cells incubated in the absence (–) or presence (+) of 100 μmol/L N^G-nitro-L-arginine methylester (L-NAME, 30 minutes) or 100 μmol/L sepiapterin (24 hours) (see Methods). *Lower panel*: Total eNOS/β-actin ratio densitometries normalized to 1 in MPH in the absence of L-NAME and sepiapterin. **B**. *P*~Ser^{1177}-eNOS/total eNOS ratio densitometries normalized to 1 in MPH as in A. **C**. *P*~Thr^{495}-eNOS/total eNOS ratio densitometries normalized to 1 in MPH as in A. **P*<0.05 vs corresponding values in MPH. Values are mean ± S.E.M. (*n* = 6).

Figure 4. MSPH–modulation of ROS generation. A. Reactive oxygen species (ROS) in primary cultures of human umbilical vein endothelial cells (HUVECs) from pregnancies where the mother showed maternal physiological hypercholesterolemia (MPH) or maternal supraphysiological hypercholesterolemia (MSPH). Relative fluorescence units (RFU) was measured in cells preloaded with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate in the absence (–) and/or presence (+) of 100 μmol/L N^G-nitro-L-arginine methylester (L-NAME, 30 minutes) or 100 μmol/L sepiapterin (24 hours) (see Methods). **B**. ROS generation expressed as the fraction of RFU inhibited by L-NAME in the absence (–) or presence (+) of sepiapterin as in A. **P*<0.05 vs corresponding values in MPH. Values are mean ± S.E.M. (*n* = 5).

Figure 5. Endothelial nitric oxide synthase monomer/dimer formation in MSPH. A. Western blots (representative blot of other 6 different umbilical veins from different patients) for endothelial nitric oxide synthase (eNOS) monomers and dimers in primary cultures of human umbilical vein endothelial cells (HUVECs) (β-actin was internal control) from pregnancies where the mother showed maternal physiological hypercholesterolemia (MPH) or maternal supraphysiological hypercholesterolemia (MSPH). Experiments were performed in cells incubated in the absence (–) or presence (+) of 100 μmol/L N^G-nitro-L-arginine methylester (L-NAME, 30 minutes) or 100 μmol/L sepiapterin (24 hours) (see Methods). **B**. eNOS monomers/β-actin ratio densitometries normalized to 1 in MPH in the absence of L-NAME and sepiapterin. **C**. eNOS dimers/β-actin ratio densitometries normalized to 1 in MPH as in B. **C**. eNOS monomer/dimer ratio densitometries normalized to 1 in MPH as in B. *P<0.05 vs all other corresponding values. †P<0.05 vs values in the presence of L-NAME. Values are mean ± S.E.M. (n = 6).

Figure 6. Biopterin availability in HUVECs from MPH and MSPH. A. Total biopterins concentration (Total) and tetrahydrobiopterin (BH_4) in primary cultures of human umbilical vein endothelial cells (HUVECs) from pregnancies where the mother exhibited maternal physiological hypercholesterolemia (MPH) or maternal supraphysiological hypercholesterolemia (MSPH) in the absence (–Sepiapterin) or in the presence (+Sepiapterin) of 100 μmol/L sepiapterin (24 hours) (see Methods). **B**. Dihydrofolate reductase (*DHFR*) and GTP cyclohydrolase 1 (*GCH1*) mRNA expression relative to *28S* rRNA (internal reference) in HUVECs from MPH and MSPH. **C**. Western blots (representative blot of other 6 different umbilical veins from different patients) for GTPCH1 in HUVECs (β-actin was internal control) from MPH and MSPH pregnancies. *Lower panel*: GTPCH1/β-actin ratio densitometries normalized to 1 in MPH. **D**. Neopterin formation in HUVECs from MPH or MSPH in the absence (–) or presence (+) of 0.7 mmol/L GTP (2 hours) or 5 mmol/L 2,4-diamino-6-hydroxypyrimidine (DAHP, 1 hour). In A, **P*<0.04 vs corresponding values in MPH, †*P*<0.01 vs corresponding values in (–Sepiapterin. In B and C, **P*<0.03 vs MPH. In D, **P*<0.02 for corresponding values in MPH, †*P*<0.04 vs all other values in MPH. Values are mean ± S.E.M. (*n* = 6–10).

Table 1. Clinical variables of pregnant women and newborns

Variables	MPH (*n* = 72)	MSPH (*n* = 35)
Maternal		
Age (years)	29.2 ± 5 (18-43)	29.7 ± 6 (17-40)
Height (m)	1.61 ± 0.05 (1.50-1.80)	1.61 ± 0.06 (1.47-1.75)
Weight (kg)		
First trimester	61.6 ± 8 (43-83)	59.8 ± 9.8 (46-90)
Second trimester	65.9 ± 7 * (51-87)	63.7 ± 8 * (46-78)
Third trimester	72.4 ± 7 † (55-92)	71.1 ± 7 † (50-80)
BMI (kg/m^2)		
First trimester	23.7 ± 4 (17-25)	22.5 ± 3 (19-25)
Second trimester	25.2 ± 3 * (20-28)	24.6 ± 3 * (19-27)
Third trimester	27.8 ± 3 † (22-31)	27.5 ± 3 † (21-31)
Systolic blood pressure (mmHg)		
First trimester	107.3 ± 9 (90-130)	104.6 ± 11 (90-122)
Second trimester	107.6 ± 11 (90-140)	106.6 ± 11 (90-120)
Third trimester	110.1 ± 9 * (92-135)	112.7 ± 13 * (90-140)
Diastolic blood pressure (mmHg)		
First trimester	64.5 ± 10(50-84)	65.6 ± 8 (58-80)
Second trimester	66.2 ± 8 (50-86)	65.7 ± 8 (50-80)
Third trimester	69.7 ± 10 * (50-120)	70.1 ± 9 * (60-88)
OGTT (mmol/L)		
Glycemia basal	4.2 ± 0.6 (3.6-5.1)	4.1 ± 0.6 (3.7-4.7)
Glycemia 2 hours after glucose	5.9 ± 1 (4.8-7.8)	5.8 ± 1 (4.2-7.8)
Newborn		
Birth Weight (gr)	3371 ± 380 (2640-4170)	3354 ± 386 (2610-4020)
Heigth (cm)	50.3 ± 2 (46-54)	49.8 ± 2 (46-54)
Gestational age (week)	38.9 ± 1 (37-41)	39 ± 1 (37-41)
Sex (female/male)	35/37	18/17

Women with maternal physiological (MPH, <7.2 mmol/L total cholesterol) or supraphysiological hypercholesterolemia (MSPH, ≥7.2 mmol/L total cholesterol) at term of pregnancy were included (see Methods). Weight, body mass index (BMI) and blood pressure were determined in the three trimesters of pregnancy. OGTT, oral glucose tolerance test. **P*<0.05 versus corresponding values at first trimester of pregnancy, †*P*<0.05 versus values at first and second trimesters of pregnancy. Data are mean ± S.D. (range).

Table 2. Lipids values in the maternal blood at term of pregnancy

	MPH (*n* = 72)	MSPH (*n* = 35)
TCh	6.2 ± 0.8 (3.7-7.2)	8.3± 0.9 * (7.3-10.8)
HDL	1.9 ± 0.4 (1-2.9)	1.9 ± 0.5 (0.7-3.8)
LDL	3.1 ± 0.7 (1.3-4.8)	5 ± 0.9 * (3.1-7.2)
vLDL	1.2 ± 0.3 (0.8-2.4)	1.3 ± 0.2 (0.7-1.9)
Triglycerides	2.7 ± 0.7 (1.8-4.6)	2.8 ± 0.6 (1.6-4.2)

Maternal blood level (mmol/L) of total cholesterol (TCh), high (HDL), low (LDL) or very low (vLDL) density lipoprotein-cholesterol and triglycerides was determined at term of pregnancy in women exhibiting maternal physiological (MPH, <7.2 mmol/L TCh) or supraphysiological (MSPH, ≥7.2 mmol/L TCh) hypercholesterolemia in pregnancy (see Methods). **P*<0.05 versus corresponding values in MPH. Values are mean ± S.D. (range).

Table 3. CGRP dilation in human umbilical vein rings

	EC_{50} (nmol/L)	
	MPH	MSPH
Without L-NAME		
Control	1.02 ± 0.02	18.4 ± 3.3 *
Sepiapterin	1.04 ± 0.02	0.02 ± 0.001 *
DAHP	1.60 ± 0.02	9.01 ± 0.5 *
With L-NAME		
Control	8.4 ± 0.84 †	*n.m.*
Sepiapterin	5.6 ± 1 †	*n.m.*
DAHP	11.8 ± 0.17 †	*n.m.*

Umbilical vein rings were obtained from term pregnancies where women exhibited maternal physiological (MPH, <7.2 mmol/L TCh) or supraphysiological (MSPH, ≥7.2 mmol/L TCh) hypercholesterolemia in pregnancy (see Methods). Vein rings were incubated in the absence (Without) or presence (With) of 100 μmol/L N^G-nitro-L-arginine methyl ester (L-NAME, 30 minutes), 100 μmol/L sepiapterin (1 hour) or 5 mmol/L 2,4-diamino-6-hydroxypyrimidine (DAHP, 1 hour) and the response to calcitonine gene related peptide (CGRP, 0.1 – 1000 nmol/L, 5 minutes) (CGRP) was assayed. CGRP half-maximal effective concentration (EC_{50}) was calculated (see Methods). *P<0.05 versus corresponding value in MPH, †P<0.05 versus corresponding values in Without L-NAME, Values are mean ± S.E.M. (n = 4 – 12). *n.m.*, not measurable.

Figure 1

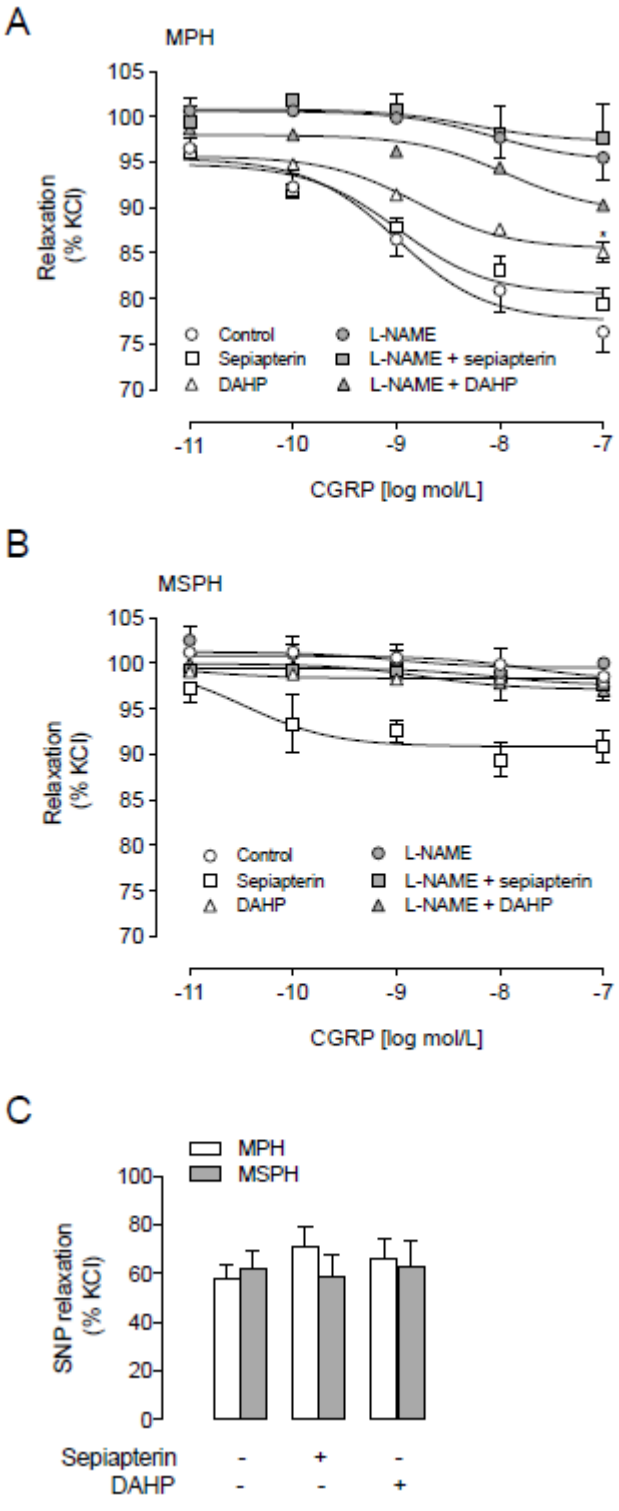
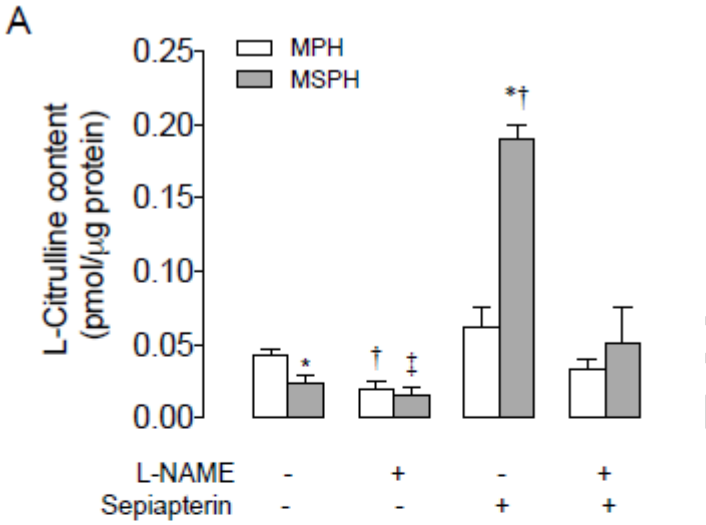


Figure 2



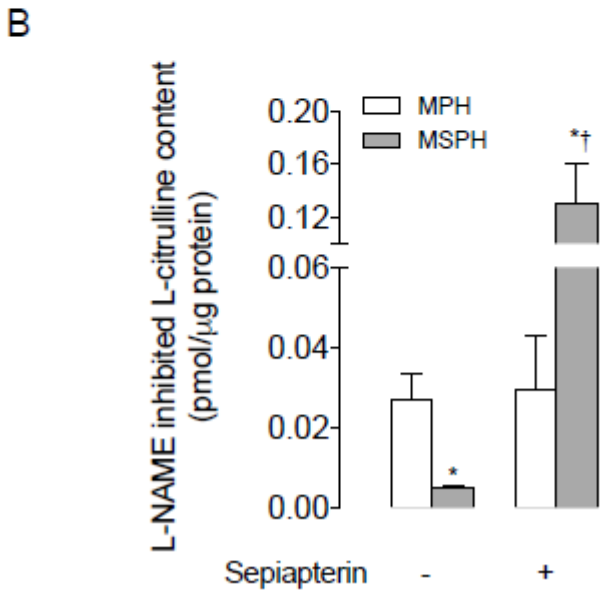


Figure 3

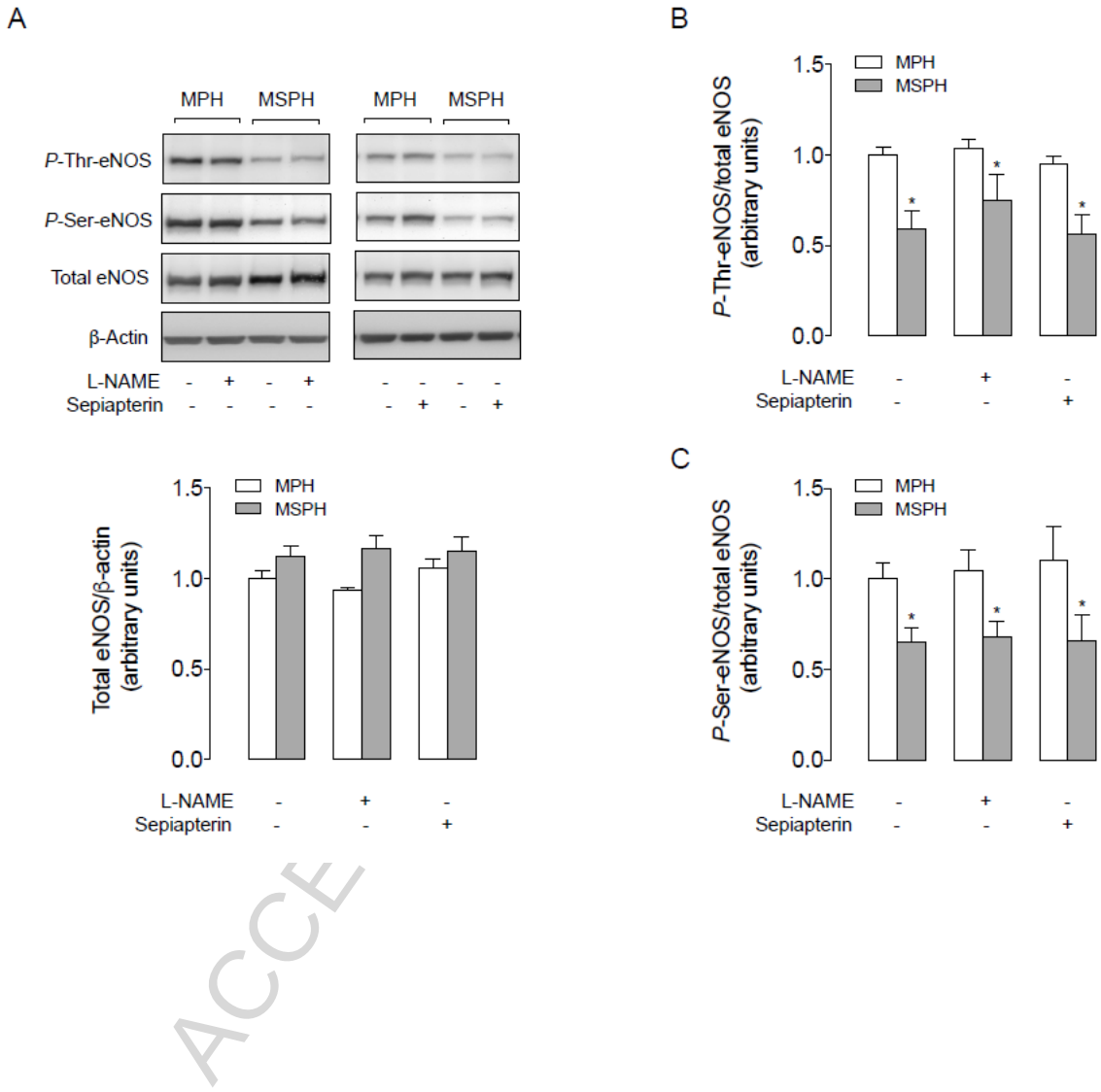


Figure 4

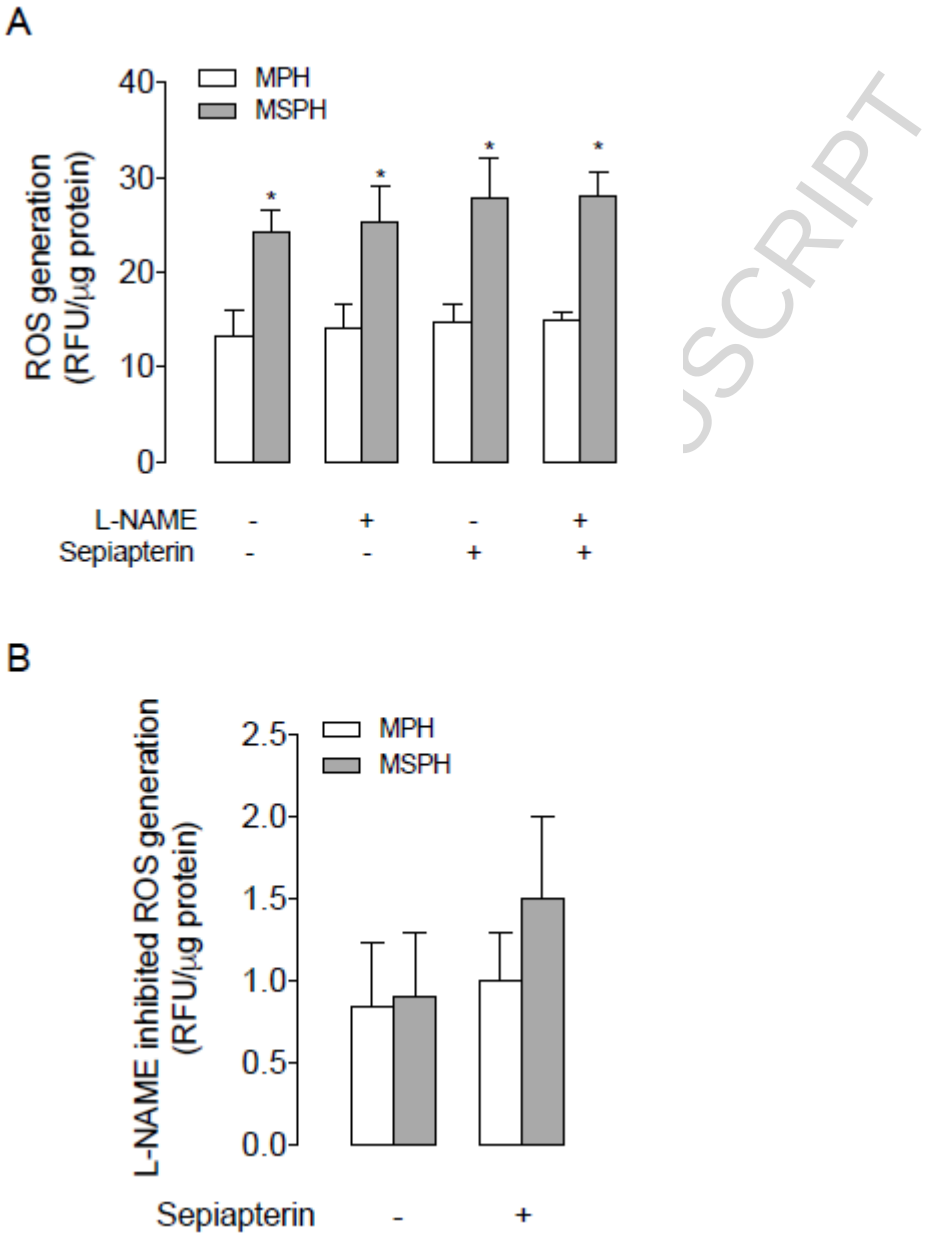


Figure 5

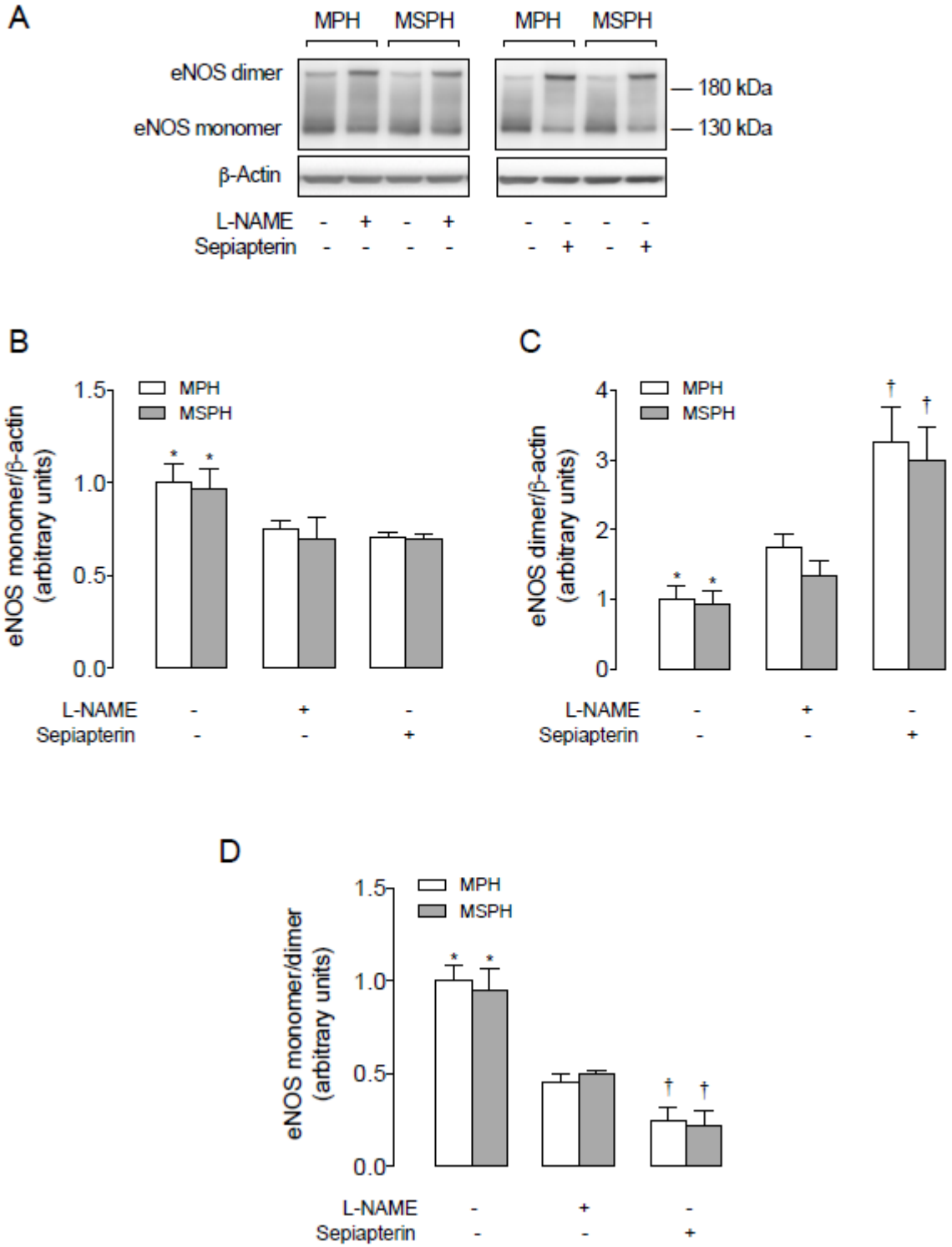
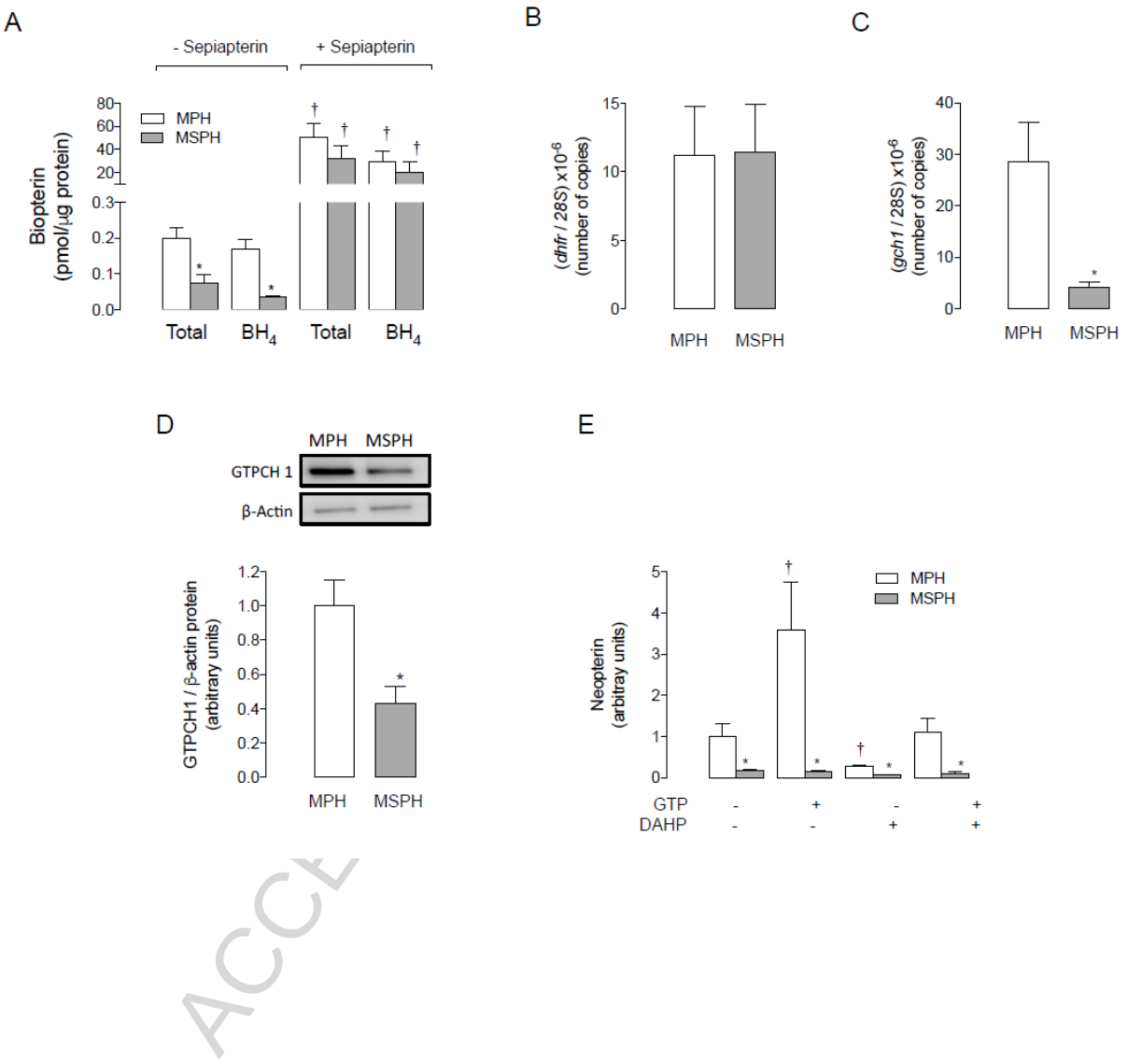


Figure 6



Highlights

1. MSPH associates with reduced umbilical vein dilation.
2. HUVECs from MSPH show reduced BH_4 synthesis.
3. The substrate for BH_4 synthesis, sepiapterin, reverses MSPH–reduced vein dilation.
4. Reduced BH_4 synthesis leads to lower NO synthesis and bioavailability in HUVECs.
5. MSPH–associated alterations may determine *in uterus* fetal vascular programming.