The Role of Nitric Oxide, ADMA, and Homocysteine in The Etiopathogenesis of Preeclampsia—Review

Weronika Dymara-Konopka * and Marzena Laskowska

Department of Obstetrics and Perinatology, Medical University of Lublin, Poland, 20-950 Lublin, Jaczewskiego 8, Poland; weronika.dymara@gmail.com
* Correspondence: melaskowska@go2.pl

Received: 19 May 2019; Accepted: 28 May 2019; Published: 5 June 2019

Abstract: Preeclampsia is a serious, pregnancy-specific, multi-organ disease process of compound aetiology. It affects 3–6% of expecting mothers worldwide and it persists as a leading cause of maternal and foetal morbidity and mortality. In fact, hallmark features of preeclampsia (PE) result from vessel involvement and demonstrate maternal endothelium as a target tissue. Growing evidence suggests that chronic placental hypoperfusion triggers the production and release of certain agents that are responsible for endothelial activation and injury. In this review, we will present the latest findings on the role of nitric oxide, asymmetric dimethylarginine (ADMA), and homocysteine in the etiopathogenesis of preeclampsia and their possible clinical implications.

Keywords: preeclampsia; asymmetric dimethylarginine; nitric oxide; homocysteine

1. Preeclampsia—Background

Preeclampsia (PE) is a serious, pregnancy-specific, multi-organ disease process of compound aetiology. It affects 3–6% of expecting mothers worldwide and it persists as a leading cause of maternal and foetal morbidity and mortality [1]. The diagnosis of PE is clinical. The diagnostic criteria were revised in 2013 and 2014: it is defined as new onset hypertension developing after 20 weeks of gestation and the coexistence of a minimum of one of the following new onset conditions: proteinuria, maternal end-organ dysfunction (including renal, hepatic, haematological, or neurological complications), or uteroplacental dysfunction reflected in foetal growth restriction (FGR) [2,3]. The disease can be further clinically classified as PE with or without severe features, as well as early-onset syndrome (presenting before 34 weeks of gestation, versus late-onset after completed 34 weeks), preterm PE (occurring from 34 + 1 but before 37 + 0 weeks), and term PE (after completed 37 weeks) [4]. The current management of the disease mainly depends on gestational age and assessment of PE severity, focusing on blood pressure control, maternal and foetal surveillance, and it aims to deliver the baby in optimal condition prolongating the pregnancy without worsening state of the mother [3]. It requires individualized calculations of risks and benefits, but unfortunately delivery still remains the only definitive treatment.

In recent times, a huge progress has been made in understanding the disease, getting scientist and doctors closer to explain biological mechanisms underlying the development of PE that possibly can be used to create new therapeutic strategies targeting them.

Within the last decade, subsequent studies confirmed the hypothesis of Roberts and colleagues from 1989, who suggested that PE clinical manifestations might be due to maternal endothelium dysfunction [5]. In fact, the hallmark features of PE result from vessel involvement and demonstrate maternal endothelium as a target tissue. However, the placenta, as the interface between mother and fetus, is also regarded a key and causative player in pathogenesis of PE. Growing evidence suggests that chronic placental hypoperfusion triggers the production and release of certain agents that are responsible for endothelial activation and injury. (Figure 1)
The development of PE involves a two-stage process [6]. The first, crucially important step is asymptomatic and it takes place during placental invasion and differentiation. While, during normal placentation, the embryo-derived cytotrophoblast properly invades the uterine wall, including the myometrium and spiral arterioles and it leads to transformation of maternal spiral arteries into large capacitance and low resistance vessels; this process is defective in preeclampsia [7–9]. The invasion of cytotrophoblast is incomplete, restricted to superficial layers of decidua that provides inadequate access to maternal oxygen and nutrients for the placenta and growing foetus. Poor placental invasion leads to diminished uteroplacental perfusion pressure and ischemia.

Abnormalities of placental invasion anticipate maternal disorder. Clinical manifestations that define PE represent the second stage of disease. Chronic placential hypoperfusion triggers abnormal production and the release of numerous bioactive factors into the maternal circulation. These circulating substances target endothelial cells resulting in widespread endotheliosis, endothelial dysfunction, generalized multi-system vasospasm, reduced plasma volume, oxidative stress, and hyperinflammatory state. Excessive expression of antiangiogenic proteins, like soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble Endoglin (sEng), which catch circulating, decreased proangiogenic substances, like vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and transforming growth factor β (TGFβ) result in an understanding of PE as an antiangiogenic state [10–13].

### Figure 1. Physiological roles of NO pathway in pregnancy and their possible influence on preeclampsia (PE) development in two-stage model of disease. NO PATHWAY ROLE: Nitric oxide (NO) pathway role; STAGES OF PE: Stages of preeclampsia (PE).

| NO PATHWAY ROLE | STAGES OF PE |
|-----------------|--------------|
| • confers autocrine/paracrine effects in the placenta | 1. Abnormal placental invasion |
| • regulates feto-placental vascular reactivity | incomplete, restricted to superficial layers of decidua |
| • main vasodilator in the placenta | inadequate access to oxygen and nutrients for placenta and fetus |
| • involved in trophoblast invasion and apoptosis, platelet adhesion in the intervillous space | reduction in uteroplacental perfusion pressure |
| • promotes embryo survival and tissue remodeling | placental ischemia/hypoxia |
| • regulates vascular angiogenesis | |
| • downstream mediator of VEGF, FGF and angiopoietins and possibly upstream regulator via HIF-1 | |
| • maintains endothelial cell barrier integrity | 2. Maternal endothelial dysfunction |
| • a key transmitter for endothelium-dependent regulation of vascular tone | endotheliosis |
| • inhibits the adhesion and activation of platelet aggregation | endothelial dysfunction |
| • acts as an anticoagulant | generalised multisystem vasospasm |
| • contributes to decrease in vascular resistance observed during early pregnancy in response to expended blood volume | reduced plasma volume |
| • supports growing need of organ perfusion during pregnancy | oxidative stress |
| • abolishes toxic activity of superoxide ions | hyperinflammatory and antiangiogenic state |
| • correlates with concentrations of anti and proangiogenic molecules | |

The development of PE involves a two-stage process [6]. The first, crucially important step is asymptomatic and it takes place during placental invasion and differentiation. While, during normal placentation, the embryo-derived cytotrophoblast properly invades the uterine wall, including the myometrium and spiral arterioles and it leads to transformation of maternal spiral arteries into large capacitance and low resistance vessels; this process is defective in preeclampsia [7–9]. The invasion of cytotrophoblast is incomplete, restricted to superficial layers of decidua that provides inadequate access to maternal oxygen and nutrients for the placenta and growing foetus. Poor placental invasion leads to diminished uteroplacental perfusion pressure and ischemia.

Abnormalities of placental invasion anticipate maternal disorder. Clinical manifestations that define PE represent the second stage of disease. Chronic placential hypoperfusion triggers abnormal production and the release of numerous bioactive factors into the maternal circulation. These circulating substances target endothelial cells resulting in widespread endotheliosis, endothelial dysfunction, generalized multi-system vasospasm, reduced plasma volume, oxidative stress, and hyperinflammatory state. Excessive expression of antiangiogenic proteins, like soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble Endoglin (sEng), which catch circulating, decreased proangiogenic substances, like vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and transforming growth factor β (TGFβ) result in an understanding of PE as an antiangiogenic state [10–13].
Defective trophoblast invasion is an early event in preeclampsia development. However, it has not been resolved whether it is the reason or result of another underlying problem. It remains unclear why trophoblast invasion is interrupted, but an altered immunological response at maternal-foetal interphase, genetics, and environmental factors are believed to contribute, although their role may vary between patients [14,15]. Furthermore, it is suggested that maternal susceptibility and response to placental derangements determines the onset, severity, clinical manifestations, and progression of the disease [16]. The most recent theory identifies PE as a complex disease with two distinct clinical presentations. The first, placental phenotype is associated with shallow trophoblastic invasion and restricted foetal growth, as opposed to PE associated with maternal metabolic syndrome. The second phenotype is associated with normal fetal growth and maternal low-grade inflammation, mainly due to placental oxidative stress, placental villi overcrowding, and decidual lesions [17,18].

Nitric oxide (NO) is one of the key players in the regulation of placental blood flow. It is actively engaged in cytotrophoblast endovascular invasion and development of the placenta, through its unique angiogenic and vasculogenic properties [19]. Current evidence supports altered NO production in the feto-placental unit in preeclampsia, which, by reduced bioavailability, may contribute to vasoconstriction of the placental bed, abnormal placental perfusion, and its maternal consequences, like increased blood pressure, systemic vascular resistance, and sensitivity to the pressors [20–25].

Asymmetric dimethylarginine (ADMA), which is an endogenous inhibitor of NO synthase (NOS), has also been associated with impaired endothelial function and with uterine artery flow disturbances that are characteristic for preeclampsia [24,26,27].

Homocysteine (Hcy) elevated concentrations in preeclamptic women lead to elevated ADMA levels, since Hcy has an inhibitory effect on ADMA metabolism. Hyperhomocysteinemia (HHcy) is also associated with endothelial cells lesions due to vascular fibrosis, which results in alterations in the coagulation system, enhanced platelet activation, and thrombogenesis—changes that are noted in preeclampsia [28–30].

In this review, we will present the latest findings on the role of nitric oxide, ADMA, and homocysteine in the etiopathogenesis of preeclampsia and their possible clinical implications.

2. Metabolism and Biological Role of NO, ADMA, and Homocysteine

Furchgott initially described nitric oxide as an endothelium-derived relaxant factor (EDRF) in 1980 after attributing a vasodilatory effect on vascular smooth muscle by stimulation of cholinergic nerves to the endothelium [31]. The identification of EDRF as NO was reported seven years later and was awarded a Nobel Prize in Physiology or Medicine for Furchgott, Ignarro, and Murad in 1998 for their discoveries concerning nitric oxide as key transmitter in the cardiovascular system. NO is produced through L-arginine-NO synthase pathway by converting L-arginine to L-citrulline in the presence of oxygen and the cofactor tetrahydrobiopterin or alternative enzymatic and non-enzymatic nitrate-nitrite-NO pathways [32,33].

Nitric oxide synthase (NOS) possess three different isoforms, namely neuronal NOS (nNOS) or type 1, inducible NOS (iNOS) or type 2 and endothelial NOS (eNOS) or type 3 [32]. NOS1 and NOS3 are considered as constitutive NOS. Endothelial NOS is stored in plasma membrane caveolae and its distribution and activity are regulated by numerous mechanisms [34]. Being released from endothelial cells, NO is quickly transported to the closest vascular smooth muscle cells, where it exerts its role by inducing the production of cyclic guanosine monophosphate (cGMP) as a second messenger. It may be neutralized by reactive oxygen species on the way to its target cells [35].

NO is the key transmitter for the endothelium-dependent regulation of the vascular tone that is controlled by humoral, metabolic and mechanical factors, for example, in response to increased blood flow [36]. Furthermore, NO inhibits the adhesion and activation of platelet aggregation, abolishes the toxic activity of superoxide ions, and acts as an anticoagulant and antiatherogenic substance [22,32].

It is also considered to have major effects on the gestational endothelial function as well as to play a supportive role in promoting embryo survival, tissue remodelling, immunosuppression, and
vasoregulation critical for placental nutrient transport [37–39]. The human foeto-placental vasculature lacks autonomic innervation and, therefore, NO confers autocrine and/or paracrine effects, influencing different aspects of physiological pregnancy. In particular, NO is the main vasodilator that is involved in foeto-placental vascular reactivity regulation, placental bed vascular resistance, trophoblast invasion and apoptosis, and platelet adhesion and aggregation in the intervillous space [40].

Further, the role of NO is also established in vasculogenesis, which results from the de novo formation of vessels derived from pluripotent precursor cells and angiogenesis, the formation of functional capillaries from pre-existing vasculature. Vascular endothelial growth factor (VEGF) is a key particle in these processes. Its expression is mediated by NO release and was required for initiation of vasculogenesis [41]. NO is also a critical downstream mediator of other than VEGF potent angiogenic substances, like basic fibroblast growth factor (FGF), and angiopoietin-1 [42]. The critical role of NO in angiogenesis has been shown in eNOS knockout mice [43]. NOS inhibition is accompanied by defective angiogenesis, as exemplified by deficient vascular sprouting. Interestingly, NO may also act upstream of angiogenic growth factors, because hypoxia-inducible factor-1 (HIF-1) perhaps mediated the effect of NO on VEGF production [44].

Asymmetric dimethylarginine (ADMA), which is an analogue of L-arginine, constitutes a natural metabolite that is found in human plasma. Dimethylarginines are formed as a result of the degradation of methylated arginine residues in proteins [45]. Approximately 80% of ADMA undergoes enzymatic transformation by two dimethylarginine dimethylaminohydrolases (DDAH-1 and -2) to L-citrulline and dimethylamine, whereas kidneys excrete the rest. ADMA is endogenous competitive inhibitor of L-arginine for all three isoforms of NOS. Elevated levels of ADMA block NO synthesis and limit the cellular uptake of L-arginine, thereby contributing to oxidative stress and disrupting further NO biogenesis. In this way, ADMA impairs the endothelial function and thus promotes atherosclerosis. Therefore, it is recognized as a biomarker of endothelial disorders. The ADMA levels are found to be elevated in patients with various cardiovascular and metabolic conditions, such as hypercholesterolemia, atherosclerosis, hypertension, chronic heart or renal failure, diabetes mellitus, stroke, and hyperhomocysteinemia [29,45–47]. ADMA has been shown to increase systemic vascular resistance in humans, as an endogenous inhibitor of NOS [45].

Homocysteine (Hcy) is a sulfur-containing amino acid that is produced during the conversion of essential amino acid methionine (Met) to cysteine (Cys) [48]. Its synthesis occurs in the transsulfuration of dietary methionine, which is abundant in animal protein, but it can also occur in demethylation that is related to fasting conditions. Hcy is metabolized by one of the two following pathways: remethylation to methionine, which requires the addition of a methyl group from 5-methyltetrahydrofolate (5-methyl THF) and the cofactor vitamin B12 (or betaine in an alternative reaction, restricted to the liver and independent of vitamin B12); and, transsulfuration to cystathionine; and finally, to cysteine, which requires vitamin B6 as a cofactor [49]. Methionine derivative, S-adenosyl methionine, is a cofactor that serves as a most important methyl donor of the body, whereas cysteine is used for glutathione synthesis or it is metabolised into taurine.

5-methyltetrahydrofolate (5-MTHF), which is the predominant circulating form of folate is the result of a reduction of 5,10-methylenetetrahydrofolate (5,10-MTHF) catalysed by the MTHFR (methylene tetrahydrofolate reductase) enzyme, coded by MTHFR gene, whose locus is on chromosome 1 at the end of the short arm (1p36.6) [50,51]. Polymorphisms of the MTHFR gene play a significant role in the pathogenesis of hyperhomocysteinemia.

The definition of hyperhomocysteinemia (HHcy), generally understood as increased homocysteine in the blood, differs between authors [52]. The total fasting concentration of Hcy in plasma of healthy patients is low and its level is 5.0–12.0 μmol/l when the immunoassay methods are used or between 5.0 and 15.0 μmol/L when assessed with the use of HPLC (high-performance liquid chromatography) [53]. Moderate HHcy is diagnosed if the levels are within the range of 16 to 30 μmol/L, 31–100 μmol/L is considered to be intermediate and a value above 100 μmol/L is classified as severe hyperhomocysteinemia [54].
The main causes of elevations in homocysteine levels are vitamin deficiency (B6, B12, folate), aforementioned genetic defects in enzymes that are involved in its metabolism (cystathionine-β-synthase deficiency and MTHFR), and disease conditions that interfere in the metabolism of cofactor levels, disturbing the transsulphuration and remethylation processes. In the general population, higher values of Hcy are observed in men than in women, although the discrepancy diminishes with age and in postmenopausal patients who tend to have higher Hcy levels [55].

In general, we can divide HHcys in two types: severe, but rare forms due to major genetic defects (individuals with the rare homocystinuria typically have levels of >100 µmol/L) and more common, moderately elevated homocysteine levels that are related to a pathogenesis, such as genetic and environmental factors, which is observed in up to 5% to 12% of the general population [52, 56].

The most common cause of severe hyperhomocysteinemia and classic homocystinuria (congenital homocystinuria) is considered to be the homozygous deficiency of CβS (cystathionine-β-synthase). This defect is responsible for an increase as much as up to 40-fold in fasting total homocysteine. Other not often observed genetically conditioned states of HHcy are the homozygous deficiency of MTHFR, deficiency of methionine synthase, and impaired activity of methionine synthase due to impaired vitamin B12 metabolism [54].

However, the most common genetic deficiency, which occurs at large rates in various populations, is single nucleotide polymorphism of MTHFR that has been associated with mild and moderate (25–60 µmol/L) hyperhomocysteinemia [57]. A point mutation C-to-T substitution at nucleotide 677 (677C→T) in the gene for MTHFR causes a thermolabile variant of the enzyme and has half-reduced enzyme function [54]. Another point mutation, called MTHFR A1298C, leads to 60% of normal enzyme function. Double heterozygous (1 abnormal MTHFR C677T gene plus 1 abnormal MTHFR A1298C gene) results in decreased reductase activity as those homozygous for the C677T polymorphism [58].

However, the leading cause of HHcy is folate, vitamin B12, and less commonly, B6 deficiency due to low supply, malabsorption, and treatment with substances, such as cyclosporin, methotrexate, fibrates, Levodopa (L-DOPA), and carbamazepine that interfere with the metabolic paths of these vitamins [59, 60]. High Hcy levels have been also associated with impaired renal function, high plasma creatinine, smoking, coffee consumption, and alcoholism [52].

HHcy is generally recognized as an independent risk factor for coronary, cerebral, and peripheral atherosclerosis, which was first reported by McCully in 1969 and later confirmed in a meta-analysis of numerous additional studies [61–63]. An extend meta-analysis suggested that an increment of homocysteine of 5 mmol/L is comparable to the increase in the risk of coronary artery disease caused by cholesterol elevation of 0.5 mmol/L [62]. An association between HHcys and cardiovascular disease, as well as some age-related pathologies, like stroke, Alzheimer’s disease, Parkinson’s disease, chronic renal failure, and osteoporosis is widely described [61–72]. There are ongoing efforts to understand if HHcy observed in vascular diseases is a causative factor or a consequence of endothelial activation [73].

3. NO, ADMA and Homocysteine in Pregnancy

During uncomplicated pregnancy, increased NOS activity in human uterine artery leads to higher NO levels [74]. NOS3 expression raises primarily in the syncytiotrophoblasts and NOS2 activity grows throughout pregnancy, with a peak around mid-gestation [38, 39, 75, 76]. Physiological reduction of blood pressure during pregnancy may greatly rely on the vasodilatory action of NO. NO contributes to the vasodilatation of blood vessels and the decrease in vascular resistance observed during early pregnancy, when maternal blood volume expands, while systemic vascular resistance and systemic blood pressure both decline [22, 25, 75–77].

In normal pregnancy, also levels of cGMP, a second messenger of NO signalling is particularly increased during the first trimester in plasma and urine [78]. Furthermore, a NO-cGMP pathway is present in the human uterus and it may be responsible for maintaining its relaxation. Spontaneous contractility in vitro was enhanced by the NOS inhibitor L-NAME (nitro-L-arginine methyl ester) and
decreased by NO. Thus, uterine reduced the responsiveness to nitric oxide at term may play a role in the initiation of labour [79].

Different studies on total NO in pregnancy gave conflicting results. The measurement of its relatively stable metabolites, nitrate, and nitrite (NOx) is often employed as an indicator of NO production and as a marker of NOS enzyme activity because NO is highly labile molecule [80]. Still, the plasma level is influenced, not only by the production, but also by the clearance of NO derivatives [79]. Some studies found that NO production increases with gestational age during normal pregnancy, especially in the second trimester, and it peaks in the third trimester [81–83]. However, contrary results were also published reporting that maternal circulating nitrite level decreased with advancing gestation [84], or even that there were no changes in NO production when compared to the nonpregnant state [85,86].

Likewise, studies investigating the circulating levels of NO in preeclampsia have also reported conflicting results [87]. These observations suggest that the status of NO biosynthesis in women during normal pregnancy and preeclampsia remains to be defined.

3.1. ADMA in Pregnancy

In normotensive pregnancy, the maternal plasma ADMA levels are generally reduced when comparing to non-pregnant group. The lowest concentration of ADMA is described during the first trimester, when the early fall in blood pressure is accompanied by a significant fall in ADMA concentration. The ADMA levels increase with gestational age in the second and third trimester [24,26]. These findings lead to a conclusion that, in early pregnancy, the reduction in ADMA and concomitant increase in NO are responsible for previously described hemodynamic adaptation, a higher need of organ perfusion in pregnancy, and uterine relaxation. In advanced pregnancy, physiologically increased ADMA levels thus help to prepare the uterine muscle fibers for the higher contractile activity before the labour. This is reflected by the higher ADMA concentrations after caesarean birth when compared with vaginal delivery and it may contribute to decreased nitric oxide production and bioavailability in neonatal vascular beds [88].

3.2. Homocysteine and Pregnancy

The homocysteine plasma levels fall in normal pregnancy [89,90]. An increase in plasma volume and associated haemodilution, glomerular hyperfiltration and postulated raised foetal need for methionine are mechanisms considered to contribute to this effect [91]. The importance of homocysteine to early foetal metabolism is demonstrated in a number of studies [92].

The reference values for HHcys in pregnancy that are proposed in one study were established as: higher than 7.7 mmol/L in the second trimester, and 10.5 mmol/L in the third trimester [93], although different authors usually defined their own cut-off values.

In the pathology of pregnancy, the disturbance of maternal homocysteine metabolism has been linked with recurrent pregnancy loss, deep venous thrombosis, foetal neural tube defects, and various conditions characterized by placental vasculopathy, such as preeclampsia, foetal growth restriction, and abruption [94–98].

4. NO, ADMA, and Homocysteine in Preeclampsia

4.1. NO Pathway Dysfunction in PE

In preeclamptic patients, like in normotensive pregnancies, the measurements of total NO concentration have shown variable results, ranging from decreased [81,99,100], unchanged [101], and increased [102,103] levels of circulating NO metabolites. The dietary intake of these substances could also influence the disparity, although a study that was conducted on women with PE, subjected to a reduced nitrate/nitrite diet, did not show decreased endogenous NO production [78]. In fact, NO measurements may be difficult to interpret, since they reflect the total activity of all three isoforms of
NOS, not just endothelial. While the whole body NO may not change in PE, in view of the evidence for reduced endothelial NO signalling and decrease in vascular relaxation in PE, tissue-specific differences in NOS expression and NO bioavailability could be expected. For instance, in late pregnant rats, renal eNOS decreases by 39%, while iNOS and nNOS increase by 31% and 25%, respectively [104].

PE is associated with abnormalities eNOS-NO pathway that probably exists at different stages of signal transduction process. There is not one particular defect, but multiple changes in key regulatory aspects in NO signaling. In studies where serum from PE women was placed on isolated vessels, nitric oxide-mediated vasorelaxation appeared to be absent [105].

Some studies have indicated that measuring plasma nitrite levels may reflect endogenous NO formation because NO is rapidly oxidized to nitrite. This is because 70% of plasma nitrites derive from NO synthase activity in the endothelium and its inhibition was associated with corresponding decreases in plasma nitrite concentrations [106,107]. By only using nitrite levels (which may be a better measure than total nitrite + nitrate), a reduction from 40 to 60% of total whole blood or plasma nitrite concentration was reported in PE women [108–110].

There is clinical evidence for the link between impaired NO formation and antiangiogenic factors overexpression in preeclampsia. Significant negative correlation between two antiangiogenic factors: sEng and sFlt-1, and nitrite concentrations was described [110], which suggested a possible inhibitory effect caused by these substances on the production of NO in patients with preeclampsia. Experimental studies have shown that NO increases proangiogenic VEGF and PIGF and it decreases sFlt-1 in hypoxic human trophoblast cells [111].

Using the nitrate reductase assay to measure NO, also a correlation between reductions in plasma NO with disease severity was identified, such that the levels were about 30% lower in severe PE vs. healthy pregnant controls [112].

Attempts to assess eNOS activity in PE led to the conclusion that it is still unknown whether eNOS deficiency plays a causal role there. In the murine model, chronic NOS inhibition reversed systemic vasodilation and glomerular hyperfiltration in pregnancy, which suggested its role for endothelial damage and decreased NO in the pathogenesis of preeclampsia [113]. However, different study with the use of eNOS knockout mice showed reduced uterine artery diameter, spiral artery length, and, as a consequence, diminished uteroplacental blood flow, resulting in elevated markers of placental hypoxia in the junctional zone. Even so, interestingly, sFlt-1 concentration was not elevated in the eNOS knockout mice [43].

Data from PE women is quite limited and without consensus on eNOS expression, as higher, lower, and unchanged levels of mRNA or enzyme have been reported. Several human studies failed to detect any significant differences in the circulating levels of eNOS [114,115]. One of the earliest studies on eNOS activity found an increase in eNOS expression in syncytiotrophoblast, foetal terminal villous capillary, and stem villous vessel endothelium, whereas the lack of eNOS expression in vascular terminal villi and weak expression in endothelial cells of villous vessels in placenta from normal pregnancy was noted [116]. These results are supported with a recent finding that caveolar eNOS expression is increased in PE placentas [117]. However, this stays in contrast to the observed similar placental levels of eNOS activity in PE patients [118], and to a more recent study in which placental syncytiotrophoblast eNOS expression was even decreased [119]. Apart from these confusing findings, altered placental eNOS levels may not directly relate to peripheral vascular endothelial function.

The reduced availability of eNOS substrate (L-Arginine) or the competitive inhibition by ADMA constitute other factors that may contribute to dysfunctional endothelial NO signalling in PE. One of proposed animal PE models involves administering the aforementioned L-NAME, a competitive inhibitor of arginine, which leads to maternal hypertension and proteinuria, and reduced foetal weight in a dose-dependent way [120]. L-NAME-induced hypertension and high circulating levels of sFlt-1 could be attenuated by the administration of exogenous sodium nitrite, and in this way restoring NO bioavailability [121].
In pregnancies that are complicated by PE, the ADMA levels are significantly higher than in both normotensive gestational age-matched and the nonpregnant control group [24,115,122–124]. Even higher concentration of ADMA in patients with early-onset PE may suggest a relationship between disease severity and determining the time of PE clinical manifestation [125,126]. Moreover, ADMA may have a predictive value in PE, since its modestly (+26%) elevated concentrations were observed as early as in the first trimester [127] and significantly elevated during second trimester in pregnancies that developed PE in more advanced gestational age [128]. Another hypothesis is that increased ADMA concentration may contribute to development of PE in early pregnancy, leading to impaired placenta and its consequences [126]. The association of abnormal uterine artery Doppler waveforms with elevated ADMA levels [129,130] supports the role of endogenous NOS inhibitors adversely affecting maternal vasodilation and blood pressure.

It has been postulated that hyperhomocysteinemia (HHcy) may contribute to the development of PE, as it leads to endothelial dysfunction and accumulation of ADMA [29,30].

4.2. Homocysteine in PE

The association of hyperhomocysteinemia and preeclampsia has initially been suggested by Decker et al. [131], and all authors have not confirmed it. However, the majority of evidence suggests a positive correlation.

Higher maternal plasma homocysteine concentrations in preeclamptic pregnancies as compared to normotensive were widely reported [93,125,130,132–137]. Overall, these differences seem to be present at all of the investigated time points across the gestation, starting from early pregnancy before 20 weeks [138–142] and in both severe and non-severe forms of PE. Comprehensively, this finding led to a conclusion that high homocysteine in early pregnancy constitutes a risk factor for PE. Therefore, attempts to introduce Hcy measurement into a screening test for PE to improve the prediction model, for example, by combining it with uterine artery Doppler test in the second trimester, resulted in being valuable [140]. However, there are also studies that rejected an association between elevated Hcy in early second trimester and subsequent PE [143–145]. This could result from differences in study designs, laboratory techniques, and disease definitions, among other reasons, which may be further investigated in a systematic review.

Still, most of the papers focus on the third trimester [93,130,132–135,137], where the differences between HHcys in severe and non-severe PE are marked. Pregnant women complicated with severe preeclampsia displayed significantly higher serum Hcy levels than with non-severe form [93,112,132,134,136,146]. Thus, homocysteine concentrations positively correlating with the clinical presentation of disease may constitute a marker of severity of preeclampsia.

Besides elevated Hcy concentrations in maternal plasma, the majority of authors analyse the possible association with either folate or vitamin B12 deficiencies, as well as NO pathways in preeclamptic patients. Here, contrary results are presented. Folate and vitamin B12 serum levels were described as unchanged [132,134,135] in PE when comparing to normotensive healthy women with uncomplicated pregnancies. However, opposite results indicating these vitamins deficiencies were also reported [135,146]. Nevertheless, further studies are needed to confirm whether the prescription of these vitamins could decrease serum homocysteine, thereby possibly reducing the risk of preeclampsia or (if it occurs) its severity.

Interesting association between HHcy and NO signalling pathway was reported. Homocysteine inhibits the expression and activity of dimethylamino dimethyl hydrolase (DDAH), which is the enzyme degrading ADMA to citrulline and dimethylamine [22,147–149]. It has been suggested that elevated ADMA is a mediator of endothelial dysfunction in hyperhomocysteinemia due to this metabolic relation [148,150]. HHcy, leading to accumulation of ADMA, may contribute to eNOS blockade and NO deficiency in the presence of general appropriate concentration of its synthase. The reduced release of NO by endothelial cells in HHcy was observed, suggesting the impairment of the eNOS pathway by DDAH inhibition [29].
The hypothesized mechanisms of disease linking homocysteine to preeclampsia are complex and still incompletely understood.

To date, vascular endothelial cell dysfunction that is provoked by an elevated level of homocysteine (Hcy) is suggested to be the most important connection. However, some authors questioned whether mild HHcy observed in PE, with Hcy values that are similar to those found in normotensive non-pregnant women, can provoke damage of the vascular endothelium. They postulate that this damage can be mediated rather by oxidative stress, as endothelium of pregnant women might be more vulnerable to oxidative injury [151]. Hyperhomocysteinemia is also associated with lesions in endothelial cells, due to vascular fibrosis, which results in alterations in coagulation system, enhanced platelet activation, and thrombogenesis—changes that are noted in preeclampsia [28–30].

On the other hand, metabolism in the kidney is the major route by which homocysteine is cleared from plasma. The association between Hcy and glomerular filtration rate (GFR) seems linear and it is present, even in the hyperfiltrating range [152,153]. Thus, this route of elimination may be affected by the already established preeclamptic changes in the kidney and secondarily lead to increased Hcy concentrations in plasma [154].

5. Therapeutic Potential of NO Pathway during Pregnancy

Enhancing the NO signalling in pregnancy is still thought to be an attractive option in both preeclampsia prevention in high-risk groups of women and treatment. There are attempts to prolong the pregnancy with the use of NOS substrates (L-arginine and L-citrulline), as well as NO-donors (glyceryl trinitrate, S-nitrosoglutathione, isosorbide mononitrate), natural derivatives, or vasodilators, such as sildenafil citrate [155–158]. NO donors and substrates have been also used for the management of other pregnancy disorders, like recurrent abortions, treatment of preterm labour, and dysmenorrhea [155]. Unfortunately, the analysis of use of these substances in PE gives conflicting results.

In vitro, nitric oxide synthase activity is inhibited by intracellular ADMA and is rescued by L-arginine [159]. Therefore, L-arginine supplementation in pregnancy could possibly overcome NOS blockage and its consequences. A study of intravenous infusion of L-arginine in pregnant women showed a significant reduction in blood pressure—an effect that was greater in women with preeclampsia [157,160]. Additionally, a significant reduction in PE incidence was described in high risk women who received L-arginine and vitamins C and E (as antioxidants reducing oxidative stress implicated in the pathophysiology of PE) before 24 weeks of gestation, when comparing to placebo and only vitamin group, although the effects of L-arginine alone were not studied [161]. However, last year, meta-analysis showed that combined vitamin C and E supplementation has no influences on the occurrence of preeclampsia [162], although an interaction between these vitamins and L-arginine is not well explained. Supplementation of L-arginine for pregnant women with chronic hypertension showed less need for antihypertensive drugs use, but no reduction in the incidence of superimposed preeclampsia [163]. Interestingly, women who go on to develop PE have been found to have higher, not lower, plasma L-arginine concentrations [129], which can counteract the raised ADMA values, but other vasoconstrictor effects may persist in women who are vulnerable to preeclampsia.

The Cochrane database systematic review, including six trials demonstrates, that there is insufficient evidence to draw reliable conclusions about whether NO donors and precursors prevent PE or its complications. Another 13 papers are still waiting to be assessed [164]. Conclusions from the review are limited mainly due to the fact that too few women have been studied. Adverse effects that were described during supplementation of NO donors included mainly headaches, often sufficiently severe to stop medication. However, more recent studies clearly show that isosorbide mononitrate and L-arginine are both effective in prevention of PE [165,166]. Besides the significant reduction of PE incidence in the treatment groups, there was also a reduction in FGR and improvement in foetal outcome, including less neonatal admissions to the intensive care unit.

An inorganic nitrate, NO3, with a capacity to increase the bioavailability of NO has been recently implemented in a clinical trial with beetroot juice as a norganic nitrate (NO3−) rich dietary
supplement [167]. It was the first trial to investigate effects of nitrate supplementation on blood pressure in human pregnancy, according to the authors. The treatment group consisted of pregnant women with chronic hypertension. In this group, the administration of NO3− donors significantly increased plasma and salivary nitrate/nitrite when compared with placebo, but there was no overall reduction in blood pressure. However, there was a highly significant correlation between changes in plasma nitrite and only lowering diastolic blood pressure in the nitrate-treated arm [168].

Sildenafil exerts its role by inducing vasodilatation, via inhibiting phosphodiesterase and thereby maintaining the availability of cGMP, the effector of NO activity within the cell [169]. In vivo studies of sildenafil citrate in rat models of preeclampsia have shown a significant reduction in the production of antiangiogenic molecules sFlt-1 and sEng [170], as well as an improvement in blood pressure, proteinuria, uteroplacental and foetal perfusion after treatment [171]. Lately, a randomized controlled trial to evaluate PE therapy with sildenafil showed that, when compared to controls (receiving a placebo), therapy with sildenafil resulted in four days prolongation of pregnancy [158].

Glyceryl trinitrate (GTN), which is commonly known as nitroglycerin, is a widely used organic nitrate in clinical practice, particularly for the treatment of angina pectoris. Transdermal nitroglycerin patches have been the focus of various studies, for both the prevention and management of PE and related disorders. Studies of both forms: transdermal [172,173] and sublingual GTN [156] in preeclamptic patients consistently showed a significant reduction in blood pressure and resistance in the uterine artery without an adverse effect on the foetal Doppler parameters. Nonetheless, these studies have highlighted the potential use of GTN as an antihypertensive agent in PE. However, it remains to be established whether GTN offers any competitive advantage over already existing treatment options. Certainly, the major disadvantage of organic nitrates, in general, and GTN, in particular, is the development of tolerance upon continuous dosing, necessitating the requirement of regular ‘nitrate-free’ intervals.

6. Conclusions

New therapeutic options emerge as our understanding of preeclampsia improves. Enhancing NO pathway, by overcoming eNOS block and neutralizing raised ADMA values and by homocysteine reduction, may be a perspective goal in the treatment and prevention of PE.

Still new, prospective clinical trials are needed to safely develop effective management strategies with the use of pharmacological modulators of the NO system, which seem to hold promise for the treatment of preeclampsia.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abalos, E.; Cuesta, C.; Grosso, A.L.; Chou, D.; Say, L. Global and regional estimates of preeclampsia and eclampsia: A systematic review. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2013, 170, 1–7. [CrossRef] [PubMed]
2. Tranquilli, A.L.; Dekker, G.; Magee, L.; Roberts, J.; Sibai, B.M.; Steyn, W.; Zeeman, G.G.; Brown, M.A. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens.* 2014, 4, 97–104. [CrossRef] [PubMed]
3. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in Pregnancy. Report of the American College of Obstetricians and Gynecologists’ Task Force on Hypertension in Pregnancy. *Obstet. Gynecol.* 2013, 122, 1122–1131.
4. Tranquilli, A.L.; Brown, M.A.; Zeeman, G.G.; Dekker, G.; Sibai, B.M. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertens.* 2013, 3, 44–47. [CrossRef] [PubMed]
5. Roberts, J.M.; Taylor, R.N.; Musci, T.J.; Rodgers, G.M.; Hubel, C.A.; McLaughlin, M.K. Preeclampsia: An endothelial cell disorder. *Am. J. Obstet. Gynecol.* 1989, 161, 1200–1204. [CrossRef]
6. Roberts, J.M.; Hubel, C.A. The two stage model of preeclampsia: Variations on the theme. *Placenta* 2009, 30, S32–S37. [CrossRef] [PubMed]

7. Cross, J.C.; Werb, Z.; Fisher, S.K. Implantation and the placenta: Key pieces of the development puzzle. *Science* 1994, 266, 1508–1518. [CrossRef] [PubMed]

8. Brosens, I.A.; Robertson, W.B.; Dixon, H.G. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet. Gynecol. Annu.* 1972, 1, 177–191. [CrossRef]

9. Huppertz, B. Placental origins of preeclampsia: Challenging the current hypothesis. *Hypertension* 2008, 51, 970–975. [CrossRef]

10. Maynard, S.E.; Min, J.Y.; Merchant, J.; Lim, K.H.; Li, J.; Mondal, S.; Libermann, T.A.; Morgan, J.P.; Sellke, F.W.; Stillman, I.E.; et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt-1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J. Clin. Invest.* 2003, 111, 649–658. [CrossRef]

11. Chaiworapongsa, T.; Romero, R.; Kim, Y.M.; Kim, G.J.; Espinoza, J.; Bujold, E.; Goncalves, L.; Gomez, R.; Edwin, S.; et al. Plasma soluble vascular endothelial growth factor receptor -1 concentration is elevated prior to the clinical diagnosis of preeclampsia. *J. Matern. Fetal Neonatal Med.* 2005, 17, 3–18. [CrossRef] [PubMed]

12. Venkatesha, S.; Toporsian, M.; Lam, C.; Hanai, J.; Mammo, T.; Kim, Y.M.; Bdolah, Y.; Lim, K.H.; Yuan, H.T.; Libermann, T.A.; et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat. Med.* 2006, 12, 642–649. [CrossRef] [PubMed]

13. Dymara-Konopka, W.; Laskowska, M.; Blazewicz, A. Angiogenic Imbalance as a Contributor of Preeclampsia. *Curr. Pharm. Biotechnol.* 2018, 19, 797–815. [CrossRef] [PubMed]

14. Harihana, N.; Shoemker, A.; Wagner, S. Pathophysiology of hypertension in preeclampsia. *Clin. Pract.* 2016, 13, 33–37.

15. Karumanchi, S.A.; Maynard, S.E.; Stillman, I.E.; Epstein, F.H.; Sukhatme, V.P. Preeclampsia: A renal perspective. *Kidney Int.* 2005, 67, 2011–2113. [CrossRef] [PubMed]

16. Roberts, J.M.; Escudero, C. The Placenta in Preeclampsia. *Pregnancy Hypertens* 2012, 2, 72–83. [CrossRef] [PubMed]

17. Ferrazzi, E.; Zullino, S.; Stampalija, T.; Vener, C.; Cavoretto, P.; Gervasi, M.T.; Vergani, P.; Mecacci, F.; Marozio, L.; Oggè, G.; et al. Bedside diagnosis of two major clinical phenotypes of hypertensive disorders of pregnancy. *Ultrasound Obstet. Gynecol.* 2016, 48, 224–231. [CrossRef]

18. Redman, C.W.; Sargent, I.L.; Staff, A.C. IFPA Senior Award Lecture: Making sense of pre-eclampsia. *Placenta* 2014, 35, S20–S25. [CrossRef]

19. Huang, L.T.; Hsieh, C.S.; Chang, K.A.; Tain, Y.L. Roles of nitric oxide and asymmetric dimethylarginine in pregnancy and fetal programming. *Int. J. Mol. Sci.* 2012, 13, 14606–14622. [CrossRef]

20. Baylis, C.; Beinder, E.; Suto, T.; August, P. Recent insights into the roles of nitric oxide and renin-angiotensin in the pathophysiology of preeclamptic pregnancy. *Semin. Nephrol.* 1998, 18, 208–230.

21. Lowe, D.T. Nitric oxide dysfunction in the pathophysiology of preeclampsia. *Nitric Oxide* 2000, 4, 441–458. [CrossRef] [PubMed]

22. Demir, B.; Demir, S.; Pasa, S. The role of homocysteine, asymmetric dimethylarginine and nitric oxide in preeclampsia. *J. Obstet. Gynaecol. Res.* 2012, 38, 525–528. [CrossRef] [PubMed]

23. Khalil, R.A.; Granger, J.P. Vascular mechanisms of increased arterial pressure in preeclampsia: Lessons from animal models. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2002, 283, R29–R45. [CrossRef] [PubMed]

24. Fickling, S.A.; Williams, D.; Vallance, P.; Nussey, S.S.; Whitley, G.S. Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and preeclampsia. *Lancet* 1993, 342, 242–243. [CrossRef]

25. Speer, P.D.; Powers, R.W.; Frank, M.P.; Harger, G.; Markovic, N.; Roberts, J.M. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants. *Am. J. Obstet. Gynecol.* 2008, 198, 112 e111–112 e117. [CrossRef]

26. Holden, D.P.; Fickling, S.A.; Whitley, G.S.; Nussey, S.S. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *Am. J. Obstet. Gynecol.* 1998, 178, 551–556. [CrossRef]

27. Pettersson, A.; Hedner, T.; Milsom, I. Increased circulating concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet. Gynecol. Scand.* 1998, 77, 808–813. [CrossRef]
28. Aubard, Y.; Darodes, N.; Cantaloube, M. Hyperhomocysteinemia and pregnancy—Review of our present understanding and therapeutic implications. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2000**, *93*, 157–165. [CrossRef]

29. Stühlinger, M.C.; Tsao, PS.; Her, J.H.; Kimoto, M.; Balint, R.F.; Cooke, J.P. Homocysteine impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine. *Circulation* **2001**, *104*, 2569–2575. [CrossRef]

30. Herrmann, W.; Isber, S.; Obeid, R.; Herrmann, M.; Jouma, M. Concentrations of homocysteine, related metabolites and asymmetric dimethylarginine in preeclamptic women with poor nutritional status. *Clin. Chem. Lab. Med.* **2005**, *43*, 1139–1146. [CrossRef]

31. Ignarro, L.J. Nitric oxide. A novel signal transduction mechanism for transcellular communication. *Hypertension* **1990**, *16*, 477–483. [CrossRef] [PubMed]

32. Ramadoss, J.; Pastore, M.B.; Magness, R.R. Endothelial caveolar subcellular domain regulation of endothelial nitric oxide synthase. *Clin. Exp. Pharmacol. Physiol.* **2013**, *40*, 753–764. [CrossRef] [PubMed]

33. Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and pathophysiology. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 579–684. [CrossRef] [PubMed]

34. Teerlink, T.; Luo, Z.; Palm, F.; Wilcox, C.S. Cellular ADMA: Regulation and action. *Pharmacol. Res.* **2003**, *47*, 82–90. [CrossRef]

35. Furchgott, R.F.; Zawadzki, J.V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **1980**, *288*, 373–376. [CrossRef] [PubMed]

36. Vallance, P.; Leiper, J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 1023–1030. [CrossRef] [PubMed]

37. Grubisic, T.M. Genetics of homocysteine metabolism and associated disorders. *Braz. J. Med. Biomed. Res.* **2010**, *43*, 1–7. [CrossRef]

38. Bailey, L.; Gregory, J. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: Metabolic significance, risks and impact on folate requirement. *J. Nutr.* **1999**, *129*, 919–922. [CrossRef]
51. Goyette, P.; Sumner, J.; Milos, R.; Duncan, A.; Rosenblatt, D.; Matthews, R.; Rozen, R. Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping, and mutation identification. *Nat. Genet.* 1994, 7, 195–200. [CrossRef] [PubMed]

52. Faeh, D.; Chiolero, A.; Paccaud, F. Homocysteine as a risk factor for cardiovascular disease: Should we (still) worry about it? *Swiss Med. Wkly.* 2006, 136, 745–756. [PubMed]

53. Baszzczuk, A.; Kopczynski, Z. Hyperhomocysteinemia in patients with cardiovascular disease. *Postepy Hig. Med. Dosw.* 2014, 68, 579. [CrossRef] [PubMed]

54. Hankey, G.J.; Eikelboom, J.W. Homocysteine and vascular disease. *Lancet* 1999, 354, 407–413. [CrossRef]

55. Clarke, R.; Woodhouse, P.; Ulvik, A.; Frost, C.; Sherliker, P.; Refsum, H. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. *Clin. Chem.* 2010, 44, 102–107.

56. Thrombosis Interest Group of Canada. Thrombophilia: Homocysteinemia and Methylene Tetrahydrofolate Reductase. Available online: http://thrombosiscanada.ca/?page_id=18 (accessed on 17 June 2015).

57. Curro, M.; Gugliandolo, A.; Gangemi, C.; Risitano, R.; Ientile, R.; Caccamo, D. Toxic effects of mildly elevated homocysteine concentrations in neuronal-like cells. *Neurochem. Res.* 2014, 39, 1485–1495. [CrossRef] [PubMed]

58. Weisberg, I.S.; Jacques, P.F.; Selhub, J.; Bostom, A.G.; Chen, Z.; Curtis Ellison, R.; Eckfeldt, J.H.; Rozen, R. The 1298A→C polymorphism in methylenetetrahydrofolate reductase (MTHFR): In vitro expression and association with homocysteine. *Atherosclerosis* 2001, 156, 409–415. [CrossRef]

59. Stanger, O.; Herrmann, W.; Pietrzik, K.; Fowler, B.; Geisel, J.; Dierkes, J.; Weger, M. Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. *Z. Kardiol.* 2004, 93, 439–453. [CrossRef]

60. Ntaios, G.; Savopoulos, C.; Grekas, P.; Hatzitolios, A. The controversial role of B-vitamins in cardiovascular risk: An update. *Arch. Cardiovasc. Dis.* 2009, 102, 847–854. [CrossRef]

61. McCully, K.S. Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. *Am. J. Pathol.* 1969, 56, 111.

62. Boushey, C.J.; Beresford, S.A.; Omenn, G.S.; Motulsky, A.G. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995, 274, 1049–1057. [CrossRef] [PubMed]

63. Refsum, H.; Ueland, P.M.; Nygard, O.; Vollset, S.E. Homocysteine and cardiovascular disease. *Annu. Rev. Med.* 1998, 49, 31–62. [CrossRef] [PubMed]

64. Guilland, J.; Ueland, P.M.; Nygard, O.; Vollset, S.E. Homocysteine and cardiovascular disease. *Annu. Rev. Med.* 1998, 49, 31–62. [CrossRef] [PubMed]

65. Wang, Y.; Wang, X.; Kong, W. Hyperhomocysteinaemia and vascular injury: Advances in mechanisms and drug targets. *Br. J. Pharmacol.* 2017, 175, 1173–1189. [CrossRef] [PubMed]

66. Morris, M.S. Homocysteine and Alzheimer’s disease. *Lancet Neurol.* 2003, 2, 425–428. [CrossRef]

67. McIlroy, S.P.; Dynan, K.B.; Lawson, J.T.; Patterson, C.C.; Passmore, A.P. Moderately elevated plasma homocysteine, methylenetetrahydrofolate reductase genotype, and risk for stroke, vascular dementia, and Alzheimer disease in Northern Ireland. *Stroke* 2002, 33, 2351–2356. [CrossRef] [PubMed]

68. Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P.F.; Rosenberg, I.H.; D’Agostino, R.B.; Wilson, P.W.F.; Wolf, P.A. Plasma total homocysteine is associated with abdominal aortic aneurysm and aortic diameter in older men. *J. Vasc. Surg.* 2013, 58, 364–370. [CrossRef] [PubMed]

69. Fu, Y.; Wang, X.; Kong, W. Hyperhomocysteinaemia and vascular injury: Advances in mechanisms and drug targets. *Br. J. Pharmacol.* 2017, 175, 1173–1189. [CrossRef] [PubMed]

70. Herrmann, M.; Widmann, T.; Herrmann, W. Homocysteine—a newly recognised risk factor for osteoporosis. *Clin. Chem. Lab. Med.* 2005, 43, 1111–1117. [CrossRef]

71. Perna, A.F.; Sepe, I.; Lanza, D.; Pollastro, R.M.; De Santo, N.G.; Ingrosso, D. Hyperhomocysteinemia in chronic renal failure: Alternative therapeutic strategies. *J. Ren. Nutr.* 2012, 22, 191–194. [CrossRef] [PubMed]

72. Brattström, L.; Wilcken, D.E. Homocysteine and cardiovascular disease: Cause or effect? *Am. J. Clin. Nutr.* 2000, 72, 315–323. [CrossRef] [PubMed]
74. Nelson, S.H.; Steinsland, O.S.; Wang, Y.; Yallampalli, C.; Dong, Y.L.; Sanchez, J.M. Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy. Circ. Res. 2000, 87, 406–411. [CrossRef] [PubMed]
75. Stefano, G.B.; Kream, R.M. Reciprocal regulation of cellular nitric oxide formation by nitric oxide synthase and nitrite reductases. Mol. Sci. Monit. 2011, 17, RA221–RA226. [CrossRef] [PubMed]
76. Sanghavi, M.; Rutherford, J.D. Cardiovascular physiology of pregnancy. Circulation 2014, 130, 1003–1008. [CrossRef] [PubMed]
77. Leiva, A.; Fuenzalida, B.; Barros, E.; Sobrevia, B.; Salsoso, R.; Sáez, T.; Villalobos, R.; Silva, L.; Chiarello, L.; Toledo, F.; et al. Nitric oxide is a central common metabolite in vascular dysfunction associated with diseases of human pregnancy. Curr. Pharm. Pharmacol. 2016, 14, 237–259. [CrossRef]
78. Conrad, K.P.; Kerchner, L.J.; Mosher, M.D. Plasma and 24-h NO(x) and cGMP during normal pregnancy and preeclampsia in women on a reduced NO(x) diet. Am. J. Physiol. 1999, 277, F48–F57.
79. Buhimschi, I.; et al. Involvement of a nitric oxide-cyclic guanosine monophosphate pathway in control of human uterine contractility during pregnancy. Am. J. Obstet. Gynecol. 1995, 172, 1577–1584. [CrossRef]
80. Baylis, C.; Vanceall, P. Measurement of nitrite and nitrate levels in plasma and urine: What does this measure tell us about the activity of the endogenous nitric oxide system? Curr. Opin. Nephrol. Hypertens. 1998, 7, 59–62. [CrossRef]
81. Choi, J.W.; Im, M.W.; Pai, S.H. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. Ann. Clin. Lab. Sci. 2002, 32, 257–263.
82. Jo, T.; Takachi, Y.; Nakajima, Y.; Fukami, K.; Kosaka, H.; Terada, N. Maternal or umbilical venous levels of nitrite/nitrate during pregnancy and at delivery. In Vivo 1998, 12, 523–526. [PubMed]
83. Shaamash, A.H.; Elsnosy, E.D.; Makhlouf, A.M.; Zakhari, M.M.; Ibrahim, O.A.; EL-dien, H.M. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, pre-eclampsia and eclampsia. Int. J. Gynecol. Obstet. 2000, 68, 207–214. [CrossRef]
84. Hata, T.; Hashimoto, M.; Kanenishi, K.; Akiyama, M.; Yanagihara, T.; Masumura, S. Maternal circulation nitrite levels are decreased in both normal normotensive pregnancies and pregnancies with preeclampsia. Gynecol. Obstet. Invest. 1999, 48, 93–97. [CrossRef] [PubMed]
85. Brown, M.A.; Tibben, E.; Zammit, V.C.; Cario, G.M.; Carlton, M.A. Nitric oxide excretion in normal and hypertensive pregnancies. Hypertens. Pregnancy 1995, 14, 319–326. [CrossRef]
86. Smarason, A.K.; Allman, K.G.; Young, D.; Redman, C.W.G. Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with preeclampsia. Br. J. Obstet. Gynecol. 1997, 104, 538–543. [CrossRef]
87. Shah, D.A.; Khalil, R.A. Bioactive factors in uteroplacental and systemic circulation link placental ischemia to generalized vascular dysfunction in hypertensive pregnancy and preeclampsia. Biochem. Pharmacol. 2015, 95, 211–226. [CrossRef]
88. Vida, G.; Sulyok, E.; Erli, T.; Martens-Lobenhoffer, J.; Bode-Böger, S.M. Birth by cesarean section is associated with elevated neonatal plasma levels of dimethylarginines. Pediatr. Int. 2012, 54, 476–479. [CrossRef] [PubMed]
89. Andersson, A.; Hultberg, B.; Brattström, L.; Isaksson, A. Decreased serum homocysteine in pregnancy. Eur. J. Clin. Chem. Clin. Biochem. 1992, 30, 377–379.
90. Hague, B.; Whiting, M.; Tallis, G. South Australian experience with hyperhomocysteinaemia as a risk factor in obstetrics. Time for a trial? Neth. J. Med. 1997, 52, S24.
91. Malinow, M.R.; Rajkovic, A.; Duell, P.B.; Hess, D.L.; Upson, B.M. The relationship between maternal and neonatal umbilical cord plasma homocysteine suggests a potential role for maternal homocysteine in fetal metabolism. Obstet. Gynecol. 1998, 178, 228–233.
92. Steegers-Theunissen, R.; Wathen, N.; Eskes, T.; Van Raaij-Selten, B.; Chard, T. Maternal and fetal levels of methionine and homocysteine in early human pregnancy. Br. J. Obstet. Gynecol. 1997, 104, 20–24. [CrossRef]
93. Lopez-Quesada, E.; Vilaseca, M.A.; Laila, J.M. Plasma total homocysteine in uncomplicated pregnancy and in preeclampsia. Eur. J. Obstet. Gynecol. Reprod. Biol. 2003, 108, 45–49. [CrossRef]
94. Bergen, N.E.; Jaddoe, V.W.; Timmermans, S.; Hofman, A.; Lindemans, J.; Russcher, H.; Raat, H.; Steegers-Theunissen, R.P.; Steegers, E.A. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: The Generation R Study. BJOG 2012, 119, 739–751. [CrossRef] [PubMed]
95. Mills, J.L.; McPartlin, J.M.; Kirke, P.N.; Lee, Y.J.; Conley, M.R.; Weir, D.G.; Scott, J.M. Homocysteine metabolism in pregnancies complicated by neuraltube defects. *Lancet* 1995, 345, 149–151. [CrossRef]  
96. Chen, H.; Yang, X.; Lu, M. Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: A systematic review and meta-analysis. *Arch. Gynecol. Obstet.* 2016, 293, 283–290. [CrossRef]  
97. De Falco, M.; Pollio, F.; Scaramelino, M.; Pontillo, M.; Lieto, A.D. Homocysteinemia during pregnancy and placental disease. *Clin. Exp. Obstet. Gynecol.* 2000, 27, 188–190. [PubMed]  
98. Steegers-Theunissen, R.P.; Van Iersel, C.A.; Peer, P.G.; Nelen, W.L.; Steegers, E.A. Hyperhomocysteinemia, pregnancy complications, and the timing of investigation. *Obstet. Gynecol.* 2004, 104, 336–343. [CrossRef]  
99. Seligman, S.P.; et al. The role of nitric oxide in the pathogenesis of preeclampsia. *Am. J. Obstet. Gynecol.* 1994, 171, 944–948. [CrossRef]  
100. Mutlu-Turkoglu, U.; Aykac-Toker, G.; Ibrahimoglu, L.; Ademoglu, E.; Uysal, M. Plasma nitric oxide metabolites and lipid peroxide levels in preeclamptic pregnant women before and after delivery. *Gynecol. Obstet. Invest.* 1999, 48, 247–250. [CrossRef]  
101. Silver, R.K.; et al. Evaluation of nitric oxide as a mediator of severe preeclampsia. *Am. J. Obstet. Gynecol.* 1996, 175, 1013–1017. [CrossRef]  
102. Schiessl, B.; Strasburger, C.; Bidlingmaier, M.; Mylonas, I.; Jeschke, U.; Kainer, F.; Friese, K. Plasma- and urine concentrations of nitrite/nitrate and cyclic Guanosinemono phosphate in intrauterine growth restricted and preeclamptic pregnancies. *Arch. Gynecol. Obstet.* 2006, 274, 150–154. [CrossRef]  
103. Pathak, N.; et al. Estimation of oxidative products of nitric oxide (nitrates, nitrites) in preeclampsia. *Aust. N. Z. J. Obstet. Gynaecol.* 1999, 39, 484–487. [PubMed]  
104. Alexander, B.T.; Miller, M.T.; Kassab, S.; Novak, J.; Reckelhoff, J.F.; Kruckeberg, W.C.; Granger, J.P. Differential expression of renal nitric oxide synthase isoforms during pregnancy in rats. *Hypertension* 1999, 33, 435–439. [CrossRef]  
105. Walsh, S.K.; English, F.A.; Johns, E.J.; Kenny, L.C. Plasma-Mediated Vascular Dysfunction in the Reduced Uterine Perfusion Pressure Model of Preeclampsia: A Microvascular Characterization. *Hypertension* 2009, 54, 345–351. [CrossRef]  
106. Kleinbongard, P.; Dejam, A.; Lauer, T.; Rassaf, T.; Schindler, A.; Picker, O.; Scheeren, T.; Goddecke, A.; Schrader, J.; Schulz, R.; et al. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic. Biol. Med.* 2003, 35, 790–796. [CrossRef]  
107. Kelm, M.; Preik-Steinhofer, H.; Preik, M.; Strauer, B.E. Serum nitrite sensitively reflects endothelial NO formation in human forearm vasculature: Evidence for biochemical assessment of the endothelial L-arginine-NO pathway. *Cardiovasc. Res.* 1999, 41, 765–772. [CrossRef]  
108. Pimentel, A.M.; Pereira, N.R.; Costa, C.A.; Mann, G.E.; Cordeiro, V.S.; de Moura, R.S.; Brunini, T.M.C.; Mendes-Ribeiro, A.C.; Resende, Á.C. L-arginine-nitric oxide pathway and oxidative stress in plasma and platelets of patients with pre-eclampsia. *Hypertens. Res.* 2013, 36, 783–788. [CrossRef]  
109. Eleuterio, N.M.; Palei, A.C.; Machado, J.S.R.; Tanus-Santos, J.E.; Cavalli, R.C.; Sandrim, V.C. Relationship between adiponectin and nitrite in healthy and preeclampsia pregnancies. *Clin. Chim. Acta.* 2013, 423, 112–115. [CrossRef]  
110. Zeng, Y.; Li, M.; Chen, Y.; Wang, S. Homocysteine, endothelin-1 and nitric oxide in patients with hypertensive disorders complicating pregnancy. *Int. J. Clin. Exp. Pathol.* 2015, 8, 15275–15279. [CrossRef]  
111. Cadnapaphornchai, M.A.; Ohara, M.; Morris, K.G.; Knotek, M.; Rogachev, B.; Ladtkow, T.; Carter, E.P.; Schrier, R.W. Chronic NOS inhibition reverses systemic vasodilation and glomerular hyperfiltration in pregnancy. *Am. J. Physiol. Ren. Physiol.* 2001, 280, 592–598. [CrossRef]
114. Laskowska, M.; Laskowska, K.; Oleszczuk, J. The relation of maternal serum eNOS, NOSTRIN and ADMA levels with aetiopathogenesis of preeclampsia and/or intrauterine fetal growth restriction. J. Matern. Fetal. Neonatal. Med. 2015, 28, 26–32. [CrossRef]
115. Laskowska, M.; Laskowska, K.; Oleszczuk, J. PP135. Maternal serum levels of endothelial nitric oxide synthase and ADMA, an endogenous ENOS inhibitor in pregnancies complicated by severe preeclampsia. *Pregnancy Hypertens.* 2012, 2, 312. [CrossRef]

116. Myatt, L.; Eis, A.L.; Brockman, D.E.; Greer, I.A.; Lyall, F. Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies. *Hum. Reprod.* 1997, 12, 167–172. [CrossRef]
117. Smith-Jackson, K.; Hentschke, M.G.; Schlembach, D.; Fischer, T.; Sterzel, R.B.; Lang, N.; Baylis, C. Nitric oxide synthase activity and Doppler parameters in the feto-placental and uteroplacental circulation in preeclampsia. *Hypertens. Pregnancy* 1999, 18, 115–127. [CrossRef]
118. Orange, S.J.; et al. Placental endothelial nitric oxide synthase localization and expression in normal human pregnancy and preeclampsia. *Clin. Exp. Pharmacol. Physiol.* 2003, 30, 376–381. [CrossRef]
119. Marshall, S.A.; Hannan, N.J.; Jelinic, M.; Nguyen, T.P.; Girling, J.E.; Parry, L.J. Animal models of preeclampsia: Translational failings and why. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2018, 314, R499–R508. [CrossRef]
120. Beinder, E.; Mohaupt, M.G.; Schlembach, D.; Fischer, T.; Sterzel, R.B.; Lang, N.; Baylis, C. Nitric oxide synthase activity and Doppler parameters in the feto-placental and uteroplacental circulation in preeclampsia. *Hypertens. Pregnancy* 1999, 18, 115–127. [CrossRef]
121. Gonçalves-Rizzi, V.H.; Possomato-Vieira, J.S.; Graça, T.U.S.; da Costa, B.P.; Kurlak, L.O.; Pipkin, F.B.; Czajkad, A.; Mistry, H. D. Placental expression of eNOS, iNOS and the major protein components of caveolae in women with preeclampsia. *Placenta* 2015, 36, 607–610. [CrossRef]
122. Khalil, A.A.; Tsikas, D.; Akolekar, R.; Jordan, J.; Nicolaides, K.H. Asymmetric dimethylarginine, arginine and homocysteine at 11-13 weeks’ gestation and preeclampsia: A case-control study. *J. Hum. Hypertens.* 2013, 27, 38–43. [CrossRef] [PubMed]
123. Lópe-Aracón, M.; Montalvo-Velarde, I.; Vital-Reyes, V.S.; Hinojosa-Cruz, J.C.; Leaños-Miranda, A.; Martínez-Basila, A. Serial determinations of asymmetric dimethylarginine and homocysteine during pregnancy to predict pre-eclampsia: A longitudinal study. *BJOG* 2015, 122, 1586–1592.
124. Zheng, J.J.; Wang, H.O.; Huang, M.; Zheng, F.Y. Assessment of ADMA, estradiol, and progesterone in severe preeclampsia. *Clin. Exp. Hypertens.* 2016, 38, 347–351. [CrossRef] [PubMed]
125. Laskowska, M.; Laskowska, K.; Terbosh, M.; Oleszczuk, J. A comparison of maternal serum levels of endothelial nitric oxide synthase, asymmetric dimethylarginine, and homocysteine in normal and preeclamptic pregnancies. *Med. Sci. Monit.* 2013, 19, 430–437.
126. Alpoim, P.N.; Godoi, L.C.; Freitas, L.G.; Gomes, K.B.; Dusse, L.M. Assessment of L-arginine asymmetric 1 dimethyl (ADMA) in early-onset and late-onset (severe) preeclampsia. *Nitric Oxide* 2013, 33, 81–82. [CrossRef]
127. Bian, Z.; Shixia, C.; Duan, T. First-trimester maternal serum levels of sFLT1, PGF and ADMA predict preeclampsia. *PLoS One* 2015, 10, e0124684. [CrossRef]
128. Rizos, D.; Elefftheriades, M.; Batakis, E.; Rizou, M.; Halisassos, A.; Hassiakos, D.; Botsis, D. Levels of asymmetric dimethylarginine throughout normal pregnancy and in pregnancies complicated by preeclampsia or had a small for gestational age baby. *J. Matern. Fetal. Neonatal. Med.* 2012, 25, 1311–1315. [CrossRef]
129. Savvidou, M.D.; Hingorani, A.D.; Tsikas, D.; Frolich, J.C.; Vallance, P.; Nicolaides, K.H. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet* 2003, 361, 1511–1517. [CrossRef]
130. Kim, M.W.; Hong, S.C.; Choi, J.S.; Han, J.Y.; Oh, M.J.; Kim, H.J.; Nava-Ocampo, A.; Koren, G. Homocysteine, folate and pregnancy outcomes. *J. Obstet. Gynaecol. Res.* 2012, 38, 520–524. [CrossRef]
131. Dekker, A.G.; DeVries, J.I.P.; Doelitzsch, P.M.; Huiggens, P.C.; von Blomberg, B.M.; Jakobs, C.; van Geijn, H.P. Underlying disorders associated with severe early onset preeclampsia. *Am. J. Obstet. Gynecol.* 1995, 173, 1042–1048. [CrossRef]
132. Acilmis, Y.G.; Dikensoy, E.; Kutlar, A.I.; Balat, O.; Cebesoy, F.B.; Ozturk, E.; Cicek, H.; Pence, S. Homocysteine, folic acid and vitamin B12 levels in maternal and umbilical cord plasma and homocysteine levels in placenta in pregnant women with preeclampsia. *J. Obstet. Gynaecol. Res.* 2011, 37, 45–50. [CrossRef] [PubMed]
133. Mujawar, S.A.; Patil, V.V.; Daver, R.G. Study of serum homocysteine, folic Acid and vitamin B(12) in patients with preeclampsia. *Indian J. Clin. Biochem.* 2011, 26, 257–260. [CrossRef] [PubMed]

134. Guven, M.A.; Coskun, A.; Ertas, I.E.; Aral, M.; Zencirci, B.; Oksuz, H. Association of maternal serum CRP, IL-6, TNF-alpha, homocysteine, folic acid and vitamin B12 levels with the severity of preeclampsia and fetal birth weight. *Hypertens. Pregnancy* 2009, 28, 190–200. [CrossRef] [PubMed]

135. Makedos, G.; Papanicolaou, A.; Hitoglou, A.; Kalogiannidis, I.; Makedos, A.; Vrazioti, V.; Goutzioulis, M. Homocysteine, folic acid and B12 levels in relation with preeclampsia. *Arch. Gynecol. Obstet.* 2007, 275, 121–124. [CrossRef]

136. Patrick, T.E.; Powers, R.W.; Daftary, A.R.; Ness, R.B.; Roberts, J.M. Homocysteine and folic acid are inversely related in black women with preeclampsia. *Hypertension* 2004, 43, 1279–1282. [CrossRef] [PubMed]

137. Sanchez, S.E.; Zhang, C.; Rene Malinow, M.; Ware-Jauregui, S.; Larrabure, G.; Williams, M.A. Plasma folate, vitamin B(12), and homocyst(e)ine concentrations in preeclamptic and normotensive Peruvian women. *Am. J. Epidemiol.* 2001, 153, 474–480. [CrossRef] [PubMed]

138. Wadhwani, N.S.; Patil, V.V.; Mehdendale, S.S.; Wagh, G.N.; Gupte, S.A.; Joshi, S.R. Increased homocysteine levels exist in women with preeclampsia from early pregnancy. *J. Matern. Fetal. Neonatal. Med.* 2016, 29, 2719–2725. [CrossRef] [PubMed]

139. Dodds, L.; Fell, D.B.; Dooley, K.C.; Armson, B.A.; Allen, A.C.; Nassar, B.A.; Joseph, K.S. Effect of Homocysteine Concentration in Early Pregnancy on Gestational Hypertensive Disorders and Other Pregnancy Outcomes. *Clin. Chem.* 2008, 54, 326–334. [CrossRef]

140. Maged, A.M.; Saad, H.; Meshaal, H.; Salah, E.; Abdelaziz, S.; Omran, E.; Katta, M. Maternal serum homocysteine and uterine artery Doppler as predictors of preeclampsia and poor placentation. *Arch. Gynecol. Obstet.* 2017, 296, 475–482. [CrossRef]

141. Cotter, A.M.; Molloy, A.M.; Scott, J.M.; Daly, S.F. Elevated plasma homocysteine in early pregnancy: A risk factor for the development of severe preeclampsia. *Am. J. Obstet. Gynecol.* 2001, 185, 781–785. [CrossRef]

142. Cotter, A.M.; Molloy, M.; Scott, J.M.; Daly, S. Elevated plasma homocysteine in early pregnancy: A risk factor for the development of nonsevere preeclampsia. *Am. J. Obstet. Gynecol.* 2003, 189, 391–396. [CrossRef]

143. Raijmakers, M.T.; Zusterzeel, P.L.; Steegers, E.A.; Peters, W.H. Hyperhomocysteinaemia: A risk factor for preeclampsia? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2001, 95, 226–228. [CrossRef]

144. Hietala, R.; Turpeinen, U.; Laatikainen, T. Serum homocysteine at 16 weeks and subsequent preeclampsia. *Obstet. Gynecol.* 2001, 97, 527–529. [PubMed]

145. Hogg, B.B.; Tamura, T.; Johnston, K.E.; Dubard, M.B.; Goldenberg, R.L. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. *Am. J. Obstet. Gynecol.* 2000, 183, 805–809. [CrossRef] [PubMed]

146. Shahbaziyan, N.; Mohammad Jafari, R.; Haghnia, S. The evaluation of serum homocysteine, folic acid, and vitamin B12 in patients complicated with preeclampsia. *Electron. Physician* 2016, 8, 3057–3061. [CrossRef] [PubMed]

147. Stuhlinger, M.C.; Oka, R.K.; Graf, E.E.; Schmöller, I.; Upson, B.M.; Kapoor, O.; Szuba, A.; Malinow, M.R.; Wascher, T.C.; Pachinger, O.; et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: Role of asymmetric dimethylarginine. *Circulation* 2003, 108, 933–938. [CrossRef]

148. Ray, J.G.; Laskin, C.A. Folic acid and homocysteine metabolic defects and the risk of placental abruption, preeclampsia and spontaneous pregnancy loss: A systematic review. *Placenta* 1999, 20, 519–529. [CrossRef]

149. Lentz, S.R. Mechanisms of homocysteine-induced atherothrombosis. *J. Thromb. Haemost.* 2005, 3, 1646–1654. [CrossRef]

150. Mao, D.; Che, J.; Li, K.; Han, S.; Yue, Q.; Zhu, L.; Li, L. Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia. *Arch. Gynecol. Obstet.* 2010, 282, 371–375. [CrossRef]

151. Powers, R.; Evans, R.; Majors, A.; Ojima, J.; Ness, R.; Crombleholme, W.R.; Roberts, J.M. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. *Am. J. Obstet. Gynecol.* 1998, 179, 1605–1611. [CrossRef]

152. Wollesen, F.; Brattstrom, L.; Refsum, H.; Ueland, P.M.; Berglund, L.; Berne, C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int.* 1999, 55, 1028–1035. [CrossRef] [PubMed]
153. Veldman, B.A.; Vervoort, G.; Blom, H.; Smits, P. Reduced plasma total homocysteine concentrations in Type 1 diabetes mellitus is determined by increased renal clearance. *Diabet. Med.* 2005, 22, 301–305. [CrossRef] [PubMed]

154. Bostom, A.G.; Lathrop, L. Hyperhomocysteinemia in end-stage renal disease: Prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int.* 1997, 52, 10–20. [CrossRef] [PubMed]

155. Maul, H.; Longo, M.; Saade, G.R.; Garfield, R.E. Nitric oxide and its role during pregnancy: From ovulation to delivery. *Curr. Pharm. Des.* 2003, 9, 359–380. [CrossRef] [PubMed]

156. Luzi, G.; Caserta, G.; Iammarino, G.; Clerici, G.; Di Renzo, G.C. Nitric oxide donors in pregnancy: Fetomaternal hemodynamic effects induced in mild pre-eclampsia and threatened preterm labor. *Ultrasound Obstetrics Gynecol.* 1999, 14, 101–109. [CrossRef] [PubMed]

157. Ledingham, M.-A.; Denison, F.C.; Kelly, R.W.; Young, A.; Norman, J.E. Nitric oxide donors stimulate prostaglandin F2α and inhibit thromboxane B2 production in the human cervix during the first trimester of pregnancy. *Mol. Hum. Reprod.* 1999, 5, 973–982. [CrossRef] [PubMed]

158. Trapani, A., Jr.; Goncalves, L.F.; Trapani, T.F.; Vieira, S.; Pires, M.; Pires, M.M. Perinatal and hemodynamic evaluation of Sildenafil citrate for preeclampsia treatment: A randomized controlled trial. *Obstet. Gynecol.* 2016, 128, 253e9. [CrossRef] [PubMed]

159. Cardounel, A.J.; Cui, H.; Samouilov, A.; Johnson, W.; Tsai, A.L.; Berka, V.; Zweier, J.L. Evidence for the pathophysiological role of endogenous methylarginines in regulation of endothelial NO production and vascular function. *J. Biol. Chem.* 2007, 282, 879–887. [CrossRef] [PubMed]

160. Facchinetti, F.; Longo, M.; Piccinini, F.; Neri, I.; Volpe, A. L-arginine infusion reduces blood pressure in preeclamptic women through nitric oxide release. *J. Soc. Gynecol. Investig.* 1999, 6, 202–207.

161. Vadiillo-Ortega, F.; Perichart-Perera, O.; Espino, S.; Avila-Vergara, M.A.; Ibarra, I.; Ahued, R.; Strauss, J.F. Effect of supplementation during pregnancy with L-arginine and antioxidant vitamins in medical food on pre-eclampsia in high risk population: Randomised controlled trial. *BMJ* 2011, 342, d2901. [CrossRef]

162. Fu, Z.; Ma, Z.; Liu, G.; Wang, L.; Guo, Y. Vitamins supplementation affects the onset of preeclampsia. *J. Formos. Med. Assoc.* 2018, 117, 6–13. [CrossRef] [PubMed]

163. Neri, I.; Monari, F.; Sgarbi, L.; Berardi, A.; Masellis, G.; Facchinetti, F. L-arginine supplementation in women with chronic hypertension: Impact on blood pressure and maternal and neonatal complications. *J. Matern. Fetal. Neonatal. Med.* 2010, 23, 1456–1460. [CrossRef] [PubMed]

164. Meher, S.; Duley, L. Nitric oxide for preventing pre-eclampsia and its complications. *Cochrane Database Syst. Rev.* 2007, 2, CD006490. [CrossRef] [PubMed]

165. Camarena Pulido, E.E.; García Benavides, L.; Panduro Baron, J.G.; Pascoe Gonzalez, S.; Madrigal Saray, A.J.; García Padilla, F.E.; Totsuka Sutto, S.E. Efficacy of L-arginine for preventing preeclampsia in high risk pregnancies: A double-blind randomized clinical trial. *Hypertens. Pregnancy* 2016, 35, 217–225. [CrossRef] [PubMed]

166. Abdelrazik, M.; ElBerry, S.; Abosereah, M.; Edris, Y.; Sharafeldeen, A. Prophylactic treatment for preeclampsia in high risk teenage primigravida with nitricoxide donors: A pilot study. *J. Matern. Fetal. Neonatal. Med.* 2016, 29, 2617e20.

167. Coles, L.T.; Clifton, P.M. Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: A randomised, placebo-controlled trial. *Nutr. J.* 2012, 11, 106. [CrossRef]

168. Ormesher, L.; Myers, J.E.; Chmiel, C.; Wareing, M.; Greenwood, S.L.; Tropea, T.; Cottrell, E.C. Effects of dietary nitrate supplementation, from beetroot juice, on blood pressure in hypertensive pregnant women: A randomised, double-blind, placebo-controlled feasibility trial. *Nitric Oxide* 2018, 80, 37–44. [CrossRef]

169. Moreland, R.B.; Goldstein, I.L.; Kim, N.N.; Traish, A. Sildenafil Citrate, a Selective Phosphodiesterase Type 5 Inhibitor. *Trends Endocrinol. Metab.* 1999, 10, 97–104. [CrossRef]

170. Ramesar, S.V.; Mackraj, I.; Cathiram, P.; Moodley, J. Sildenafil citrate decreases sFlt1- and sEng in pregnant l-NAME treated Sprague-Dawley rats. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2011, 157, 136–140. [CrossRef]

171. Herrera, S.; Pellicer, B.; Serra, V.; Culi, O.; Cortijo, J.; Felipo, V.; Pellicer, A. Sildenafil citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG* 2012, 119, 1394–1402. [CrossRef]
172. Cacciatore, B.; Halmesmäki, E.; Kaaja, R.; Teramo, K.; Ylikorkala, O. Effects of transdermal nitroglycerin on impedance to flow in the uterine, umbilical, and fetal middle cerebral arteries in pregnancies complicated by preeclampsia and intrauterine growth retardation. *Am. J. Obstet. Gynecol.* 1998, 179, 140–145. [CrossRef]

173. Trapani, A., Jr.; Gonçalves, L.F.; Pires, M.M. Transdermal nitroglycerin in patients with severe pre-eclampsia with placental insufficiency: Effect on uterine, umbilical and fetal middle cerebral artery resistance indices. *Ultrasound Obstet. Gynecol.* 2011, 38, 389–394. [CrossRef] [PubMed]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).