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High-altitude hypoxia and preeclampsia

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1. ABSTRACT

The influence of hypoxia (lowered arterial blood and/or tissue PO_2) on fetoplacental development and the role of hypoxia in preeclampsia are major research foci in perinatal biology. While animal and cell models are of utility, we do not know whether artificial hypoxic stimuli mimic the pathological conditions attributed to hypoxic stress *in vivo*; we cannot distinguish the effects of hypoxia from under- or overlying pathologies. High altitude (>2700 m) is the natural experiment we can use to distinguish pathology from adaptation in human pregnancy. The two best known impacts of high altitude on pregnancy outcome are reduced fetal growth and an increased incidence preeclampsia. This review focuses on the mechanisms by which altitude increases maternal risk for the development of preeclampsia. The review first considers the evidence that placental hypoxia is causally involved in the development of preeclampsia. It then focuses on how data from studies of pregnant women at high altitude support (or do not support) etiological models of preeclampsia. Considered are the theories that reduced uteroplacental blood flow, circulating factors of placental origin, placental oxidative stress and increased maternal vascular reactivity are etiological in preeclampsia. The data suggest that oxidative stress and endothelial dysfunction have pathophysiological origins that are independent of placental hypoxia. We conclude that altitude shifts the individual risk for the development of preeclampsia because of impacts on multiple physiological systems, no one of which can be specifically pointed to as causal.

2. HIGH ALTITUDE AND PREECLAMPSIA

The ancient Greeks gave eclampsia (lightning) its name because the disease struck quickly and without warning. Pre-eclampsia, the prodromal state, was characterized by racing pulses, a symptom that would now be recognized as hypertension. After more than 2000 years, the cause of preeclampsia remains unknown.

Residence at high altitude (>2700 m) is the only external environmental factor that has been consistently linked with an increased incidence of preeclampsia (1-4). Despite the utility of experimental animal and cell culture models employing hypoxic stimuli, high altitude is the only *in vivo* human model with which to compare the results of *in vitro* and animal experimentation. To date we do not know whether artificial hypoxic stimuli mimic the pathological conditions attributed to hypoxic stress *in vivo.* This review evaluates how the data from high altitude support or do not support etiological theories of preeclampsia. We have used a variety of research designs, all with appropriate Institutional Review Board Approvals and participants' informed consent, including cohort studies, birth-certificate analyses and prospective longitudinal physiological analyses. The data have consistently shown anywhere from a two- to a four-fold elevation in the incidence of preeclampsia at high altitude using both strict criteria (primiparas with documented proteinuria and hypertension that resolved following delivery) and less strict, but clinically relevant criteria (e.g. hypertension plus evidence of other organ system

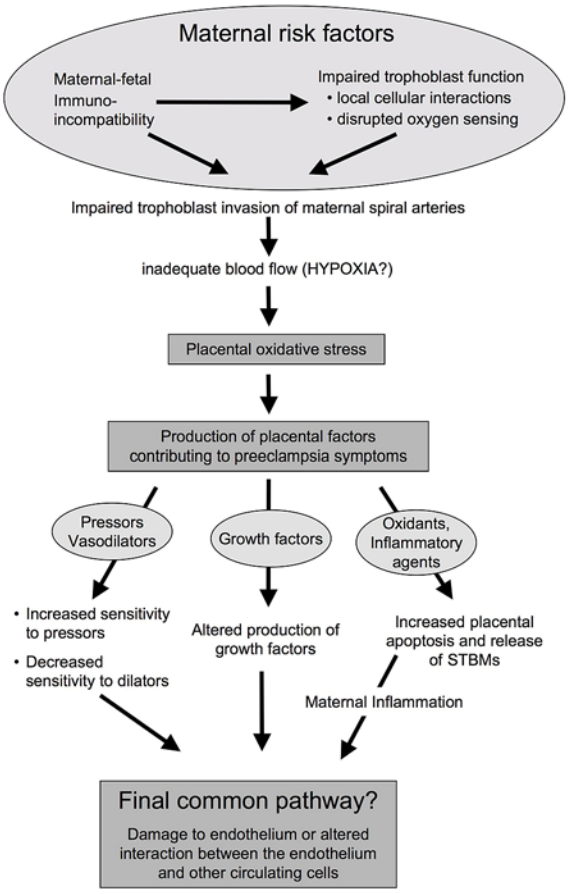

Figure 1. Individual susceptibility is a prominent contributor to the development of preeclampsia; obesity, variation in thresholds for inflammatory response, vascular sensitivity, insulin sensitivity or other characteristics can contribute to increased susceptibility. At the top of the model we show that impaired trophoblast invasion is a common feature of the disease, and while as yet the cause of impaired invasion is unknown there is good evidence to support disrupted oxygen sensing and immunological interactions as playing a role (these issues are reviewed elsewhere in this volume). At high altitude trophoblast invasion is impaired and uterine blood flow is reduced. This review considers how these two observations may translate into specific features of preeclampsia, such as placental oxidative stress, altered production of growth factors and increased vascular responsiveness

involvement, such as neurological symptoms, abnormal liver function or platelet consumption). Increased preeclampsia at high altitude is a global phenomenon, being observed in North and South America, the middle east and anecdotally among Chinese migrants to Tibet. It is not due to an altitude-associated increase in known maternal risk factors (e.g. obesity). The effect of altitude is independent of other risk factors, including socioeconomic status (5, 6). Since the most obvious effect of high altitude is lowered arterial oxygen tension (hypoxemia, lowered PO_2), the increased incidence of preeclampsia and IUGR at high altitude supports that hypoxia contributes to the development of preeclampsia. Further support derives from the disease literature: women with congenital heart diseases associated with poor cardiac output or impaired lung transfer of oxygen to blood also have a markedly increased risk for preeclampsia (7).

A plethora of animal studies have investigated regulation of vascular tone and reactivity, uterine artery structure and growth and maternal physiology in pregnancy under hypoxic conditions (reviewed in 8-10). They converge in revealing how subtle changes in multiple physiological systems likely contribute to an increased risk for the development of preeclampsia at high altitude. The following section of this review examines some of the evidence that hypoxia is involved in the etiology of preeclampsia. We then examine 4 well-known etiological models of preeclampsia and consider to what extent data from high altitude are consistent with the etiological model.

2.1. Hypoxia and preeclampsia

The model presented in Figure 1 is only one of many possibilities but will serve for this review as an organizational schema.

The first question that must be answered if we are to consider high altitude a useful model for understanding the pathophysiology of preeclampsia is whether the evidence supports that preeclampsia is characterized by fetoplacental hypoxia. Hypoxia and/or ischemia-reperfusion injury are often invoked as a mechanism contributing to preeclampsia (11-17). Many studies since the early 1980s, pioneered by Stuart Campbell in the UK (uterine arteries) and Warwick Giles in Australia (umbilical arteries), have demonstrated that increased resistance to blood flow is present before the onset of symptoms in women who eventually develop preeclampsia and/or intrauterine growth restriction (18-20). Increased resistance implies reduced blood flow, though this correlation has not been directly tested. We found that reduced uterine artery blood flow is present at high altitude (21) and that reduced blood flow precedes the onset of symptoms in preeclampsia (22). The high-altitude fetus is thus subjected to the double insult of hypoxemia of the blood entering the placental intervillous space and decreased uteroplacental blood flow. This supports the idea that reduced oxygen delivery and/or PO_2 contribute to the development of preeclampsia and IUGR at any altitude, and likely augments the risk at high altitude.

But does what is observed at high altitude mirror what is seen in preeclampsia? Both morphological and molecular evidence support that high altitude placentas resemble preeclampsia in some, but not all features. Fox, (23, 24) showed that hypoxia was associated with increased cytotrophoblast proliferation, a finding mirrored in high altitude placentas by an increased proportion of cytotrophoblast relative to other trophoblast cell populations (reviewed in 25). There is decreased

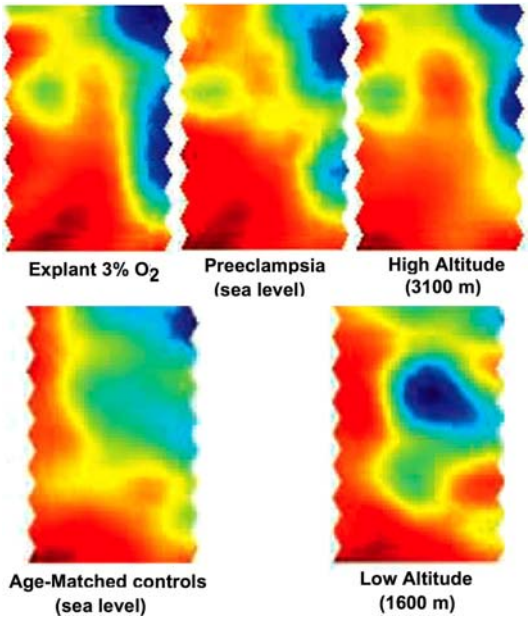

Figure 2. Shown here are the results of microarray analysis of pooled placental samples from term explants cultured under 3% oxygen, preeclamptics, high altitude, age-matched controls for the preeclamptic patients and low altitude controls for the high altitude patients. This analysis is based on self-organizing maps. Of the 1700 genes represented on the microarray plate, areas of increased gene expression are shown in red and areas of decreased gene expression are shown in blue. The methodological details of the analysis, the results from a targeted analysis of the maximally different areas of these microarrays, the list of individual gene differences and the validation of differences in selected individual genes between altitude, explants, preeclampsia and controls are in reference 31. This figure is reprinted here to highlight the broad similarity in the pattern of gene expression between the *in vitro* and *in vivo* models of placental hypoxia (3% O_2, preeclampsia and high altitude) and their divergence from the controls (AMC at sea level and low altitude). Reprinted by permission from 31.

remodeling of the uteroplacental arteries at the level of the basal plate in preeclampsia (26) and at high altitude, although not to the same extent as in preeclampsia. Increased vascularity (27) or a more tortuous and dense distribution of blood vessels at the level of the maternal myometrium is observed in preeclampsia, and in high altitude placentas (28). In contrast increased fibrin deposition and other evidence of syncytial damage is common in preeclampsia, but consistently decreased at high altitude (25). Placental expression of markers of hypoxia, such as erythropoietin receptor (29) and sFlt-1 (30) are increased at high altitude and/or in preeclampsia. Global profiles of gene expression were similar in placentas exposed to high altitude hypoxia, preeclampsia at sea level and term placental explants cultured under 3% oxygen conditions (31) (Figure 2). Confirmation of the changes in a number genes by Soleymanlou and colleagues (qPCR) indicated that the high altitude placenta retains an immature phenotype (consistent with lower oxygen tension) relative to the low altitude placenta. There is increased expression of Integrin alpha6, a marker of immature trophoblast phenotype, and increased markers of proliferation and hypoxia (e.g. VEGF). These molecular data are consistent with the morphological data (increased cytotrophoblast and vascular development). Correlation between the gene expression profiles demonstrated in Figure 2 was strong in preeclampsia versus high altitude ($r=.5$, $p<.001$), and greater still in preeclampsia versus term explants cultured under 3% oxygen ($r=.6$, $p<.001$) (31). There is thus good evidence to support the involvement of hypoxia in preeclampsia. However, hypoxia is neither necessary nor sufficient to cause preeclampsia. A doubling of the incidence at high altitude in the absence of differences in epidemiological risk factors indicates that hypoxia increases risk, but other factors must come into play in orderto tip one individual versus another over her particular tolerance into the preeclampsia syndrome. This is where the high altitude model is of special utility.

3. ALTITUDE AND ETIOLOGICAL MODELS OF PREECLAMPSIA

3.1. Etiological model #1 - Reduced blood flow

The idea that placental ischemia or hypoxia 'causes' preeclampsia has a long and contentious history. Beker, in 1929, wrote that 'an inadequate blood supply to the uterus may be the cause of toxaemia' (32). Browne and Veall countered, in 1953, that 'placental ischemia is the result, and not the cause of hypertension in toxaemia' (33), a theory modified by Clemetson, who proposed that reduced or slowed cord blood flow causes fetal hypoxia (markedly lower umbilical artery PO_2) and this in turn causes placental 'hypoxia' via the blood return through the umbilical arteries to the placental circulation (34). To date, the chicken and egg question of whether decrement in blood flow precedes the onset of symptoms remains contentious.

A majority of investigators believe impaired placental invasion of the maternal vasculature, possibly due to fetoplacental-maternal immuno-incompatibility (35), or to aberrant oxygen sensing (12, 36) is the primary initiating factor in the disease and 'causes' the reduced blood flow and resultant placental hypoxia (Figure 1). Table 1 summarizes the relevant publications on uteroplacental blood flow in normal and pathological human pregnancy. While many clinicians feel that uteroplacental blood flow cannot be reliably or accurately measured, data across 50 years and multiple techniques converge on an average blood flow of ~700 ml/min in late pregnancy. The ranges in Table 1 are noteworthy; normotensive women without complications have blood flows that could overlap with those noted in preeclampsia (Table 1), suggesting lowered blood flow alone is not causal in preeclampsia.

The underlying cause of reduced blood flow in preeclampsia is thought to be a relative failure of trophoblast to fully invade and remodel the maternal spiral

Table 1. Reported values for uteroplacental blood flow in normal and abnormal near-term human pregnancy

Group	Sample size	Technique	Range (ml/min)	Blood flow (ml/min)	Reduction versus normal	Reference
Normal	8	Isotopic	NR	600		33
Chronic HTN	3	Isotopic	NR		-50%	33
Chronic HTN+PE	2	Isotopic	NR		-70%	33
Mild PE	2	Isotopic	NR		-60%	33
Normal	7	Nitrous oxide	610-925	750		107
Normal	13	Nitrous oxide	175-840	492±188		108
Normal	8	Nitrous oxide	255-480	500		109
Normal	5	Nitrous oxide	308-651	465±136		110
Chronic HTN	1	Nitrous oxide	NR		-12%	110
Normal	50	Isotopic	NR			111
Chronic HTN	31	Isotopic	NR		-20%	111
Severe PE	11	Isotopic	NR		-29%	111
Normal	37	Isotopic	NR			116, 117
Mild PE	17	Isotopic	NR		-35%	116, 117
Severe PE	8	Isotopic	NR		-67%	116, 117
PE+IUGR	10	Isotopic	NR		-54%	116, 117
PE w/o IUGR	15	Isotopic	NR		-40%	116, 117
IUGR w/o HTN	19	Isotopic	NR		-61%	116, 117
Normal	26	Isotopic	NR	1140		118
IUGR w/o HTN	13	Isotopic	NR		-57%	118
Normal	24	Doppler	NR	825		112
Normal	18	Doppler	NR	921		113
Low altitude normal	18	Doppler	NR	921		113, 21
High altitude normal	23	Doppler	NR		-33%	21
Mild PE	7	Doppler	NR		-67%	22
Normal	18	Doppler	NR	830±284		114

NR = not reported; PE=preeclampsia; IUGR = Intrauterine growth restriction; HTN = hypertension

arteries (37-43). Abnormal Doppler findings correlate with other placental morphological characteristics, such as reduced tertiary branching of the villous vascular tree, that would logically be associated with reduced blood flow or intermittent ischemia (13, 19, 40). We examined the decidual ends of uteroplacental arteries in placentas from high (3100 m) vs. low (1600 m) altitude. We found that while individually variable, remodeling was absent in 67% of all arteries examined in the high altitude placentas vs. 27% of the arteries examined in low altitude placentas (28). The latter contrasts with findings using the same technique at sea level, wherein 100% of arteries from normal placentas were remodeled (26). Other studies suggest that a gradient of remodeling is a much more likely scenario in normal human placentas (38). Thus there may be exquisite sensitivity of the trophoblast to oxygen tension such that even very small differences in tissue PO_2 can effect the invasion process. To that end, a number of investigators are now testing their *in vitro* models across a range of oxygen concentrations, e.g. 1-8%, which would likely reflect the high and low extremes of blood circulating in the intervillous space near term (44-46).

The high altitude data are consistent with a variety of studies indicating that failed remodeling contributes to reduced uteroplacental blood flow. But there are nonetheless troubling inconsistencies in the data. The occurrence of placental ischemia (<50% reduction in blood flow) without hypertension or IUGR is noted in the literature (reviewed in Table 1). A small subset of women have impaired trophoblast invasion of the maternal spiral arteries, but develop neither preeclampsia nor IUGR, while other subsets of women have impaired invasion and develop IUGR, but not preeclampsia (37, 39, 42). Moreover having normal resistance indices does not necessarily mean that blood flow is normal. We found no evidence for increased vascular resistance in the uterine artery at high altitude, despite the relative lack of uteroplacental arterial remodeling, and despite lower uterine blood flow (47). Another study at even greater altitude (4300 m) found lower vascular resistance in the uterine artery (48). This means that resistance does not directly translate into volumetric flow: there can be a marked reduction in overall uteroplacental blood flow, which would reduce both oxygen and substrate delivery, without any obvious differences in resistance. In summary, with caveats regarding indirect measures of blood flow, the literature on preeclampsia and from high altitude are consistent in showing that reduced blood flow is present more often than in normal pregnancy. It seems reasonable to infer that reduced uteroplacental blood is not sufficient to produce the disease, but that reduced blood flow may increase the risk for development of preeclampsia in women who are otherwise susceptible.

3.2. Etiological model #2 - Circulating factors of placental origin

The idea that the hypoxic/ischemic or otherwise stressed placenta produces something toxic to the mother (toxaemia) is also a theory with a long history. Even the idea that oxidative mechanisms are involved can be traced back as far as 1949, when Thompson & Tickner proposed that 'a mono-amine oxidase in the placenta is inactivated by placental ischemia; there is evidence that it can destroy vaso-constrictor amines and thus ischemia may cause vasoconstriction' (49). Innumerable "factors" have since been suggested and tested (50, 51). Today's equivalent of yesterday's abnormal coagulation cascade is the angiogenic growth factors (reviewed in this volume), currently under scrutiny as a causal culprit (52). However it is worth noting that without exception, no circulating factor has ever been found where the ranges reported in preeclampsia do not

overlap with those of normotensive women. Similarly, in those studies wherein serial samples were collected and retrospectively analyzed for the factor(s) of interest, sensitivity and specificity have not attained a consistency that would permit clinical use. Factors that do change prior to the onset of hypertension and other symptoms also overlap with the ranges observed in women who remain normotensive.

In this respect the data from high altitude are still being explored. We have shown greater maternal circulating concentrations and placental expression of the circulating anti-angiogenic growth factor sFlt-1 (30), favored as a potential cause of endothelial dysfunction (53). We have also shown greater expression of placental VEGF and circulating total VEGF (31, 54) at high altitude. However, additional data indicate that a significant portion of the sFlt-1 (and VEGF) measured in serum may actually be of platelet or other circulating cell origin. This means the circulating concentrations reported often reflect variation in sample collection and storage as opposed to reflecting the *in vivo* circulatory state (55-57). How local platelet or other circulating cells' secretion of growth factors or cytokines may influence the development of preeclampsia is a difficult area of study, but one that merits greater attention (58-61). It is at least possible that our collective failure to find "the" circulating factor of placental origin that causes preeclampsia is first, that no single factor exists, and second, that virtually all the circulating factors reported to date as being "associated" with preeclampsia, whether prior to or during the course of the disease, are released (or not) to a variable degree after the blood has been collected. They therefore may reflect risk or susceptibility, but not the true "*in vivo*" circulating milieu. This would account for the lack of consistency between studies, and the substantial overlap that exists between what is observed in preeclampsia vs. normotensive pregnancy.

Nonetheless, one of the breakthroughs in theoretical models of preeclampsia was the idea that the plethora of symptoms, along with the inter-individual variability in symptoms, might be accounted for by a systemic dysfunction localized to a cell type as opposed to specific organ, i.e. disruption of the vascular endothelium (62). With respect to markers of endothelial cell activation or dysfunction, the circulating factors that have been studied thus far in high altitude pregnancy (other than sFlt-1 and total VEGF) are pro- versus anti-inflammatory cytokines, and endothelial cell adhesion molecules.

Increased Th1 (pro-inflammatory) cytokine activity in pregnancy is associated with preeclampsia (63-66). More proinflammatory cytokines are produced by lymphocytes from preeclamptic women than from women with normal pregnancies (67-69). This again emphasizes the idea that no one circulating factor is really important. Rather, local release of factors that interact with the endothelium, potentially escalating the cascade of preeclampsia symptoms, are more important. We found that maternal circulating concentrations of the pro-inflammatory cytokines IL-6, TNF-alpha, and IL-8 were all elevated late in normal pregnancy in women residing at high altitude, but did not differ even marginally in the non-pregnant state. The same subjects failed to increase their levels of anti-inflammatory (Th-2) IL-10 during pregnancy, causing a marked reduction in circulating concentrations relative to low altitude controls that was most pronounced in the third trimester when pregnancy complications develop (70). We suspect that the overall profile of cytokine production during pregnancy at high altitude is altered by sympathoadrenal activation secondary to the interaction of hypoxia and pregnancy (a general stress, see the section on vascular reactivity below). Alternatively, altered cytokine production or degradation may reflect underlying mechanisms that contribute both to the observed alterations in circulating concentrations, and to the development of preeclampsia, without one necessarily causing the other.

Elevation of pro-inflammatory cytokines such as IL-6 have been linked with an increase in circulating concentrations of endothelial cell adhesion molecules in preeclampsia (71). We investigated circulating concentrations of vascular cell adhesion molecule (VCAM-1), E-Selectin and platelet-endothelial cell adhesion molecule (PECAM). Not only were none of these circulating markers elevated relative to low altitude (71) but VCAM-1 was reduced at high compared with low altitude in normal pregnancies (72). The discordance between our findings at high altitude and in preeclampsia suggests that placental hypoxia is unlikely to be the cause of elevated circulating VCAM-1 concentrations in preeclampsia. Likewise, since prior data link an increase in pro-inflammatory cytokines with elevated VCAM-1 in preeclampsia, the high altitude data suggest that increased inflammation does not necessarily increase endothelial cell adhesion molecules under *in vivo* conditions of hypoxia. Insofar as these limited data permit us to infer, endothelial cell activation does not seem to be a generalized effect of maternal hypoxemia, or of mild placental hypoxia, and therefore may be uniquely associated with the pathophysiology of preeclampsia.

3.3. Etiological model #3 - Placental oxidative stress

In this theoretical model (Figure 1) there is an imbalance between the cellular generation of reactive oxygen species (ROS) and the capacity of anti-oxidants to prevent oxidative damage. This has been suggested as playing a pivotal role in preeclampsia (reviewed in 73). In this etiological model, placental oxidative stress is often considered as the event precipitating the increased placental apoptosis observed in preeclampsia (74-76). Increased apoptosis, in turn, is thought to increase the deportation of apoptotic syncytiotrophoblast fragments (STBMs) into the maternal circulation, and, for reasons that are not clear, such fragments are not cleared by the lungs as in normal pregnant women, and circulate in the mother. These fragments then increase inflammatory stress and endothelial cell damage (77). The oxidative stress model

Table 2. Differences in concentrations of pro- versus anti-oxidant enzyme precursors, in enzyme activity levels and in measures of oxidative stress in low altitude vs. high altitude and preeclamptic vs. control placentas

Markers of oxidative status	Preeclampsia (n=20) vs. age matched control (n=18)	High (n=18) vs low altitude (n=8)
Thioredoxin (ng/mg protein)	-15% [1]	-34% [2]
Thioredoxin Reductase Activity (U/g protein)	-48% [1]	-46% [2]
Glutathione Peroxidase Activity (moles/min/mg protein)	-34% [1]	-27% [2]
Superoxide Dismutase Activity (U/mg protein)	-20% [1]	-30% p=0.05
Lipid Peroxidation (4-HNE/MDA uM/mg protein)	+291% [1]	-47% [2]
Protein Carbonyl (U/mg protein)	+18%	-34% [2]

Data calculated from 78, 83. [1] $p<.05$ preeclampsia versus gestational age matched control, [2] $p<.05$ low versus high altitude. 4HNE = 4-hydroxy-2(E)-nonenal, MDA = malondialdehyde

has both a maternal and a placental component. The maternal model suggests that reduced anti-oxidants (e.g. vitamins A, C, and E) in the mother increase her placental/endothelial susceptibility to oxidative stress, while the placental model argues that the placenta's ability to buffer oxidative stress is either diminished or overwhelmed. The high altitude data can thus far address only the placental model. Antioxidant enzymes are markedly reduced in preeclamptic placentas (78) and oxidative stress is therefore increased (Table 2). Some studies show that there are compensatory mechanisms for oxidative stress in preeclamptic placentas (79) while others show specific defects that could limit the placenta's ability to cope with normal levels of oxidative stress or which would contribute to unusually high burdens of oxidative stress (80, 81). Along with oxidative stress is increased nitrative stress (82), specifically of the syncytiotrophoblast and this too, is thought to contribute to increased apoptosis. Table 2 summarizes the results generated from the same laboratory with respect to preeclampsia and gestational age-matched controls (78) and normal pregnancies at high altitude versus low altitude (83). Clearly high-altitude placentas are similar to preeclamptic placentas in having a diminished anti-oxidant capacity, but unlike preeclampsia, they show no evidence of increased lipid peroxidation or protein carbonylation (Table 2). Lipid peroxidation is reduced by 47% in high-altitude relative to low-altitude placentas, and thus increased oxidative stress in preeclamptic placentas is unlikely to be due to chronic mild hypoxia. Moreover, we evaluated apoptosis in the high versus low altitude placentas (83), and there was no difference between altitudes; the values obtained were similar to other published data using similar techniques (84). Surprisingly, given the lack of lipid peroxidation and protein carbonylation in the high altitude placentas, we found that there were increased nitrotyrosine residues in the syncytiotrophoblast, a feature that has been consistently reported in preeclampsia (75). Thus the primary conclusion of these studies was that hypoxia does not necessarily increase oxidative stress, nor apoptosis. A second conclusion was that hypoxia may increase nitrative stress, likely via increased nitric oxide scavenging of oxygen radicals, but increased nitrative stress does not appear to contribute to apoptosis (83). Again, these data suggest that mechanisms other than lowered tissue PO_2 contribute to the increased oxidative stress observed in preeclampsia.

3.4. Etiological model #4 - Altered vascular sensitivity

Altered vascular responsiveness in preeclamptic women began to be investigated in the 1950s (85-87). The seminal publication in 1973 by Norman Gant, in which primigravid adolescents showed an increased vasopressor response to angiotensin II long before the onset of symptoms (88) further promoted the idea that an underlying, perhaps constitutional aberration in vascular sensitivity is present in women predisposed to develop preeclampsia. While compelling, this idea is hampered by the fact that the rate of repeat preeclampsia in subsequent pregnancies is only ~30%; the increased incidence of the disease in primiparous women argues against a constitutional predisposition of any great impact. Instead vascular sensitivity may vary from pregnancy to pregnancy, even within the same woman, due to the interaction of other pregnancy-related physiological changes. A substantial literature supports that human pregnancy is characterized by attenuated systemic vascular response to a number of pressor agents, as well as enhanced response to vasodilators, which contributes to the fall in systemic vascular resistance that, in turn, facilitates the normal increase in cardiac output and redistribution of blood flow to favor the uteroplacental circuit (88-92). But this varies considerably from individual to individual.

Enhanced pressor and systemic vascular resistance response to not only angiotensin II, but also catecholamines are noted in hypertensive pregnancy (87, 93, 94). Circulating norepinephrine and epinephrine levels correlate with elevated blood pressure, reduced plasma volume and elevated heart rate in preeclamptic, but not normotensive pregnant women (94). Directly measured sympathetic neural outflow (muscle sympathetic nerve activity) is greater in women who develop preeclampsia (95, 96). Moreover a preeclampsia like syndrome can be induced in animals by inducing sympathetic over-reactivity (97), and eclamptic seizures can occur in preeclamptic women given anticholinergics (98). These data imply that diminution of para-sympathetic activity potentiates an already hyper-reactive sympathetic vascular stimulation – one which may be constitutional or pregnancy-induced. This particular theory concerning the etiology of preeclampsia waxes and wanes in popularity. Nonetheless the evidence for SNS dysregulation and an exaggerated stress response in preeclampsia is too great to simply ignore the possibility (95, 99-103). Taken together, the data support that alpha-sympathetic activity in preeclamptic women is enhanced compared with normal pregnant

Table 3. Maternal physiological changes in normal, high altitude and preeclamptic pregnancy

Changes	Sea Level Normal	High Altitude	Preeclampsia
Blood pressure	Declines to mid trimester, then rises	Gradual rise	Decline followed by sharp rise
Plasma volume	Increases	Increases, but lower baseline	No increase
Catecholamines	Decreased sensitivity	Higher concentrations, greater sensitivity	Increased sensitivity
Cytokines	Increased anti- vs. pro-inflammatory	Increased pro- vs. anti-inflammatory	Increased pro—vs. anti-inflammatory
Trophoblast Invasion	Virtually complete	Diminished	Further diminished
Uterine blood flow	Normal	~30% lower	~70% lower

Abstracted from references 4, 21, 22, 28, 70, 115

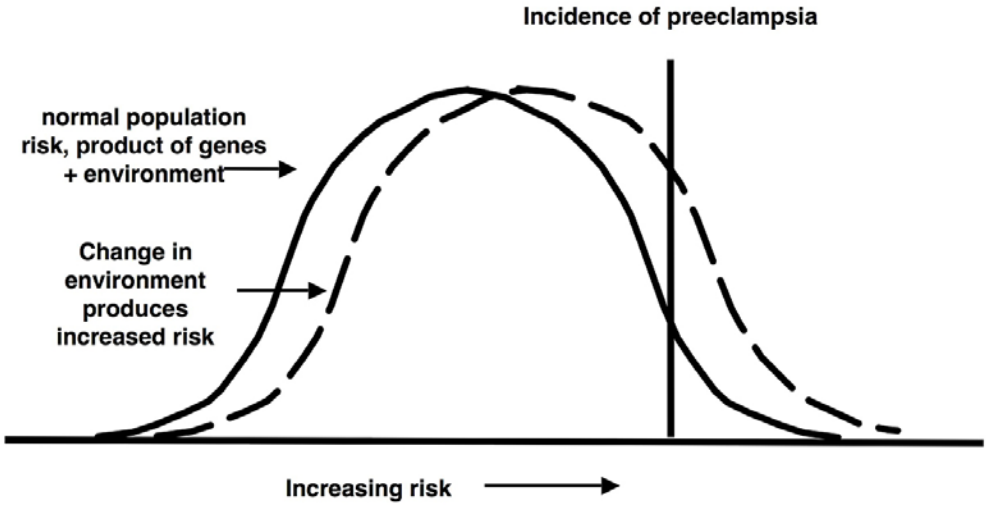

Figure 3. Model for how chronic mild hypoxemia due to high-altitude residence, operating on multiple maternal physiological characteristics, may right-shift the population-wide risk of preeclampsia.

women. The high altitude-data support that SNS activity may contribute to an increased risk for the disease. Urinary excretion of catecholamines was elevated in pregnancy at high altitude (70). Altitude-associated differences in both norepinephrine and epinephrine were most pronounced early in pregnancy (46% and 109% greater, respectively), although even non-pregnant values were 37% (norepinephrine) and 47% (epinephrine) greater among the high altitude women.

The idea that altered vascular reactivity is present in high altitude pregnancy has been vigorously pursued in a series of animal studies by several different laboratories (see the excellent review in 8). Taken together the data suggest that there is altered vascular reactivity in pregnancy at high altitude that favors increased vasoconstrictor over vasodilator responses. Increasingly sophisticated methods to explore the effects of shear stress, intraluminal pressure and other variables are being systematically applied to pregnancy- related vascular reactivity under conditions of normoxia and hypoxia (104-106). But the importance of altered vascular reactivity is central to each of the 3 hypotheses evaluated in sections 3.1-3.3 above, and may be the underlying correlate of the numerous alterations in maternal physiology present in high altitude pregnancy. These physiological studies are summarized in Table 3. The take-home message of the human data (reviewed in 10) is that maternal physiological adjustment to pregnancy is altered under conditions of chronic mild hypoxemia to a state that is intermediate between normal pregnancy and preeclampsia. The underlying cause of such systemic alterations can only be vascular.

4. CONCLUSIONS AND PERSPECTIVES

These 4 broad theories, reduced blood flow, circulating factors, placental oxidative stress and altered vascular function, are, of course, related and subject to numerous permutations in terms of the direction of causal arrows shown in Figure 1. The natural experiment of voluntary residence at high altitude clearly supports the idea that multiple systems are involved in preeclampsia. It also supports that while extremes of variation may be present in one individual's measurements, there is virtually always overlap with the normal range when a large enough population is considered. The question that remains to be answered is if there is a single cause, or a final common pathway by which preeclampsia is induced, why would it be more common at high altitude? This rhetorical question implies the answer – there cannot be a single cause. Rather, the likely explanation for the link between maternal hypoxemia and an increased risk for preeclampsia lies in the impact of hypoxia on multiple physiological systems (Figure 3, Table 3). In this model it is not just one effect of hypoxia that 'causes' preeclampsia, rather it is the impact of hypoxia on several important adjustments to pregnancy that shifts the general population risk such that more women eventually develop the disease (Figure 3). In the model presented in Figure 3, we suggest that most physiological variables have a normal

distribution, and that perturbation of the environment (e.g. by lowered oxygen pressure) can shift a greater proportion of individuals into a higher risk category for the development of a disease such as preeclampsia. None of the variables discussed above and listed in Tables 2 or 3, are sufficient to cause preeclampsia. Rather they are correlates of the disease, not markers of a single underlying cause, but far more likely to represent the range of variability present in human pregnancy. Altitude simply shifts the risk.

5. ACKNOWLEDGEMENTS

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