Placenta (2004), **25**, Supplement A, Trophoblast Research, Vol. 18, S60–S71
doi:10.1016/j.placenta.2004.01.008

# Maternal Adaptation to High-altitude Pregnancy: An Experiment of Nature—A Review

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Paper accepted 2 January 2004

A long and productive history of studies at high altitude has demonstrated that chronic hypoxia plays a key role in the aetiology of intrauterine growth restriction (IUGR) and pre-eclampsia. Susceptibility to altitude-associated IUGR varies among high-altitude populations in relation to their duration of altitude exposure, with multigenerational residents demonstrating one-third the birth weight fall present in shorter-resident groups. Higher uteroplacental blood flow during pregnancy in multigenerational high-altitude residents suggests that such population differences are due, at least in part, to differences in maternal vascular responses to pregnancy. We hypothesize that natural selection acting on hypoxia-inducible factor (HIF)-targeted or -regulatory genes has enabled maternal vascular adaptation to pregnancy in long-resident high-altitude groups. Preliminary evidence in support of this hypothesis demonstrates that the potent HIF-targeted vasoconstrictor, endothelin-1 (ET-1), is differentially regulated by pregnancy and chronic hypoxia in Andean vs European residents of high altitude. Andeans show the normal, pregnancy-associated fall in ET-1 levels previously reported at low altitude, whereas Europeans have higher ET-1 levels and little pregnancy-associated change, like pre-eclamptic women. Single nucleotide polymorphisms (SNPs) in the ET-1 gene also differ in Andeans compared with low-altitude populations. We conclude that high altitude serves as an experiment of nature for elucidating genetic factors underlying susceptibility to complications of pregnancy and fetal life. Such studies may be important for identifying persons at risk for these complications at any altitude. 
Placenta (2004), **25**, Supplement A, Trophoblast Research, Vol. 18, S60–S71

## INTRODUCTION

Hypoxia is a frequent complication of prenatal life. When prolonged, it is associated with intrauterine growth restriction (IUGR) (Table 1) and increased perinatal mortality and morbidity. Whereas persons are subject to chronic hypoxia in utero at all elevations, the largest single group of persons at risk is the 140 million worldwide residents of high altitude (>2500 m or 8000 ft) [1]. There has been a long and productive history of studies of pregnancy, fetal and neonatal life at high altitude. The initial observations on a population level that fetal growth restriction and preterm delivery were separable causes of low birth weight were made there nearly 50 years ago ([2] reviewed in [3]). Not only was this crucial in terms of our current understanding of the causes of low birth weight but was followed by continued investigations demonstrating the utility of charting newborn birth weight in relation to gestational age as a predictor of infant mortality [4]. This system continues to be used worldwide; its introduction constitutes one of the truly great public health advances of our time. The first recognition that chronic hypoxia was part of the aetiology of pre-eclampsia was also made at high altitude [5]. Continuing investigations by several investigative groups [6–9] are rapidly expanding our knowledge of the cellular and molecular mechanisms by which hypoxia influences the maternal, placental and fetal responses required to successfully produce the next generation.

Here we begin with a brief review of the magnitude and cause of the altitude-associated increase in IUGR. Because studies have been conducted at a range of elevations on three continents (North America, South America, Asia), we ask whether the decline in infant birth weight varies among populations and if so, what mechanisms are likely involved. We then move to a consideration of the physiological factors controlling uteroplacental blood flow since altered uteroplacental blood flow is a core predictor of pregnancy abnormalities [10]. After reviewing recent research addressing the effects of chronic hypoxia on uteroplacental blood flow, we ask whether these effects vary among populations or species in relation to

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**Table 1**. Key to abbreviations

| | | | |
|---|---|---|---|
| ARNT | ADP-ribosylation factor domain protein 1 | PHD | Prolyl hydroxylase domain |
| EI | External iliac | PlGF | Placenta like growth factor |
| ET-1 | Endothelin 1 | sFlt-1 | Membrane bound VEGF receptor 1 |
| HIF | Hypoxia-inducible factor | SNP | Single nucleotide polymorphism |
| HRE | Hypoxia responsive element | SVR | Systemic vascular resistance |
| IUGR | Intrauterine growth restriction | UA | Uterine artery |
| LSBL | Locus-specific branch length | VEGF | Vascular endothelial growth factor |
| NO | Nitric oxide | VHL | von Hippel Lindau |
| NOS | Nitric oxide synthase | | |

the altitude-associated increase in IUGR. Finally, we consider the role that genetic factors may play in the population and species differences observed in altitude-associated IUGR, hypothesizing that variation in hypoxia-sensitive genes might be involved. We review the role played by the hypoxia-inducible factor (HIF) pathway since it is responsible for regulating most of the oxygen-sensitive genes. After surveying the HIF-regulated and regulatory genes differentially affected by pregnancy and chronic hypoxia, we consider the possibility that such genes may have been acted upon by natural selection in populations long resident at high altitude. We conclude with some future directions for research aimed at advancing our understanding of the genetic mechanisms regulating maternal physiological responses to human pregnancy.

## ALTITUDE-ASSOCIATED IUGR

### Magnitude and cause of the birth weight decline

In Colorado and elsewhere, infant birth weight declines with increasing altitude, averaging a 100 g fall per 1000 m altitude gain (nearly a quarter of a pound per 3000 ft) [11,12]. While convenient to express in this fashion, the decline in birth weight is actually curvilinear with the 'breakpoint' occurring about 2000 m or 6600 ft [13], consistent with the shape of the haemoglobin-oxygen dissociation curve. The entire distribution of birth weights is shifted to lower values, rather than only a subset of lower birth weight babies being affected [2]. This leftward shift has the net effect of increasing the proportion of low birth weight infants (<2500 g) by more than 50 per cent [11]. In Colorado, the effect of high altitude on birth weight is as great or greater than that associated with low maternal weight gain, smoking, primiparity or pre-eclampsia [11].

The decline in birth weight is principally due to a slowing of fetal growth in the 3rd trimester. Gestational age is, on average, 0.5 week shorter at elevations over 2744 m (9000 ft) in Colorado, but this is not sufficient to explain the 240 g birth weight fall [11]. Comparisons of weights of babies born prematurely suggest that fetal growth begins to slow after 28–31 weeks' gestation [14]. Such findings have been recently confirmed by Krampl and co-workers who found a reduction in fetal biometry dimensions from 25–29 weeks' onwards, with abdominal circumference being more affected than head circumference [15].

The incidence of pre-eclampsia is increased at high altitude and this likely contributes to the altitude-associated birth weight decline [5,11,16,17]. In fact, the greater incidence of pre-eclampsia accounts for about half the birth weight decline in our recent studies in Bolivia [17]. Consistent with the concept that not just a subset of women but the population of pregnant women is affected, even normotensive women in Colorado fail to show the normal pregnancy-associated blood pressure fall suggesting a generalized disorder in maternal vascular adjustment to pregnancy [16].

The increased incidence of IUGR and pre-eclampsia at high altitude likely contributes to a rise in perinatal and infant mortality and morbidity. In a large chart-review of women receiving prenatal care at similar kinds of health care facilities at low (300 m) vs high (3600 m) altitude in Bolivia, the combination of high-altitude residence and pre-eclampsia raised the frequency of stillbirths 3-fold [17]. Not only were IUGR and pre-eclampsia more common but all the other pregnancy, fetal and newborn complications surveyed were more frequent at the high- than the low-altitude site [17,18]. Infant mortality increases proportionally to the rise in IUGR in nearly all studies [2,19–23]. In the one report in which such an increase did not occur [14], women from high altitudes used specialized medical services to a greater extent than did the low-altitude residents. Thus, the increased incidence of IUGR and pre-eclampsia at high altitude appear to raise infant mortality. Whether a different standard should be applied for determining IUGR at high vs low altitude, as has been called for recently [15], awaits determination of the birth weight-specific infant mortality (and morbidity) risks at high altitude vs low altitude, with adequate controls for gestational age and other key factors such as medical care. Such an assessment is especially important for the developing regions of South America where infant mortality rates are among the highest in the western hemisphere [24] in order for the standard to be able to identify as many high-risk infants as possible.

### Interpopulational variation in the altitude-associated birth weight decline

Population variation in the magnitude of the birth weight reduction at high altitude was first noted in Andean vs

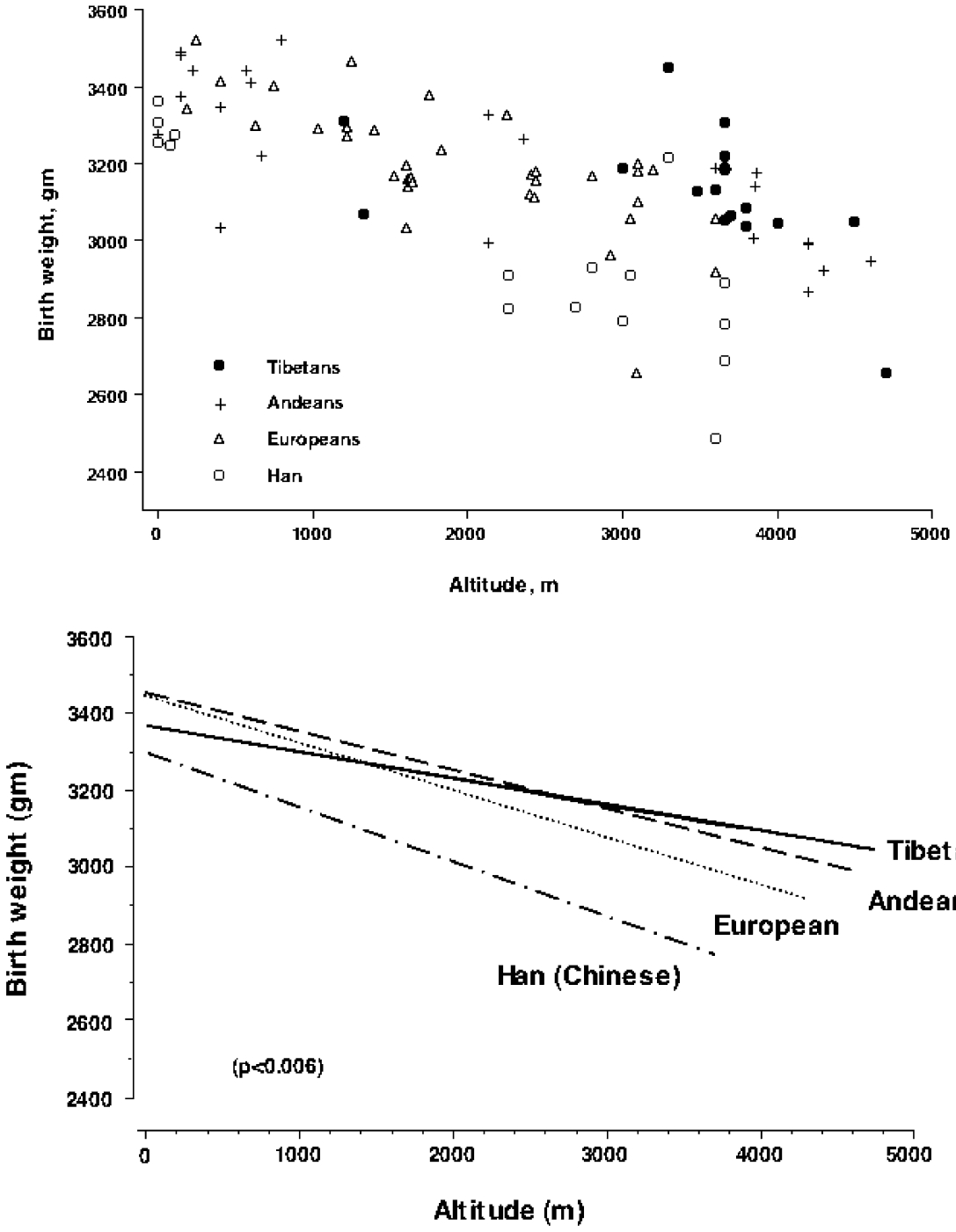


**Figure 1.** (a) Data points are average values for ~4 million births occurring in the populations and altitudes represented. The Tibetan population is likely to have lived the longest at high altitude, followed by Andeans, Europeans and then Han. For original data sources, see [26]. (b) Best fit regression lines for the data presented in 1a, weighted by sample size and variance, demonstrate that the magnitude of altitude-associated birth weight decline varies inversely with the duration (in generations) of altitude residence ($P<0.0006$).

European women in La Paz, Bolivia (3600 m) [25]. In a large review of some 4 million births, we found that while the general pattern was for birth weight to decline, the birth weights observed at a given altitude were highly variable (Figure 1A) [26]. But when grouped by population ancestry (Figure 1B), a consistent pattern emerged.

Specifically, Tibetans and Andeans who have lived at high altitudes for 10 000 years (Andeans) to 20 000 years (Tibetans) show one-third the birth weight reduction present in European or Han ('Chinese') populations that have resided at high altitudes for <500 years (i.e. <400 years in South America, <150 years in North America, and ~50 years in western China

**Table 2**. Pre-natal and post-natal mortality (deaths per 1000 pregnancies or livebirths) in women of low vs high socioeconomic status living in La Paz, Bolivia (elevation 3600 m) evaluated at 2.67 gravidity and 2.20 parity

| | Income group | Unadjusted means | Adjusted means | Mean difference | *F* | *P* value |
|---|---|---|---|---|---|---|
| Pre-natal mortality | High | 120.0 | 137.0 | −26.0 | 7.796 | 0.005 |
| | Low | 127.8 | 111.0 | | | |
| <20 weeks | High | 113.0 | 129.0 | −34.3 | 14.8 | 0.000 |
| | Low | 109.7 | 94.3 | | | |
| ≥20 weeks | High | 7.7 | 9.3 | 8.9 | 4.817 | 0.028 |
| | Low | 18.1 | 18.2 | | | |
| Post-natal mortality | High | 5.9 | 10.9 | 25.7 | 27.706 | 0.000 |
| | Low | 42.7 | 36.6 | | | |

[26]). Thus, for example, we found babies born to Tibetan mothers weighed 310 g more at 2700–3000 m (95 per cent CI=126, 494 g; $P<0.01$) and 530 g more at 3000–3800 m (210, 750 g; $P<0.01$) than babies born to Han mothers residing in the Tibet Autonomous Region of southwestern China. When viewed across their respective altitude ranges, Tibetans living at 2700–4800 m demonstrated a 15 g reduction in birthweight per 1000 m altitude gain whereas Han residing at 2700–3800 m had a 45 g/1000 m birthweight fall [27]. Such interpopulational variation does not appear attributable to differences in maternal body size, nutrition, or health care [25,26]. Since the Andean and Tibetan women are likely to have been born and raised at high altitude whereas the European and Han women may have moved there more recently, it is possible that some of these population differences are due to factors stemming from the woman's own altitude of birth and development. However, in our and others' Colorado studies, lifelong high-altitude residence does not appear to protect against altitude-associated IUGR [28,29]. It is likely that population differences in altitude-associated IUGR influence infant mortality. In the high-altitude regions of Tibet where Tibetan and Han populations live under conditions of similar and, in communist China, no-cost health care, not only were Tibetans protected from altitude-associated birthweight declines but they also had lower estimated pre- and post-natal mortality rates than the Han residing at the same altitudes [27]. Clearly, socioeconomic and health care characteristics are also key factors affecting infant mortality but these observations suggest that population-specific, genetic factors may also be involved.

In Bolivia we used surnames to compare the altitude-associated increase in IUGR among Andean, mestizo ('mixed'), and European surnamed segments of the population. Surnames as a means for assessing group-level population ancestry has been validated by comparison with genetic markers in the Bolivian population [30]. In approximately 1000 consecutive births to women receiving prenatal care and living at low (300 m, Santa Cruz), medium (2500 m, Cochabamba), or high (3600 m, La Paz and Oruro) altitude, we found that babies of Andean ancestry had only one-third as much altitude-associated increase in IUGR as babies of European ancestry [31]. Such findings help to explain why babies born to the higher-socioeconomic and more often European segment of the population at high altitude weigh less than those of the Andean, often lower-socioeconomic sector [12].

To evaluate the effects of socioeconomic vs population-ancestry characteristics on birth weight and on pre- and post-natal survival in Bolivia, we compared 1602 deliveries to high-altitude (3600 m) residents from households with above average vs below average monthly incomes (>$500 and <$500/month, $n=817$ and 785 respectively). Similar to previous reports [12], the frequency of low birth weight (<2500 g) was greater in the high- than low-income households (10.3 vs 7.0 per cent, $P<0.02$). Using logistic regression to take into account the effects of maternal education (another socioeconomic indicator), high income increased the likelihood of having a low birth weight baby [OR=1.69 (1.08, 2.64) 95 per cent confidence intervals] whereas Andean ancestry reduced the risk [OR=0.35 (0.14, 0.86)]. We also estimated pre-natal mortality from the medical records where deaths in utero were coded as 'spontaneous abortions' (abortos) if they occurred before week 20 or 'stillbirths' (nacidos muertos) if after week 20. Postnatal mortality was estimated from information concerning the numbers of previous livebirths and children currently alive. Because the low-income women had higher gravidity and parity than the high-income women (gravidity=3.0 vs 2.4 pregnancies and parity=2.6 vs 2.0 livebirths respectively) and thus greater opportunity for a pre- or post-natal loss, we adjusted the mortality estimates to a common gravidity or parity. We found greater pre-natal mortality in the high than low-income group (Table 2), and this was due entirely to more deaths before week 20. Post-natal mortality, however, was markedly reduced in the high- vs low-income groups. We concluded that the Andean population was protected during prenatal life from the effects of chronic hypoxia, possibly due, in part, to factors also protecting them from altitude-associated IUGR, but that socioeconomic disadvantages outweighed these protective effects so as to raise mortality after birth.

## PHYSIOLOGICAL FACTORS AFFECTING UTEROPLACENTAL $O_2$ DELIVERY

### Normoxic pregnancy

Pregnancy affects all the determinants of $O_2$ delivery to the uteroplacental circulation. Ventilation rises, although this does

not normally change arterial $O_2$ saturation since values are already nearly maximal. Haemoglobin declines due to greater plasma than red cell mass expansion and reduces arterial $O_2$ content. Thus a rise in blood flow is entirely responsible for increasing $O_2$ delivery to the uteroplacental circulation.

The rise in uteroplacental blood flow is due, in turn, to higher cardiac output and redistribution of blood flow to favour the uteroplacental circulation. Cardiac output rises as a result of a fall in systemic vascular resistance (SVR, afterload) and a rise in blood volume (preload). The SVR fall begins in the luteal phase immediately following conception [32], most likely as the result of primary systemic vasodilation. Nitric oxide (NO) production increases [33], sympathetic tone declines, and circulating levels of the potent vasoconstrictor endothelin-1 (ET-1) fall [34]. Another key regulator is vascular endothelial growth factor (VEGF). Continual but low VEGF levels are required for endothelial cell survival [35]. But in pre-eclampsia, high levels of the membrane bound VEGF receptor 1 (sFlt-1) bind VEGF and placental like growth factor (PlGF), decrease VEGF availability, inhibit endothelial cell proliferation, and reduce vasorelaxation responses in renal arteries [36].

Redistribution of blood flow to favour the uteroplacental circulation stems primarily from profound changes which are confined to that vascular bed. Vascular resistance falls as a result of the anastomoses which develops between the ovarian branch and the main uterine artery (UA), as well as the growth and enlargement of existing vessels. Because the fall in vascular resistance in the uteroplacental bed is greater than that occurring in vessels supplied by the external iliac (EI) artery, the UA progressively 'steals' EI blood flow. Unilateral UA blood flow therefore rises from a nonpregnant value of $\sim$10 ml/min to $\sim$350 ml/min near term [37], a change which is greater than that experienced by any organ system following birth. In species with haemochorial placentae (e.g. most primates, rodents and guinea pigs), the vessels determining uteroplacental vascular resistance reside largely outside the uterus whereas in epitheliochorial species, the uteroplacental vessels comprise the major site of vascular resistance [38,39]. Specifically, in haemochorial species, two-thirds of the uteroplacental vascular resistance resides in the mesometrial, main UA and ovarian arteries and only one-third is located in uteroplacental channels. Since the UA makes a demonstrable contribution to uteroplacental vascular resistance in the haemochorial species under study and is the smallest vessel which can be reliably visualized in humans, our studies have focused on the UA.

UA enlargement in haemochorial species is due to alterations in its responsiveness to circulating and locally produced vasoconstrictors and vasodilators, UA distensibility and remodelling. Pregnancy raises the production of NO and other substances to augment UA vasodilator response to pharmacological agonists as well as to flow [40–42]. We and others have shown that the guinea pig UA vasoconstrictor response to alpha-adrenergic agonists is reduced [43,44]. Distensibility is also enhanced [45]. A key factor for enlarging lumenal diameter is UA remodelling, accompanied by compositional changes, hyperplasia and hypertrophy in all layers of the vessel wall as well [45–47]. Of note, the increase in guinea pig cellular proliferation occurs before the greatest rise in UA blood flow, suggesting that UA enlargement is a prerequisite for the flow increase to occur. The factors prompting UA growth likely involve pregnancy hormones and growth factors [48]. Since growth is more pronounced in vessels supplying the pregnant than the nonpregnant uterine horn, venous to arterial transfer of fetoplacentally derived growth factors may also be important [49]. Increased flow itself may be an important growth stimulus given that the UA's characteristic outward hypertrophic growth resembles that occurring in response to flow rather than pressure [50]. The ability of VEGF and other growth factors to stimulate NO [51] suggests that interactions between vasoactive and growth factors are likely crucial for raising UA blood flow.

## Chronically hypoxic pregnancy

At high altitude, the pregnancy-associated rise in alveolar ventilation and increase in arterial $O_2$ saturation nearly restores arterial $O_2$ content to sea level values [52,53] and both relate positively to fetal weight [29,54,55]. But since birth weights are generally lower than at sea level, it is likely that reduced uteroplacental blood flow rather than diminished arterial $O_2$ content is chiefly responsible for the hypoxia-associated IUGR observed.

Our and others' studies show that chronic hypoxia alters systemic and uteroplacental vascular adjustments to pregnancy. In pregnant women, guinea pigs or sheep, cardiac output is lower at high vs low altitude, probably as the result of lower blood volume and/or higher SVR [56–59]. Blood volume expands normally during pregnancy at high altitude, but begins from lower non-pregnant levels such that blood volume is lower near term at high than at low altitude [58]. The higher SVR in high vs low-altitude pregnant humans or experimental animals contributes to the higher maternal blood pressures observed [16,57]. Such an increase could, in turn, be due to greater myogenic tone, altered production of local regulators of vascular tone (more vasoconstrictors and/or less vasodilators), and/or a lack of the compensatory organ remodelling that occurs in a normal pregnancy.

Factors operating both at the systemic level and within the uteroplacental circulation are likely to be important. Acute (hours) and more chronic (weeks) high-altitude exposure raise sympathetic nervous system activity and systemic catecholamine levels in nonpregnant women [60]. In addition, circulating ET-1 levels are elevated by chronic hypoxia [61]. ET-1 induced vasoconstriction appears to be an especially important contributor, given the ability of endothelin-A receptor blockade to prevent hypoxia-associated IUGR and the accompanying reduction in uteroplacental blood flow in rats [62,63].

Concerning the uteroplacental circulation, UA blood flow is one-third lower near term in pregnant high (3100 m) vs low

(1600 m) altitude residents as a result of a lesser increase in UA diameter [64]. The portion of common iliac blood flow diverted to the UA is less in pre-eclamptic women compared to normal women, suggesting even lower UA blood flows [65]. Given the importance of vessels outside the uterus in the determination of uteroplacental vascular resistance in haemochorial species, the causes of the lower UA blood flow are likely to involve the main UA, mesometrial or arcuate vessels. Of note, chronic hypoxia opposes the effects of normal pregnancy on flow vasodilation in the guinea pig UA. Thus, rather than pregnancy increasing the vasodilator response to flow, UA from chronically hypoxic animals vasoconstrict at high flow [42], resembling myometrial arteries from pre-eclamptic women in which enhanced flow vasodilation also fails to occur [66]. Chronic hypoxia also inhibits guinea pig UA growth such that there is only half as much rise in DNA synthesis as in vessels from normoxic animals [67]. Both these flow and growth alterations may stem from a lack of pregnancy-associated increase in NO. Chronic hypoxia reduces NO-dependent vasorelaxation to acetylcholine in isolated guinea pig UA rings and inhibits the pregnancy-associated increase in endothelial NOS protein (NOS III) in whole vessel homogenates [41,68]. Unknown is whether hypoxia also affects the pregnancy-associated alterations in other vasodilators (e.g. endothelial-derived hyperpolarizing factor), growth (e.g. VEGF, PlGF) or vasoactive factors (e.g. ET-1, catecholamines).

Unlike the guinea pig, a specie in which hypoxia-associated IUGR occurs [57,69], a different UA response to chronic hypoxia has been observed in sheep. Sheep vary among breeds in their susceptibility to hypoxia-associated IUGR with some but not other breeds showing birth weight reductions at high altitude [56,70]. In sheep resistant to hypoxia-associated IUGR, chronic hypoxia raised the vasodilator response to acetylcholine and increase in NO production, NOS III protein and message during pregnancy to a *greater* extent in UA from high- vs low-altitude [71–73]. The converse was seen in guinea pigs as noted above, where chronic hypoxia did not alter the magnitude of acetylcholine vasodilation and reduced NO production. There are additional differences between the effects of chronic hypoxia in sheep and guinea pigs concerning the UA vasoconstrictor response to phenylephrine. Pregnancy reduced the UA vasoconstrictor sensitivity to phenylephrine similarly in chronically hypoxic vs normoxic guinea pigs. Conversely in sheep, there was a greater reduction in the chronically hypoxic vs normoxic animals as the result of decreased alpha1-adrenergic receptor density, binding affinity and inositol phosphate 3 production [73–75]. Such species differences suggest that susceptibility to altitude-associated reductions in birth weight are due, at least in part, to genetic factors regulating the maternal systemic and uteroplacental circulatory responses to pregnancy [76].

Not only is there variation between species in uteroplacental vascular responses to pregnancy and hypoxia-associated IUGR, such variation also occurs within the human specie. Specifically, we have shown that Tibetan women whose infants

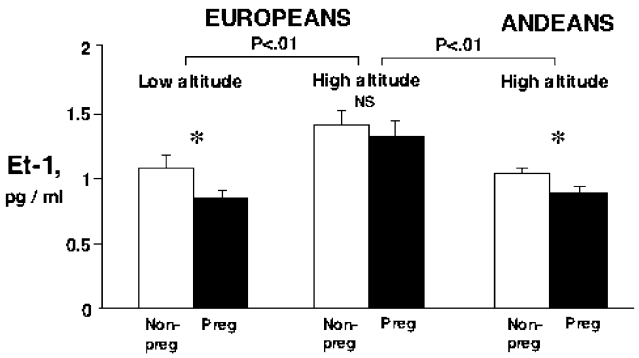


**Figure 2.** Andean women have lower ET-1 levels than European high-altitude (3600 m) residents when nonpregnant. Whereas values fall during pregnancy in the Andeans, there is no clear change in the European women. (*=$P<0.05$ for comparisons of the non-pregnant vs pregnant state, and brackets=$P<0.05$ between ancestry or altitude groups).

are protected from altitude-associated IUGR have higher UA blood flow velocity and greater lower extremity blood flow redistribution to favour the UA than Han women residing at the same elevation (3600 m) [55]. Moreover, pregnant Han women at high altitude have smaller UA diameters and lower blood flows than their low-altitude counterparts [77], like Colorado residents of high altitude [64]. Andean high-altitude pregnant women have a normal or even exaggerated fall in uteroplacental vascular resistance [78]. Preliminary data from Andean high-altitude women suggest that UA blood flow is also greater than in women of European ancestry residing at the same altitude [79]. Our preliminary data also support the hypothesis that such differences in UA blood flow may be due, in part, to differences in HIF-related genes. The potent vasoconstrictor ET-1 appears to be differentially regulated by pregnancy and chronic hypoxia in Andean vs European residents of high altitude (Figure 2). Andeans demonstrate the normal, pregnancy-associated fall in ET-1 previously reported at low altitude whereas Europeans have higher ET-1 levels and little pregnancy-associated change, like pre-eclamptic women [34]. Thus these data support the hypothesis that long-term residents of high altitude may be protected from adverse effects of chronic hypoxia on vascular responses to pregnancy via actions of HIF-targeted genes, among others.

## DO GENETIC FACTORS INFLUENCE MATERNAL VASCULAR ADAPTATION TO PREGNANCY?

Population and species differences in the magnitude of hypoxia-associated IUGR suggest the involvement of genetic factors. Such genetic involvement is consistent with other studies suggesting associations between specific genetic variants and pre-eclampsia or IUGR [80–85]. In evaluating the kinds of genetic factors that might be involved, we (and others) have been struck by the observation that many of the candidate genes thus far identified are part of the HIF

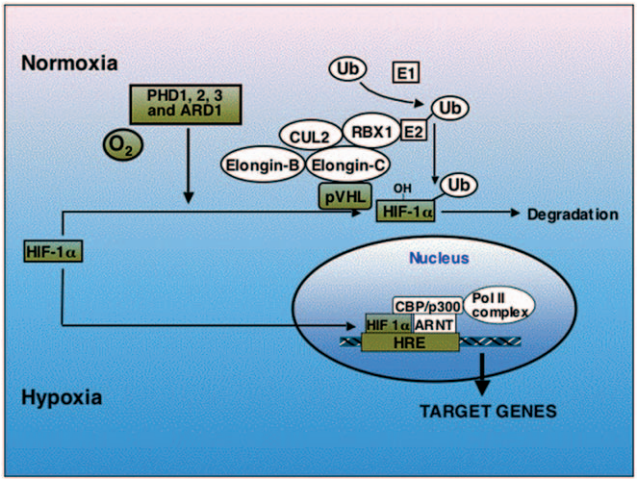


**Figure 3**. HIF 1-alpha regulation (courtesy K Stenmark).

pathway. Since this pathway regulates a majority of the hypoxia-responsive genes, we hypothesized that HIF-regulated pathways are logical targets on which selection for traits influencing variation in altitude-associated IUGR would be expected to act.

The molecular mechanisms by which HIFs influence hypoxic responses have recently been subject to extensive investigation. These studies have shown that HIF is a highly conserved heterodimer consisting of a beta subunit (the constitutive HIF1beta/ARNT complex) and one of three alpha subunits (HIF 1, 2 or 3alpha). Despite continual production, degradation is sufficiently rapid that the HIFalpha proteins are virtually undetectable in normoxia (Figure 3). This degradation requires *trans*-4-hydroxylation at proline-564 and -402, recognition and binding by the von Hippel Lindau (VHL) protein, ubiquination by a E3 ubiquitin ligase complex (consistingm of elongin C/elongin B, cullin 2, and the RING-H2 finger protein Rbx-1), and transport to the proteasome [86]. But under hypoxia and selected other circumstances (e.g., specific oncogenes, proline hydroxylase enzyme inhibition [87], presence of large divalent metal ions or iron chelators), HIFalpha escapes hydroxylation and recognition by VHL. This permits HIF protein levels to rise, translocate to the nucleus, heterodimerize, and transcriptionally activate genes containing the *cis*-acting hypoxia responsive element (HRE) 5′ACGTG(C/G)3′ [88].

Over 40 HIF-regulated or regulatory genes have been identified whose functions influence the vascular adjustments to hypoxia and/or pregnancy [89] (Table 3). This group comprises a number of candidate genes whose vascular effects can plausibly be linked to the vasoconstriction, endothelial damage and reduced uteroplacental blood flow characteristic of *pre-eclampsia* and IUGR [80–85]. Many (if not most) are polymorphic [i.e. exhibiting relatively common (>1 per cent) genetic variants]. Moreover, such allelic variation is likely functional insofar as it is associated with differences in levels of circulating gene products [90]. Supporting the likelihood that

**Table 3**. HIF genes altered by hypoxia and pregnancy

| Function | Genes | Reference |
|---|---|---|
| HIF targets | | |
| Vasoactive | ET-1, ECE | [84,99–103] |
| | Leptin | [104,105] |
| | NOS II, III | [84,99,101,103] |
| | Tyrosine hydroxylase 1,2; α1-adrenergic receptor | [106–108] |
| Growth | EPO, transferrin | [109–111] |
| | IGF (IGF2, IGFBP1-3) | [112–115] |
| | PDGF β | [116,117] |
| | TGF α | [8,118] |
| | VEGF, Flt-1, sFlt, KDR, neuropilin 1-2, PlGF | [36,102,119–126] |
| Inflammation | Interleukins 1, 6; TNF α | [127–132] |
| HIF regulatory | | |
| | HIF1-3 α, HIF1-α, ARNT2-3 | [8,133–135] |
| | ARD1 | [136] |
| | Cul2, JAB1/CSN5, RBX1 | [137–139] |
| | PHD1, 2, 3 | [140,141] |
| | VHL | [7,142] |

Notes: NOSIII is hypoxia but not HIF-regulated. Cul2 and RBX1 are constitutive. Effects of pregnancy on Cul2, RBX1, and PHD are unknown.

such genes are expressed in the uteroplacental vascular bed, HIF or HIF-regulatory gene expression is altered in placentae from pregnancies complicated by pre-eclampsia and/or IUGR compared with normal pregnancy [8,9].

## GENOMIC APPROACHES FOR IDENTIFYING CANDIDATE GENES

Genomic approaches have been infrequently applied to pregnancy complications but offer considerable power for determining interindividual variation in risk factors for complex diseases [91,92]. Such approaches include ones which take advantage of the possibility that natural selection has acted to differentiate one population, in this case Andeans, from other groups with shorter duration of high-altitude exposure with respect to genes influencing uteroplacental blood flow and fetal growth. The principle underlying these approaches stems from the observation that since geographic separation of human populations is relatively recent,[1] most genetic variation is shared among all groups with only ~15 per cent differing between major continental regions [93]. Thus, comparatively small differences in allele frequency are expected across most genes but not, importantly, those that have been acted upon by natural selection [94]. For example, the Duffy null allele

[1] 100–60 000 years ago (kya) for African/non-African, ~50 kya for Asian/European, ~20–15 kya for Asian/American separation.

**Table 4**. Allele frequencies, differentials, and LSBLs for 10k-WGA SNPs near HIF-regulated/regulatory genes

| Gene | SNP no. | Allele frequencies | | | Quechua–Nahua differential | Quechua LSBL |
|---|---|---|---|---|---|---|
| | | Quechua | Nahua | Asian | | |
| NOSII | 1 | 0.425 | 0.588 | 0.500 | 0.163 | 0.075 |
| NOSII | 2 | 0.425 | 0.650 | 0.525 | 0.225[b] | 0.100 |
| α1-adrenergic receptor | | 0.100 | 0.250 | 0.350 | 0.150 | 0.150[b] |
| endothelin | | 0.800 | 0.525 | 0.175 | 0.275[a] | 0.275[a] |
| FLT1 | 1 | 0.825 | 0.900 | 0.600 | 0.075 | 0.000 |
| TGF α | | 0.225 | 0.250 | 0.450 | 0.025 | 0.025 |
| cullin 2 | | 0.175 | 0.325 | 0.200 | 0.150 | 0.025 |
| neuropilin 2 | 1 | 0.400 | 0.421 | 0.600 | 0.021 | 0.021 |
| neuropilin 2 | 2 | 0.050 | 0.053 | 0.150 | 0.003 | 0.003 |
| neuropilin 1 | | 0.500 | 0.421 | 0.368 | 0.079 | 0.079 |
| PHD3 | 1 | 0.775 | 0.625 | 0.500 | 0.150 | 0.150[b] |
| PHD3 | 2 | 0.361 | 0.214 | 0.250 | 0.147 | 0.111 |
| PHD3 | 3 | 0.425 | 0.400 | 0.075 | 0.025 | 0.025 |

[a] $P<0.05$.
[b] $0.05<P<0.10$.

(*FY*0*) which provides protection from the *Plasmodium vivax* form of malaria has reached very high frequencies across sub-Saharan Africa in the last several thousand years, while not being present outside Africa [95]. Divergence for *FY*0* and markers near the functional site is detected as high $F_{ST}$ levels (i.e., the proportion of the total genetic variation that is due to differences among groups) [96]. We recently extended the $F_{ST}$ approach to quantify locus-specific divergence using a measure termed locus-specific branch length (LSBL) [97]. While the $F_{ST}$ approach evaluates if any one or more of the populations under consideration have undergone dramatic changes in allele frequency, the LSBL approach provides the ability to geometrically isolate the population in which the allele frequency change occurred. These $F_{ST}$ and LSBL based methods provide exciting breakthroughs for identifying candidate genes in pathways thought affected by recent directional or balancing selection [92,94,98].

As a preliminary test as to whether any of the candidate genes listed in Table 3 are implicated in the population differences observed in altitude-associated IUGR, we generated an empirical distribution for the levels of LSBL in Andean high-altitude residents and, for comparative purposes, indigenous American groups not residing at high altitude. In addition, we included East Asians as a group which is likely to share a recent (relative to other human populations) common ancestor [97]. We used a new genotyping method developed by Affymetrix (10K-WGA mapping array, Santa Clara, CA, USA) that permits whole genome amplification and generation of allele frequencies for 11 555 single nucleotide polymorphisms (SNPs) located throughout the genome. Using the Affymetrix 10K-WGA chip, we examined several populations, including East Asians and two indigenous Central and South American groups (Nahua and Andean), both of which have previously been shown to have very low (<1 per cent) African or European admixture. These empirical distributions of $F_{ST}$ and LSBL can then be used to quantify how different any particular gene is from the range of values typically seen for the population comparisons being made.

For a preliminary evaluation of branch lengths for the candidate genes listed in Table 3, we screened for genes located within 40 kb of the 11 555 SNPs inventoried on the 10K-WGA chip. Nine such genes were identified (Table 4). Shown are the allele frequencies for the SNPs near each of these nine genes, the difference in allele frequency, and the LSBL for Andeans (Quechua from Peru) and two related populations [Nahua from Mexico and East Asians (Chinese and Japanese living in the US)]. Of these nine, remarkably, nearly half (44 per cent) had one SNP which was in the highest 90th percentile of the distribution of all SNPs on the 10K-WGA. These genes were NOSII, the alpha1-adrenergic receptor, endothelin, and PHD3, all of which are important candidate genes. To phrase this differently, there is a less than 10 per cent chance that genetic variation near these four genes was within the range exhibited in the low-altitude control populations (the Nahua and East Asians) and a <5 per cent chance that variation near the ET-1 gene was within the expected range.

## DIRECTIONS FOR FUTURE STUDY

Thus, the available data suggest that UA blood flow is lower during pregnancy at high altitude in settings where IUGR is most pronounced. Further the variation in hypoxia-associated IUGR that is evident both within as well as between species suggests important avenues for future studies designed to address the contribution of genetic factors to maternal vascular adaptation to pregnancy. Future studies are required for assaying additional SNPs near the candidate, HIF-targeted genes to verify the existence of recent natural selection in Andean populations and for testing functionality. Such studies represent a novel and, as yet, relatively unexplored approach

for achieving an integrated understanding of the physiological and genetic bases for pregnancy complications. Such an approach can also be expected to yield new predictive tests as well as therapies for treatment designed to alleviate suffering at this most vulnerable period of life.

## ACKNOWLEDGEMENTS

Our appreciation is extended to the many subjects who have participated in our studies of pregnancy at high altitudes in Colorado, Peru, Tibet and Bolivia and to the technical staff at the Women's Health and Colorado High-Altitude Research Centers, the Bolivian High-Altitude Biology Institute, the Pennsylvania Department of Anthropology and the other sites which have cooperated with us in the past. We especially thank Ms Wendy MacCannell for her help with the preparation of this article and Rhonda Mouser for the conduct of the endothelin-1 assays. Grant support was provided by NIH TW01188, HL60131, HL14985.

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