Chapter 10

AN EVOLUTIONARY MODEL FOR IDENTIFYING GENETIC ADAPTATION TO HIGH ALTITUDE

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Abstract: Coordinated maternal/fetal responses to pregnancy are required to ensure continuous O_2 delivery to the developing organism. Mammals employ distinctive reproductive strategies that afford their young an improved chance of survival through the completion or the reproductive period. Thus, mortality prior to the end of the reproductive period is concentrated in the earliest phases of the lifecycle. At high altitude, fetal growth restriction reduces birth weight and likely compromises survival during the early postnatal period. Population variation in the frequency of the altitude-associated increase in intrauterine growth restriction (IUGR) demonstrates that multigenerational Tibetan and Andean high-altitude populations are protected compared with shorter duration, European or Han (Chinese) residents. This experiment of nature permits testing the hypothesis that genetic factors (a) influence susceptibility to altitude-associated IUGR, (b) act on maternal vascular adjustments to pregnancy determining uteroplacental blood flow, and (c) involve genes which regulate and/or are regulated by hypoxia-inducible factors (HIFs). Serial, studies during pregnancy as well as postpartum in Andean and European residents of high (3600 m) and low (300 m) altitude will permit evaluation of whether uteroplacental O_2 delivery is lower in the European than Andean women and, if so, the physiological factors responsible. Comparisons of HIF-targeted vasoactive substances and SNPs in or near HIF-regulatory or targeted genes will permit determination of whether these regions are distinctive in the Andean population. Studies coupling genetic and genomic approaches with more traditional physiological measures may be productively employed for determining the genetic mechanisms influencing physiological adaptation to high altitude.

Key Words: adaptation, hypoxia, hypoxia inducible factor (HIF), IUGR, natural selection, preeclampsia, uteroplacental ischemia

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INTRODUCTION

Studies of human physiological adaptation to high altitude (defined here as >2500 m or 8000 ft) have long sought to determine whether or not there are genetic factors involved. Such efforts have been hindered by the inherent difficulties in distinguishing between genetic attributes *vs*. those that are acquired as a result of prenatal, postnatal, or later-in-life influences (9). In the language of geneticists, the phenotype (P) is a function of genetic (G) and environmental (E) influences plus the interactions between them (G x E); rarely, are only genetic factors responsible for visible traits. Interaction is so ubiquitous that some have questioned whether any influence is purely genetic or environmental (47). Such interactions include the influences of age, nutrition, disease or other kinds of environmental characteristics on the expression of genetic traits. For example, at high altitude, the larger lung volumes of lifelong, Andean high-altitude residents reflect both developmental exposure and a hereditary potential for larger lung dimensions (19). In addition. Brutsaert and co-workers have shown that the extent of an individual's physical fitness -- largely a function of acquired traits such as habitual exercise levels and training -- influences the extent to which genetic factors confer protection from an altitude-associated decrement in maximal exercise capacity (8).

The purpose of this article is to describe a model for identifying the genetic and/or genomic contribution to human adaptation to high altitude. Specifically, this model lays the groundwork for applying the analytical techniques described in this volume by Shriver *et al.,* to the adaptive challenge of fetal growth restriction posed by residence at high altitude. For reasons elaborated upon below, because the risk to survival prior to the end of the reproductive period is greatest during the period of pre- and early post-natal life, we elect to focus on fetal growth restriction as our index of high-altitude adaptation. Since genetic changes occur over generations and hence require long periods of time, we also choose to compare populations with and without multigenerational exposure to high altitude, while proposing to make provision for controlling for differences between groups unrelated to high-altitude exposure.

DEFINING ADAPTATION

From a genetic perspective, evolution can be defined as change in gene (allele) frequency over time. Four factors are involved– mutation, genetic drift, gene flow and natural selection – but only one of these, natural selection, is directional. Natural selection is also the force of greatest interest to physiologists since, generally, it is the effects of genes on the organism's ability to adapt to the environment that is of paramount concern. "Adaptation" in an evolutionary context refers to the ability to live and reproduce in a given environment (17). In principle, adaptations can be grouped into those affecting fertility (the production of live offspring) and mortality (in an evolutionary context, the ability of the organism to survive until the end of its reproductive period). "Fitness" is the net result of influences on both fertility and mortality, sometimes simply referred to as reproductive success. Distinguishing between adaptations affecting fertility and mortality can be difficult, especially in mammals that, as a group, shelter their young within their mother's uterus, making early mortality difficult to detect.

Mammalian distinctions. The appearance of mammals in the paleontologic record changed natural selection from operating by an "r"- to a "k"- strategy. An "r"-based strategy is characterized by the production of a large number of young, each of which has a low probability of survival. The "k"-strategy employed by mammals relies upon the production of fewer eggs, each of which has a greater probability of being fertilized and, if fertilized, a greater probability for survival. In short, the changeover from an "r" to a "k" strategy reflects a shift from a more "quantity" to "quality" reproductive approach. Still, among mammals, probably only on the order of 20% of fertilized eggs survive until birth.

A prolonged intrauterine period greatly enhances the likelihood of survival but poses a different kind of challenge; how to house a 50% genetically dissimilar organism without the mother's immune detection and rejection? For some mammals, the answer is to protect the offspring by having a "shell" (more a leathery sort of skin) to protect the offspring from immune detection while in utero. These are "prototherian" mammals, exemplified by the duck-billed platypus. Another strategy is that employed by "metatherian" mammals, like kangaroos or wallabys, which give birth early to an extremely immature offspring that is housed in the mother's pouch where it can nurse and grow to a large enough size to be able to fend for itself. The metatherian strategy appears to have been the kind employed by the earliest mammals (105). "Eutherian" mammals' reliance on the placenta carries the "r" *vs.* "k"-approach a stage further insofar as the placenta greatly improves the conceptus' chance of survival by permiting a period of prolonged gestation for fetal growth and development. The placenta is a specialized tissue of fetal origin that not only aids in nourishing the fetus but also minimizes its detection by the mother's immune system[1].

Shape of the age vs. survival curve. The influence of the placenta on the shape of the survival curve is illustrated in Figure 1. Defining life as beginning at conception[2], mortality rates in fish are very high during hatching and then decline, as they get larger and are better able to flee from predators. The protection afforded by shelled eggs lowers mortality somewhat during early life. Protection is further enhanced in placental mammals, displacing the survival curve to the right even more. Relatively high mortality soon after conception is still present in placental mammals, likely due to implantation failure, chromosomal or other kinds of genetic abnormalities which are nearly always lethal and result in spontaneous abortion. An improved chance of survival following this early, prenatal period is carried to an extreme in humans whose smaller number of births, extended period of infancy, and social support systems reduce infantile and childhood mortality relative to other mammals. Even more remarkably in humans, survivorship extends beyond the end of the reproductive period into old age.

One point requires clarification: the difference between "lifespan" and "life expectancy." Sometime used interchangeably, "lifespan" refers to a probably, genetically-endowed maximum length of life for a given species, whereas "life expectancy" is the probability that a given organism will achieve this lifespan. For humans, the lifespan is estimated as being approximately 120 years. Life expectancy is generally calculated at birth but can, in principle, be calculated at any age. When calculated at birth, by far the greatest contributor to life expectancy is the mortality rate during infancy and childhood. If calculated at age 15, life expectancies are quite similar even when comparing societies with markedly different standards of living (21).

The point here is that humans follow a reproductive strategy in which mortality, prior to the end of the reproductive period, is concentrated in the earliest phases of the lifecycle.

In other words, once born and especially once infancy and childhood are completed, the chances of surviving through the reproductive period are very high. In relation to high altitude, the implication here is that the most important determinants of survival are likely to be those affecting intrauterine mortality or mortality soon after birth. Fertility does not seem to be adversely affected at high altitude (103); on the contrary, levels appear to be higher in some high- than low-altitude Peruvian groups (26). Therefore, the most important influences of altitude are likely to be centered on fetal growth and/or gestational age.

Importance of birth weight. For humans, the single most important predictor of neonatal mortality is small size at birth, whether due to fetal growth restriction or preterm delivery (52). This is illustrated by the continuing decline in mortality rates from the neonatal through the infant and childhood periods in Figure 1.

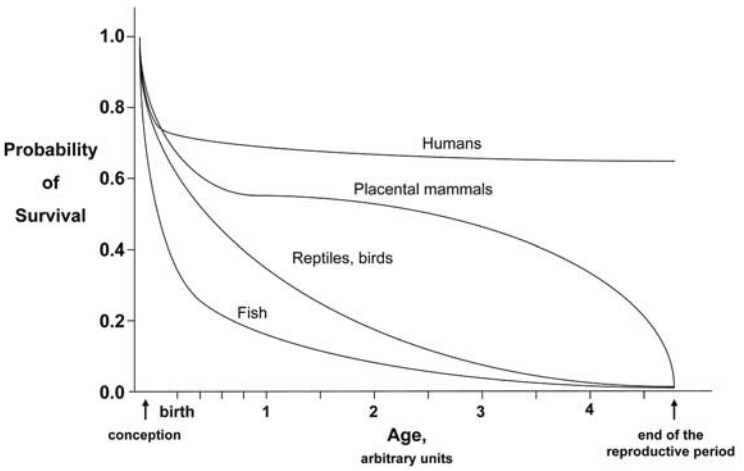

Figure 1. For fish and reptiles or birds, mortality is especially high following hatching and then gradually declines, with all individuals dying after the completion of reproduction. Placental mammals, initially, have high intrauterine mortality but an improved chance of survival relative to fish, reptiles or birds. Mortality gradually declines following birth for most placental mammals, with all deaths occurring by the end of reproduction. Humans have a distinctive pattern in which mortality is relatively low during infancy and childhood and then very low during adolesence and the adult years, with a considerable proportion surviving after the completion of the reproductive period. This graph is drawn for illustrative purposes and is not strictly to scale. Actual age-specific mortality risks vary by historical period and by society.

The contributions of gestational age and birth weight to neonatal mortality vary, depending on the particular ages and weights involved, as shown by the non-symmetric shape of the mortality boxes in the fetal growth charts first advanced by Dr. Lula Lubchenco nearly 50 years ago (Figure 2). The development of such charts, now used the world over, permits identifying whether or not a given newborn is at "risk", it ranks as one of the great public health advances of our times. Sociocultural factors also influence the effects of fetal growth and gestational age on neonatal mortality; preterm births are less likely to occur in developed countries where medical technology permits delaying labor or hastening maturation of the fetus' lungs when labor is threatened, making IUGR an especially important factor

(28). But today in the developed as well as the developing world, as well as throughout human existence, a high proportion of infant mortality is likely attributable to the influences of fetal growth restriction and preterm delivery.

While many factors contribute to IUGR[3], one of the more pervasive is uteroplacental ischemia. As elaborated below, uteroplacental blood flow increases more than 30-fold during pregnancy, fueling the exponential rise in fetal growth, and making any reductions in blood flow potentially important. While sometimes not thought of as a nutrient, oxygen can be so considered since oxygen, like other nutrients, is a source of energy (ATP supply) as well as a cofactor in numerous metabolic processes. Other nutrients required for fetal growth include glucose, amino acids, various trace minerals and vitamins.

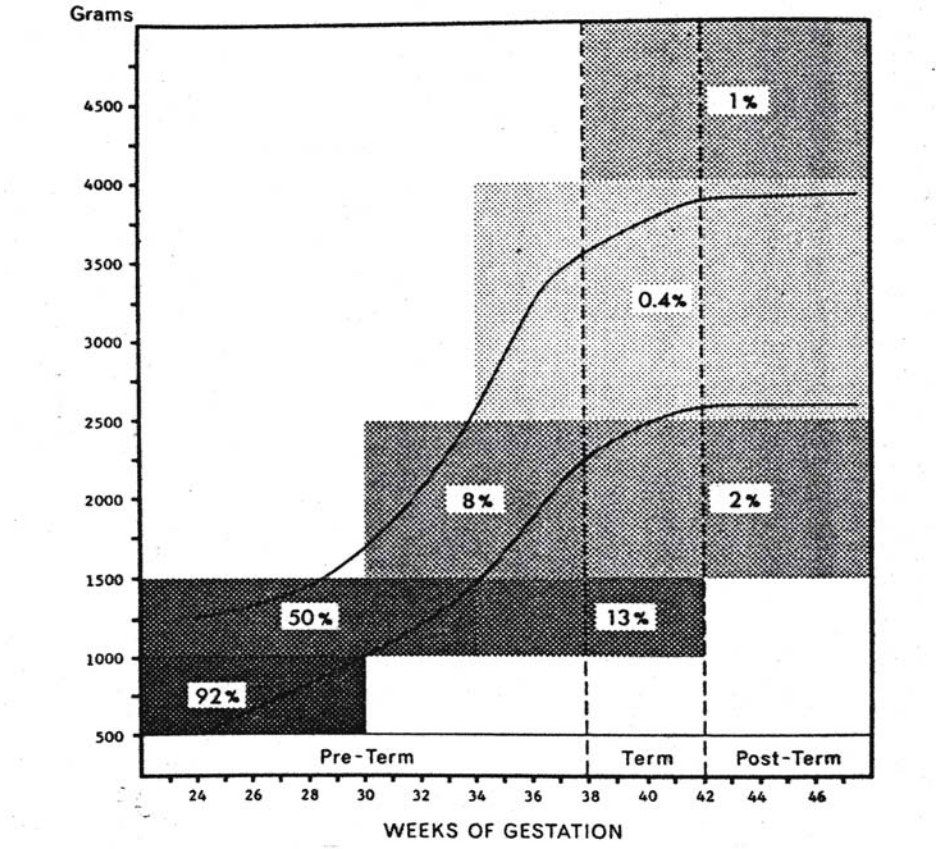

Figure 2. Birth weight (gm, y axis) and gestational age (x axis) influence neonatal mortality, shown as the percent deaths during the first 28 days of postnatal life. Mortality declines with advancing gestational age and increasing birth weight. The figure is reprinted from Lubchenco (52) who compiled data for all University of Colorado Medical Center newborn admissions from July 1, 1958 - July 1, 1968.

EFFECT OF HIGH ALTITUDE ON BIRTH WEIGHT

Epidemiological, anthropological and public health observations. High altitude occupies a special place in the history of investigating the cause(s) of low birth weight (conventionally defined as <2500 gm or 5.5 lbs). Until the 1970s, the World Health Organization and other such agencies considered any low birth weight baby to be "preterm" since gestational age information was not routinely sought. The first observations that hypoxia slows fetal growth on a population level were made at high altitude (49). In these studies, the possibility of shortened gestation was carefully considered; popular opinion held that the lack of snowplows prevented women from reaching the hospital and hence doctors delivered their patients early (65). The actual data, however, showed that gestational age did not differ from low-altitude values. Hence fetal growth restriction was identified as the primary cause in this early, as well as numerous more recent, studies (25, 37, 73). From ultrasound studies and altitude comparisons across a range of gestational ages, growth measurably begins to slow about week 30 (43, 102).

The birth weight decline averages 100 gm per 1000 m altitude gain (37). The effects of altitude are greater than those of low maternal weight gain, smoking, primiparity or preeclampsia and second only to gestational age in importance (37). Given the more than 100,000 Coloradans and 140 million persons living at high altitudes worldwide, high-altitude residents are the single largest group at risk for IUGR (41). The primary factor responsible appears to be the hypoxia associated with residence at high altitude (25, 49, 73). Additional contributors are the altitude-associated increase in preeclampsia, which accounts for about half the birth weight decline, as well as yet to be fully elucidated interactions with other factors such as smoking (37, 39, 55, 66, 78, 81).

While occurring worldwide, the magnitude of the altitude-associated birth weight reduction varies in relation to the duration (in generations) of high-altitude exposure. That is, Tibetans and Andeans who have lived at high altitudes for 10,000+ yrs show one-third the reduction present in European or Han ("Chinese") populations that have resided at high altitudes for <500 years (79). Such variation does not appear attributable to maternal body size, nutrition, health care or birth weight present at sea level (29, 64). Nor do birth weights of babies born to lifelong high-altitude residents differ from those of babies born to women moving to altitude as adults, suggesting that developmental effects are not chiefly responsible (30, 69, 106).

Given the lower birth weights present at high altitudes, the expectation would be that neonatal and, by extension, infant mortality would be increased as well. Consistent with this, Bolivia and Peru have the highest infant mortality rates in South America and, in the case of Bolivia, the highest in the western hemisphere (80). There is a proportional rise in mortality with increasing altitude within Bolivia for both urban and rural dwellers, with rural rates being higher than those seen in urban areas (7, 24). Since there is considerably greater health care access in urban than rural regions, the urban-rural differences support the importance of health care for the mortality rates observed. But since the altitude rise is present for both urban and rural residents, the data also suggest that altitude is a likely contributor. In Colorado, infant mortality was greater in the high *vs.* low-altitude regions of the state until about 1980. With the advent of specialized neonatal care services and transport systems, mortality rates fell, particularly at the higher altitudes, so that now rates are similar throughout the state or in comparison to national levels (49, 62, 102, 109). There is

continuing controversy as to whether the mortality risk for a given birth weight is modified by altitude. Recent Colorado or USA data indicate a slightly lower birth weight specific mortality risk at high than low altitude (102, 109). This may, in turn, be due to the more chronic nature of the altitude-related reduction in birth weight or to evidence supporting a greater utilization of specialized health care services by the higher-altitude women (102). The lack of a vital statistics system or gestational age data in South America, Tibet, or Ladkakh prevents assessing birth weight specific mortality risk in these regions. Nonetheless, low birth weight babies have higher mortality than do their normal weight counterparts -- not only during infancy but possibly at later ages as well (4) -- suggesting that it is not good to be "small" at high (or any) altitude.

Maternal oxygen transport adjustments to pregnancy. Pregnancy alters all the components of the maternal O_2 transport system; of these, greater uteroplacental blood flow is the most important. Arterial O_2 tension rises, due to an increase in ventilation that, in turn, reflects the central as well as peripheral, stimulatory effects of progesterone and estrogen hormones and elevated metabolic rate (68). But at low altitude, this ventilation rise does not normally influence arterial O_2 saturation since values are already nearly maximal. Blood volume expands, with the rise in plasma volume exceeding that of red cell mass, such that both hemoglobin concentration and arterial O_2 content decline. Thus increased uteroplacental O_2 delivery depends entirely on greater uteroplacental blood flow. A rise in uteroplacental blood flow is accomplished by uteroplacental as well as non-uteroplacental, systemic means. There is an early fall in systemic vascular resistance (SVR) that, together with the increase in blood volume, raises resting cardiac output some 40% (11). The fall in SVR is likely due to vasodilatory effects of increased NO production, decreased sympathetic tone, and a fall in the potent vasoconstrictor endothelin-1 (ET-1) (27, 53).

In the uterine circulation, an anastomosis develops between the ovarian branch and the main uterine artery (UA), providing the uterus with dual, bilateral arterial blood supply. Because the fall in uteroplacental vascular resistance is greater than that which occurs in the rest of the systemic circulation, a large fraction of the increased cardiac output is directed to the uteroplacental vascular bed. Most (~80%) goes through the two UA, raising unilateral flow from ~10 ml/min in the non-pregnant state to ~350 ml/min near term (82). This impressive rise in UA blood flow is due both to a doubling of intraluminal diameter (which quadruples cross-sectional area) and to increased blood flow velocity. UA vasodilation, greater distensibility as well as growth in all layers of the vessel wall are responsible for the increase in UA diameter (13). Similar changes likely occur in the mesometrial and basilar arteries, but the mechanisms by which these changes interact with the trophoblast invasion occurring downstream (at the level of the spiral arterioles) are not well understood.

An important distinction arises between species with hemochorial *vs.* epithelioral placentas (*i.e.,* humans, most other primates, rodents, and guinea pigs *vs.* sheep, dogs, cows, etc) in terms of the vessels constituting the primary site of uteroplacental vasculature resistance. In hemochorial species, trophoblasts invade approximately one-third of the way through the uterine wall, whereas trophoblast invasion is only one cell layer thick in epitheliochorial species. As a result, in hemochorial species the vessels determining uteroplacental vascular resistance reside largely outside the uterus; more precisely, 2/3^{rds} of uteroplacental vasculature resistance is located in the mesometrial, main UA and ovarian arteries with only 1/3rd being located in uteroplacental channels (63, 96). This is the opposite of that which occurs in species with epitheliochorial placentas where the uteroplacental vessels

comprise the major site of vascular resistance. Our studies have focused on the UA since it makes a demonstrable contribution to uteroplacental vascular resistance in the hemochorial species under study and can be visualized in humans.

Mechanisms by which chronic hypoxia may alter uteroplacental oxygen delivery. While in principle, the lower birth weights at high altitudes could be due to alterations in maternal and/or placental characteristics; we suspect maternal characteristics for several reasons. First, in a limited number of women in which we were able to make measurements of both UA diameter and blood flow velocity, UA volumetric blood flows were approximately one-third lower in near term women residing at high (3100 m) *vs*. low (1600 m) altitude in Colorado (112). Second, we had previously noted that not only were birth weights lower but also the maternal complication of preeclampsia was more common at high altitude. This was an accidental outgrowth of noting a higher than expected occurrence of preeclampsia in the high-altitude residents being studied physiologically. When we compared our sample to all women delivering during the study period, we found a surprisingly high incidence of preeclampsia in the population at large (67). Later, in a more systematic study, we confirmed the increased incidence of preeclampsia at high altitudes (81). Subsequent studies have replicated this finding elsewhere (39, 54). Third, placental characteristics have not, to date, been shown to be markedly different at high *vs*. low altitude, although generalized thinning has been reported (58). TissotvanPatot *et al.,* found a smaller proportion of maternal vessels had undergone trophoblast invasion and converted to a low-resistance circuit. But a greater absolute number of vessels meant that the number of "converted" vessels was the same at low and high altitude (101). Further studies are greatly needed to indicate whether or not metabolic processes are altered in potentially significant ways in high *vs*. low altitude placentas.

At high altitude, the pregnancy-associated increase in ventilation raises arterial O_2 saturation. Near-term O_2 content still falls due to greater plasma volume than red cell mass expansion (69) (61). While the extent to which the rise in ventilation is able to defend O_2 content relates positively to infant birth weight (69), heavier birth weights in long term high-altitude natives are not due to differences in arterial oxygenation since Tibetans and Andeans do not have higher arterial O_2 content than their Han or European counterparts (71). Thus, UA blood flow is likely to be the key determinant of uteroplacental O_2 delivery at high as well as at low altitude.

Non-uteroplacental as well as uteroplacental factors may be responsible for lowering uteroplacental O_2 delivery at high altitude. Cardiac output is lower, probably as the result of lower blood volume and/or higher SVR (40). The higher SVR may be due, in turn, to increased vasoconstrictor and/or reduced vasodilator production. Acute (hours) and more chronic high-altitude exposure raise sympathetic nervous system activity and catecholamine levels in non-pregnant women (60). ET-1 levels are also elevated by chronic hypoxia (72) and in preeclampsia (20). Underscoring the potential importance of ET-1, ET-1 A receptor blockade prevents the IUGR as well as the accompanying reduction in uteroplacental blood flow that is associated with hypoxia as well as NO synthase blockade (99). A third possibility -- reduced systemic NO production -- is not strongly supported by current data (108).

Concerning the uteroplacental circulation, UA blood flow near term is one-third lower in Colorado residents of high (3100 m) *vs.* low (1600 m) altitude, due to less pregnancy-associated increase in UA diameter (111). UA diameters and volumetric flows are also lower

in Han residents of high- *vs.* low altitude (12) and in our preliminary studies of European *vs.* Andean residents of high altitude (70). Our data, like those of Krampl and co-workers (42), do not indicate that the lower UA blood flows are due to higher indices of uteroplacental vascular resistance, suggesting that the factors limiting uteroplacental blood flow reside in the uterine, not primarily the placental, vessels.

Our experimental animal studies demonstrate that chronic hypoxia opposes the normal pregnancy-associated changes in the UA. During normoxic pregnancy, the UA demonsterates an increased vasodilator response to flow; this enhanced flow vasodilation is absent in UA from chronically hypoxia animals (57). Similar findings are reported in preeclampsia, where a failure of mesometrial artery flow vasodilation also occurs (44). Chronic hypoxia also inhibits UA growth such that there is only half as much rise in DNA synthesis in vessels isolated from chronically-hypoxic *vs*. normoxic animals (88). 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 NO synthase protein (NOS III) in whole vessel homogenates (107). NOS III is also known to be important for the hypertrophic outward vascular growth characteristic of pregnancy (74). Unknown is whether hypoxia also affects the pregnancy-associated alterations in other vasodilators (*e.g.,* endothelial-derived hyperpolarizing factor), growth factors (*e.g.,* VEGF, PGF) and vasoactive factors (*e.g.,* ET-1, catecholamines), although our preliminary data and limited reports in non-pregnant persons (56) suggest that it does. In contrast, in sheep which did *not* exhibit altitude-associated reductions in fetal growth or UA blood flow (40) chronic hypoxia prompts an *exaggerated* increase in vasodilator response to acetylcholine and a greater rise in NO production, NOS III protein and message than seen during pregnancy at low altitude (113). Likewise, whereas chronic hypoxia did not alter the pregnancy-associated fall in UA vasoconstrictor response to phenylephrine in the guinea pig, this was augmented in sheep UA as a result of decreased alpha1-adrenergic receptor density, binding affinity and inositol phosphate 3 production (35). Such species variation in the effects of chronic hypoxia supports an important role for genetic mechanisms in the regulation of UA vascular response to pregnancy.

THE ROLE OF THE HYPOXIA-INDUCIBLE FACTOR (HIF) SYSTEM

HIF and O_2 homeostasis. HIF plays a central role in O_2 homeostasis, regulating over 70% of hypoxia-responsive genes. Thus HIF-regulated pathways are logical targets on which selection for traits influencing susceptibility to hypoxia-related disorders could be expected to act. The molecular mechanisms by which such regulation is achieved have been the subject of intense investigation (Figure 3). HIF is a highly-conserved heterodimer consisting of class one molecules -- the constitutive HIF1beta/ARNT complex -- and one of three class two molecules -- HIF 1, 2 or 3alpha. Despite continual production, degradation is sufficiently rapid that the HIFalpha proteins are virtually undetectable in normoxia. This degradation requires trans-4-hydroxylation at proline-564 and -402, recognition and binding by the VHL protein, ubiquination by a E3 ubiquitin ligase complex (consisting elongin C/elongin B, cullin 2, and the RING-H2 finger protein Rbx-1), and transport to the

proteasome (89). But under hypoxia and selected other circumstances (*e.g.,* specific oncogenes, PHD enzyme inhibition, 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 HRE 5'ACGTG(C/G)3' (93).

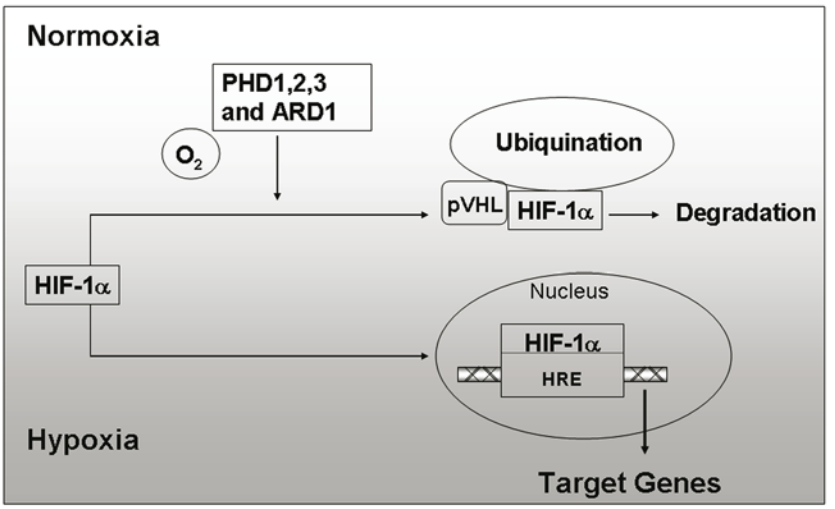

Figure 3. HIF-1 alpha regulation (schema courtesy of Kurt Stenmark).

Mechanisms and evidence of action during pregnancy. Over 40 HIF-regulated or regulatory genes have been identified whose functions influence the vascular adjustments to hypoxia and pregnancy (85; Table 1). Moreover, variation occurs in these genes and such variation alters circulating plasma levels (86). Together with previous reports demonstrating that susceptibility to preeclampsia as well as IUGR is heritable (2, 36, 50, 51, 90, 104), there is a powerful rationale for screening these genes in an effort to find those responsible for the population variation in hypoxia-associated IUGR. Additional, direct support for the involvement of HIF-regulated genes comes from recent studies showing that trophoblast cells from preeclamptic placentas over express HIF-1 and 2 alpha (10, 84). Their transcriptional targets such as ET-1 and VEGF are also likely to be altered, since both have been implicated in the characteristic vasoconstriction, endothelial damage and reduced uteroplacental blood flow of preeclampsia (18, 20, 46, 59, 95, 97-100). Vascular growth and remodeling are also likely affected; hypoxia, both in vitro and in vivo, alters the production of cytokines and other growth-related factors required for trophoblasts to transition to the invasive phenotype and remodel maternal spiral arterioles (22, 23, 101).

A genetic and genomic strategy for identifying variation in HIF-targeted or regulatory genes. As noted above, long-term (multigenerational) residents are relatively protected from the altitude-associated increase in IUGR and such protection appears due to ancestry-dependent, perhaps genetic, factors that permit normal maternal vascular adaptation to pregnancy. Thus, high-altitude populations provide a natural laboratory for determining whether genetic factors influence susceptibility to IUGR and possibly other conditions

characterized by uteroplacental ischemia and, if so, the pathways or mechanisms through which such genes act.

Logical next steps involve a search for variation in HIF-regulatory and -regulated genes, and linking such variation to the maternal vascular abnormalities in IUGR and preeclampsia. Our concept here is that genetic variation in these systems exists in all populations and may explain, in part, susceptibility to preeclampsia and IUGR. High-altitude residents are expected to have been acted upon by natural selection across many generations and thus to preferentially express those genetic variants conferring resistance.

The availability of single nucleotide polymorphisms (SNPs) from the Human Genome Project provides a crucial methodological tool. SNPs permit evaluating the contribution of multiple genes to disease susceptibility in conjunction with varying environmental exposures. SNPs have been described in the HIF-regulated genes listed in Table 1, including proteins of known functional significance during pregnancy such as ET-1, VEGF, tyrosine hydroxylase (the rate-limiting step in catecholamine synthesis) and glucose transporters (31, 32, 34, 76). SNPs also occur in HIF-regulatory genes. This is exemplified by VHL, a tumor suppressor gene, which contains SNPs in coding regions that influence whether the person develops phaeochromocytoma, hemanigoblastoma, or renal cell carcinoma (14). Such SNPs are functional insofar as when VHL is mutated at specific amino acids and expressed in mammalian cells, it can no longer interact with HIF1alpha and send it to the proteasome for degradation.

Genomic approaches have been infrequently applied to pregnancy complications but offer considerable power for identifying genetic involvement in complex diseases. Thus they are increasingly being used in studies of human genetic variation to provide information unavailable from other approaches (5, 91). Such genomic approaches stem from the recognition that since geographic separation of human populations is quite recent[4], most genetic variation is shared among all populations (48). Thus, we expect low levels of genetic differentiation across most genes but, given our overall hypothesis, greater divergence in the Andean population in those genes that are HIF-regulated or -regulatory. Such differences in divergence are readily detectable using statistically sophisticated methodologies as further described by Shriver et al. in chapter 9 of this volume.

SUMMARY AND CONCLUSION

The reduced O_2 availability of residence at high altitude restricts fetal growth and increases the frequency of preeclampsia, making high-altitude residents the single largest group at risk for these disorders of pregnancy/fetal life. The altitude-related increase appears due, in part, to alterations in maternal vascular reactivity, growth and remodeling that reduce uterine artery blood flow. Moreover previous studies demonstrate that multigenerational compared with shorter-term high-altitude residents are protected from the altitude-associated increase in IUGR, probably as the result of greater UA blood flow. Based on recent evidence demonstrating that hypoxia-inducible transcription factors (HIFs) play a central role in regulating O_2-sensitive genes, are implicated in pregnancy disorders, and our preliminary evidence that they are differentially regulated in long- *vs.* short-term populations, future studies will test the overall hypothesis that genetic variants in HIF-targeted or regulatory pathways protect multigenerational high-altitude residents from hypoxia-as-

sociated IUGR. Strategies that couple genetic and genomic approaches with more traditional physiological tools may permit the identification of genes influencing physiological responses to pregnancy and can be productively employed for investigating other health effects of high altitude as well. Such studies are relevant not only for the 140 million high-altitude residents worldwide, including more than 100,000 in Colorado, but also the larger number of persons whose health is compromised by hypoxia.

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[1] While the placenta has been subject to intense investigation, surprisingly little attention has been devoted to the comparative physiology of the placenta or its potential influences on mammalian evolution; topics worthy of future exploration.

[2] The ongoing debate concerning abortion demonstrates that the definition of "life" is subject to cultural, as well as biological interpretations.

[3] Birth weights