Maternal oxygen delivery is not related to altitude- and ancestry-associated differences in human fetal growth

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Fetal growth is reduced at high altitude, but the decrease is less among long-resident populations. We hypothesized that greater maternal uteroplacental O_2 delivery would explain increased fetal growth in Andean natives *versus* European migrants to high altitude. O_2 delivery was measured with ultrasound, Doppler and haematological techniques. Participants ($n = 180$) were pregnant women of self-professed European or Andean ancestry living at 3600 m or 400 m in Bolivia. Ancestry was quantified using ancestry-informative single nucleotide polymorphims. The altitude-associated decrement in birth weight was 418 g in European *versus* 236 g in Andean women ($P < 0.005$). Altitude was associated with decreased uterine artery diameter, volumetric blood flow and O_2 delivery regardless of ancestry. But the hypothesis was rejected as O_2 delivery was similar between ancestry groups at their respective altitudes of residence. Instead, Andean neonates were larger and heavier per unit of O_2 delivery, regardless of altitude ($P < 0.001$). European admixture among Andeans was negatively correlated with birth weight at both altitudes ($P < 0.01$), but admixture was not related to any of the O_2 transport variables. Genetically mediated differences in maternal O_2 delivery are thus unlikely to explain the Andean advantage in fetal growth. Of the other independent variables, only placental weight and gestational age explained significant variation in birth weight. Thus greater placental efficiency in O_2 and nutrient transport, and/or greater fetal efficiency in substrate utilization may contribute to ancestry- and altitude-related differences in fetal growth. Uterine artery O_2 delivery in these pregnancies was 99 ± 3 ml min^{-1}, ∼5-fold greater than near-term fetal O_2 consumption. Deficits in maternal O_2 transport in third trimester normal pregnancy are unlikely to be causally associated with variation in fetal growth.

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The factors determining the trajectory of fetal growth and the reasons for failure to achieve growth potential are major problems in perinatology. O_2 is a critical regulator of placental and fetal development (Genbacev *et al.* 1997; Lampl, 2005), and fetoplacental deficits in O_2 are commonly hypothesized as a causal agent in reduced fetal growth (intrauterine growth restriction, IUGR) and preeclampsia (Kingdom *et al.* 2000; Zamudio, 2003; Burton & Jauniaux, 2004; Soleymanlou *et al.* 2005).

Reduced fetal growth is well documented under the conditions of chronic hypoxia at high altitude in human and animal models (Yip, 1987; Han, 1993; Moore *et al.* 1998). Altitude decreases birth weight more than any other demographic or behavioural attribute (e.g. smoking) in term births; the effect is independent of socioeconomic status (Jensen & Moore, 1997; Giussani *et al.* 2001). High altitude thus provides a natural experiment to test the relationship between O_2 delivery and variation in fetal growth. We have previously shown resistance to altitude-associated IUGR in ethnic groups with a longer evolutionary history of high altitude residence (Zamudio *et al.* 1993*a*; Moore *et al.* 2001).

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A distinct disadvantage to the prior studies was that ethnic affiliation was self-stated and corroborated with language, family history and other socially determined factors. The difficulties inherent in this approach have been thoroughly reviewed (Brutsaert, 2001). A breakthrough in our ability to assess the impact of ancestral origins on outcomes of interest is the use of ancestry-informative markers (AIMs), genome-wide, single nucleotide polymorphisms (SNPs) that vary considerably between sub-Saharan Africa, Asia, Europe and the New World (Shriver *et al.* 2003; Shriver *et al.* 2005). Genetically confirmed geographical ancestry permits separation of environmental from ancestry effects in normal physiology (Brutsaert *et al.* 2003, 2005) and in factors that contribute to ethnic variation in chronic disease (Fernandez *et al.* 2003). It is particularly useful for the separation of genetic from developmental effects (Brutsaert *et al.* 2004), the latter being defined as variation due to differences in environmental exposures during growth and development. The relative importance of genetic, developmental and environmental causes for altitude-associated IUGR and the nature of their interaction remain to be elucidated; the data presented here represent a first effort towards this goal.

We have previously shown that maternal uterine blood flow and O_2 delivery are reduced by 33% at 3100 *versus* 1600 m in North America (Zamudio *et al.* 1995), but in only 5 of 23 high-altitude subjects was the critical measure required for estimating volumetric blood flow and O_2 delivery (uterine artery diameter) obtained. Even this 33% reduction may not have directly impacted fetal growth since it is well-known that maternal and fetal blood flow can decrease by as much as 50% without negative consequences for fetal growth (reviewed in Carter, 1989). Reduced O_2 delivery can be accommodated by increased fetal haemoglobin concentration and O_2 extraction as well as increased placental vascularity and diffusing capacity (reviewed in Carter, 1989; Zamudio, 2003). It is a puzzle therefore why such mechanisms do not preserve fetal growth in humans at high altitude, while doing so in some animal models (Kitanaka *et al.* 1989; Penninga & Longo, 1998).

We tested the hypothesis that women of at least three generations of Andean *versus* sea level ancestry, living at high altitude would have increased maternal O_2 delivery to the fetoplacental unit, correlating with the preservation of fetal growth. We therefore compared maternal uterine arterial O_2 delivery in women of Andean *versus* sea level ancestry, living at high (3600 m) *versus* low altitude (400 m). We quantified participants' genetic ancestry using 133 AIMs, to identify the geographical region(s) of genetic origin for each participant.

Methods

Research design, subjects and sites

A cross-sectional, prospective study design was used to evaluate ancestry, altitude and their interaction on uterine blood flow, O_2 delivery and pregnancy outcome. The Andean group was composed of women native to $\geq$ 3600 m altitude ($n = 43$) and similar Andean women who were born at high altitude but who migrated to and lived at 400 m altitude prior to and throughout the index pregnancy ($n = 46$). The European group was composed of women of sea level ancestry with no known Andean ancestors living at 400 m ($n = 44$) and similar women residing at 3600 m ($n = 37$). All participants gave written, informed consent to the protocols, which were approved by the collaborating Bolivian institution (Instituto Boliviano de Biología de Altura, Consejo Tecnico), the Bolivian National Bioethics Committee and the Institutional Review Board of the New Jersey Medical School. The study conformed to the *Declaration of Helsinki*. Inclusion criteria were good health (absence of chronic conditions that predispose to preeclampsia, e.g. hypertension, renal disease, obesity); birth places and ethnicity as either Andean or European; and conception, gestation and delivery at the altitude of study. Women were excluded for drug, alcohol or tobacco use, complications of pregnancy (gestational diabetes mellitus, pregnancy-induced hypertension, preeclampsia, intrauterine growth restriction, preterm labour or rupture of the membranes), or a positive oral glucose tolerance test.

Study sites were the Instituto Boliviano de Biología de Altura of the Universidad de San Andreas Mayor and the Hospital Materno-Infantil in La Paz, Bolivia (3600 m), the Centro Nacional de Enfermedades Tropicales, the Hospital Hernandez Vera Primer de Mayo, the Caja Petrolera de Salud and the Clinica Sirani in Santa Cruz, Bolivia (400 m).

Ancestry/AIMs

Women who indicated Asian or African ancestors or with partners whose ancestry was discordant with their own, e.g. father of sea-level origin, mother of Andean origin, were excluded. The inclusion criteria for assignment to the Andean or European groups had three layers, interview, questionnaire and assessment of genetic geographical ancestry using AIMs. Included in the Andean group were women who identified themselves and their partners as being Aymara or Quechua. These are the only two Native American groups indigenous to the highlands of Peru and Bolivia. They further indicated fluency in their indigenous language and, via questionnaire, their own and their partners' surnames, birthplaces and those of their parents and grandparents. Andean surnames differ considerably

from those of Spanish and other migrants to South America and have been shown previously to correlate with genetic data (Chakraborty *et al.* 1989). Participants who claimed Andean ethnic affiliation, language and three generations of Andean ancestry with no known European or lowland ancestors were included. The AIMs analysis was the final arbiter: Andeans had to be at least 60% Native American to be included. For Europeans, stated ethnic affiliation had to be consistent with European ancestry, i.e. women who claimed tribal affiliation with any Native American group were excluded. They further had no knowledge of an indigenous Native American language, indicated no known highlands ancestors, and could enumerate three generations of their own and their partner's parents' and grandparents' names and origins. Again AIMs were the final arbiter. Women were excluded if their percentage Native American admixture was greater than 50%. The reason for the latter criterion is that admixture with Native American populations by Hispanic populations throughout the New World is ubiquitous (Merriwether *et al.* 1997; Bonilla *et al.* 2004).

Fetal DNA was used for determination of geographical ancestry for several reasons. It eliminates problems related to verification of paternity and averages varying degrees of admixture in the parents. The placenta, tissue of fetal origin, is an arbiter of maternal physiology during pregnancy (Haig, 1993), and modulates maternal metabolism to increase nutrient availability for the fetus (Evain-Brion, 1999). Fetal DNA represents the genetic triad of mother, father and baby. DNA was isolated from cord blood or core villous placental tissue samples using the Qiagen Midi DNA isolation kit (Qiagen, Chatsworth, CA, USA). The AIMs analyses were performed by DNAPrint^{TM} (Genomics, Inc., Sarasota, FL, USA, www.dnaprint.com) using ADMIXMAP technology, a high throughput technique for screening the polymorphisms uniquely linked to the geographical regions referenced above (Shriver *et al.* 2005). SNPs are scored by a melting-curve assay in which the target sequence containing the SNP is amplified by PCR using a mismatched primer to create an artificial restriction site polymorphism. PCR products are digested with a restriction enzyme and the resulting restriction fragment length polymorphisms are scored by their melting curves following the methods originally outlined by Akey *et al.* (2001). The European group included 39 women who were born and raised in Europe, North America, Argentina, Chile, Mexico and Brazil. The remainder were born and raised at low altitude in Bolivia. The subset born outside of Bolivia did not differ from those born within Bolivia in the dependent variables presented here; the group is hereafter termed European both for convenience and because the AIMs data indicated geographical ancestry primarily derived from the European continent. No genetic data can resolve origins at the level of tribe. Therefore the Native American genetic admixture we observed in our European participants is hereafter referred to as 'other Native American', meaning non-Andean ancestry. The sample sizes we report here reflect the study population accepted for analysis after the AIMs admixture estimates.

Protocol

Prior to delivery (4 ± 1 day, range, 0–10), a medical/obstetric history, health (blood pressure, oxygen saturation, urinalysis) and ancestry screens were obtained. One millilitre of 'arterialized' (warmed hand vein) blood was obtained for measurement of blood gases, haemoglobin and haematocrit. Uterine and external iliac artery diameters and Doppler blood flows were assessed with minor modification from the protocol we have previously published (Palmer *et al.* 1992), which has been utilized by others in studies of fetal growth (Konje *et al.* 2003). At delivery samples of umbilical cord venous blood and of the placenta were taken for isolation of DNA.

Ultrasound

The ultrasound systems used were an ATL HDI model 3000 (3600 m) and 5000 (400 m) (Global Medical Imaging, Charlotte, NC, USA) for all studies. The uterine arteries were measured at the point of crossover with the external iliac artery. Our prior studies indicated that the diameters of pelvic arteries other than the uterine (common iliac, external iliac) do not change with pregnancy (Palmer *et al.* 1992). These same studies showed that flow increases progressively in the common iliac artery due to a rise in cardiac output and flow velocity, while flow decreases in the external iliac artery due to reduced flow velocity, indicating redistribution of lower extremity blood flow to favour the pregnant uterus (Palmer *et al.* 1992). Because our prior studies showed no change in serial measures of common and external iliac artery diameter during pregnancy, we examined these arteries in an effort to control for the possibility that group differences in uterine artery diameters might be due to larger or smaller average body sizes, rather than altitude or ancestry, the independent variables of interest. However, in these term pregnancies the shift in fetal position along with the enlargement of the uterus pushes the common iliac arteries deep into the pelvis, which yields unacceptably high angles of insonation (> 50 deg in $> 60\%$ of studies) and attenuated Doppler signals that underestimate flow. The values obtained, which should be roughly equivalent to the sum of uterine artery and external iliac blood flows (Palmer *et al.* 1992), were too low to be considered reliable. This was not surprising as in our prior studies, $\sim$30% of the common iliac artery studies at week 36 were excluded from analysis due to low quality (i.e. attenuated signal and high angle

of insonation). These data were thus eliminated from analysis.

External iliac and uterine arterial diameters were measured in duplicate both with and without colour, and in triplicate if values differed by ≥ 0.03 cm. The external iliac artery diameters were larger in systole *versus* diastole by $9 \pm 6\%$ and hence the equation (2 × systolic diameter + diastolic diameter)/3 was used to calculate an average diameter. Repeat measures of uterine artery diameter had a coefficient of variation (CV) of $4 \pm 1\%$ using colour-flow, $5 \pm 1\%$ without colour flow; the CV was less than 4% for the external iliac arteries both with and without colour flow. In a subset of studies ($n = 40$), uterine arterial diameters were measured up to five times (CV 2%) and with two different probe placements (at the beginning and at the end of the study, ∼45 min apart) yielding a CV of 6%. In the 42% of UA studies in which diameter could be examined in systole and diastole, there was no difference in diameter at peak systole *versus* diastole ($3 \pm 1\%$). At the same location where diameter was measured, the Doppler waveforms for time-averaged blood mean flow velocity (MFV, cm s^{-1}) within a given cardiac cycle were obtained using a 7 MHz probe. MFV was recorded from ≥ 6 cardiac cycles (mean 8 ± 1, range 6–16). Again, the CV between cardiac cycles was low (3%) and between different probe placements separated by ∼45 min of time (7%). Volumetric flow (ml min^{-1}) was calculated as the cross-sectional area of the blood vessel multiplied by the MFV × 60. Studies at 3600 m were completed by a single obstetric radiologist (LP), while two obstetrician-sonographers completed all studies at 400 m (C.R., G.H.). Studies were video-taped and a subset of 10 per group (total 40) obtained on the ATL 5000 were re-examined with an ATL 3000 to derive measures of variation between sites. The coefficient of variation (CV) for diameter was $2 \pm 1\%$ between the two machines. A Doppler angle of 40 deg or less (Palmer *et al.* 1992; Bernstein *et al.* 2002) was achieved in 90% of the uterine artery studies. Bilateral uterine artery studies were successfully completed in all but five patients (3 at high altitude, 2 at low altitude); bilateral external iliac studies were obtained in all patients. Overall the angle attained for the uterine artery was 24 ± 3 deg and for the external iliac 26 ± 2 deg. Values with and without colour did not differ and hence all measures were averaged for each participant. The CVs did not differ between altitudes or ancestry groups and are consistent with prior reports (Palmer *et al.* 1992; Bernstein *et al.* 2002; Konje *et al.* 2003).

O_2 delivery

The warmed hand vein technique was used to collect arterialized venous blood from the mother for measurement of pH and arterial blood gases. The mother's hand was wrapped in a heating pad and allowed to warm for a minimum of 30 min at 50°C. This technique underestimates saturation relative to arterial blood by 3% (Liu *et al.* 1992), while pH, P_{CO_2} and various metabolites show correlations of 0.97–0.99 (McLoughlin *et al.* 1992). P_{O_2} has a more variable relationship, with relatively low correlation (0.61; McLoughlin *et al.* 1992). We measured S_{a,O_2} both by pulse-oximetry and blood gases. Values were $3 \pm 2\%$ lower at 400 m and $3 \pm 4\%$ lower at 3600 m when comparing blood gas to pulse-oximeter derived values. Since S_{a,O_2}, not P_{O_2}, is used for calculation of O_2 delivery, the variation in P_{O_2} using the warmed hand vein technique is not relevant to the data reported in this paper. While less optimal than arterial blood gases, arterialized blood studies are safe, relatively painless, and ethically permitted in healthy pregnant women. They have been used in studies of pregnant women at high altitude before, correlating directly with measures of angiogenesis in high altitude placentae (Espinoza *et al.* 2001).

Arterialized blood was collected into heparinized blood gas syringes, which were sealed and immediately placed on ice. Blood gases were measured within 1 h of collection. Blood gases were measured using a Radiometer ABL 5000 (Copenhagen, Denmark) at high altitude and an Eschweiler ECOSYS II (Kiel, Germany) at low altitude; the machines were calibrated prior to every study using the manufacturers' standard calibration solutions. Analyses were repeated where duplicate values for pH varied by > 0.02 or P_{CO_2} by > 2 mmHg. The CV in duplicate measures did not exceed 1.7% for any blood gas parameter. Standard base excess was calculated using a modified Henderson equation (Schlichtig *et al.* 1998). Haemglobin (hgb) was measured using a Radiometer OSM 3 at 3600 m, and by the cyanomethemoglobin technique at 300 m. Hematocrit (hct) was measured in both locations by the microhematocrit technique. Haemoglobin studies were repeated if the duplicate measures varied by more than 0.3 g of haemoglobin and haematocrit if duplicate measures varied by more than 2%. Maternal arterial O_2 content (ml l^{-1}) was calculated as: ((hgb × 10) × 1.34)) × S_{aO_2}. Uterine arterial O_2 delivery was calculated as O_2 content (ml O_2 l^{-1}) × volumetric blood flow (l min^{-1}). Volumetric blood flow and O_2 delivery were examined for each uterine artery (left and right), summed for the left and right arteries within each participant, and are presented as bilateral flow and O_2 delivery.

Statistics

All continuous data presented in this report were normally distributed as indicated by the Kolmogarov–Smirnoff test. Data were analysed using SAS (version 8.0). Student's *t* test and regression models were used for analyses of birth weight and body size in the infants. Birth weight

Table 1. Maternal and infant characteristics

	European ancestry		Andean ancestry		*P*-values		
	400 m	3600 m	400 m	3600 m	Altitude	Ancestry	Interaction
Maternal characteristics							
n	44	37	46	43			
Gestational age at time of blood flow measurement	37.8 ± 0.2	37.8 ± 0.2	38.3 ± 0.2	38.0 ± 0.2	NS	NS	NS
Age (years)	27 ± 1	29 ± 1	28 ± 1	33 ± 1	< 0.001	< 0.01	NS
Parity	1.0 ± 0.2	1.0 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	NS	< 0.01	NS
Height (cm)	161 ± 1	162 ± 1	156 ± 1	152 ± 1	NS	< 0.0001	NS
Non-pregnant weight (kg)	62 ± 2	61 ± 1	59 ± 2	58 ± 2	NS	NS	NS
Non-pregnant Body Mass Index (kg m^{-2})	24 ± 1	24 ± 1	24 ± 1	25 ± 1	NS	NS	NS
Weight gain with pregnancy (kg)	11 ± 1	13 ± 1	12 ± 1	14 ± 1	< 0.05	NS	NS
Heart rate (BPM)	89 ± 2	91 ± 2	86 ± 2	84 ± 1	NS	NS	NS
Systolic BP (mmHg)	105 ± 1	112 ± 2	104 ± 1	106 ± 1	NS	NS	< 0.05
Diastolic BP (mmHg)	69 ± 1	77 ± 2	69 ± 1	74 ± 1	< 0.0001	NS	NS
Mean Arterial Pressure (mmHg)	93 ± 1	100 ± 2	92 ± 1	95 ± 1	< 0.0001	NS	< 0.05
Infant characteristics							
Birth weight (grams (unadjusted)	3401 ± 63	2993 ± 56	3550 ± 46	3316 ± 59	< 0.0001	< 0.0001	0.11
Birth weight (grams, adjusted)	3414 ± 32	2995 ± 32	3553 ± 21	3317 ± 34	< 0.0001	< 0.0001	< 0.005
Placental weight (g)	473 ± 15	478 ± 20	466 ± 13	497 ± 17	NS	NS	NS
Birth/Placental weight ratio	7.4 ± 0.2	6.5 ± 0.2	7.8 ± 0.2	6.9 ± 0.2	0.005	0.08	NS
Gestational Age (days from LMP)	270 ± 1	270 ± 1	272 ± 1	272 ± 1	NS	NS	NS
Clinically assessed gestational age at birth (weeks)	38.6 ± 0.2	38.4 ± 0.2	38.8 ± 0.1	38.6 ± 0.2	NS	NS	NS
Length at birth (cm)	50.2 ± 0.1	48.3 ± 0.1	50.9 ± 0.1	48.8 ± 0.1	< 0.0001	< 0.0001	NS
Abdominal circumference (cm)	34.0 ± 0.1	33.5 ± 0.1	34.5 ± 0.1	34.6 ± 0.1	NS	< 0.0001	< 0.001
Head circumference (cm)	34.6 ± 0.1	34.1 ± 0.1	34.9 ± 0.1	34.9 ± 0.1	NS	< 0.001	< 0.01
Sex ratio M/F	22/22	11/26	24/22	22/21	NS	NS	NS

was adjusted for maternal age and parity, infant sex and gestational age based upon these analyses. Birth length and abdominal and head circumferences were adjusted for differences in gestational age and infant sex. A two-way ANOVA with altitude and ancestry as the independent variables was used for the remaining variables and for comparison of the adjusted data on infant size and weight. Interaction between altitude and ancestry was further investigated using Sheffe's test when the interaction term carried a *P*-value of 0.15 or less. Such contrasts are reported as significant where $P < 0.05$ or as a trend where $P < 0.10$. Chi square tests were used to compare the infant sex ratio between groups. Correlation analyses were used to evaluate relationships between independent and dependent continuous variables. The data in figures and tables are presented as means ± S.E.M. unless otherwise indicated and are reported as significant where $P < 0.05$.

Results

Ancestry

Individuals who identified themselves, their partners, and their parents and grandparents as Andean had genetic ancestry profiles that were 80 ± 1% Native American and 15% ± 2 European. The European sample was 63 ± 2% European and 30 ± 2% (non-Andean) Native American ancestry. Low levels of sub-Saharan African (mean 2–4 ± 1%) and East Asian admixture (mean 2–4 ± 1%) were detected in both ancestry groups. There was no difference between altitudes in the measures of geographical ancestry, i.e. Andean individuals at low *versus* high altitude did not differ in their proportional admixture, nor did those of European ancestry between the low and high altitude sites.

Altitude effects

Note that in both Tables 1 and 2, unless otherwise indicated, a difference by altitude means that there is a difference between altitudes for both ancestry groups, and that the differences are similar in magnitude. Similarly, where a difference is indicated by ancestry, the effect is significant at both altitudes.

Residence at high altitude was associated with increased diastolic pressure in both European and Andean women (Table 1) and an increase in systolic pressure among European women only (Table 1). This resulted in a higher mean arterial pressure (MAP) for both ancestry groups

Table 2. Maternal haematology, blood gases (arterialized venous blood)

	European ancestry		Andean ancestry		*P*-values		
	400 m	3600 m	400 m	3600 m	Altitude	Ancestry	Interaction
Hemoglobin (gm dl^{-1})	12.0 ± 0.2	15.3 ± 0.2	11.1 ± 0.2	14.6 ± 0.2	< 0.0001	< 0.001	NS
Hematocrit (%)	36.7 ± 0.4	42.0 ± 0.6	34.4 ± 0.6	40.7 ± 0.6	< 0.0001	< 0.005	NS
S_{a,O_2} (%)	97.6 ± 0.1	91.4 ± 0.4	97.8 ± 0.1	91.1 ± 0.3	< 0.0001	NS	NS
P_{a,O_2} (mmHg)	90.8 ± 1.7	54.6 ± 1.2	93.7 ± 1.3	53.2 ± 1.7	< 0.0001	NS	NS
P_{a,CO_2} (mmHg)	35.2 ± 0.4	28.0 ± 0.6	35.7 ± 0.4	26.5 ± 0.4	< 0.0001	NS	< 0.05
pH	7.42 ± 0.004	7.44 ± 0.004	7.43 ± 0.004	7.44 ± 0.003	0.001	< 0.05	NS
Bicarbonate	22.3 ± 0.3	19.0 ± 0.4	23.2 ± 0.3	17.8 ± 0.3	0.0001	NS	< 0.005
Standard base excess	−0.6 ± 0.3	−3.2 ± 0.3	0.0 ± 0.2	−3.9 ± 0.2	< 0.0001	NS	< 0.05
Calculated arterial O_2 content (ml l^{-1})	157 ± 2	187 ± 2	146 ± 3	178 ± 3	< 0.0001	< 0.001	NS

at high altitude. Birth weight and length were reduced at 3600 m, regardless of ancestry (Table 1), but placental weight was similar. As expected, maternal P_{aO_2}, S_{aO_2} and P_{aCO_2} were lower at 3600 m, with a compensated respiratory alkalosis resulting in a normal pregnancy blood pH that was slightly greater at high altitude (Table 2). Standard base excess was decreased at high altitude, consistent with a compensated respiratory alkalosis due to prolonged hyperventilation. Maternal arterial O_2 content was increased at 3600 m, due to elevated haemoglobin concentrations (Table 2).

External iliac diameters were unchanged, but mean flow velocity was increased at high altitude (Fig. 1*A* and *B*). In contrast, altitude reduced uterine artery diameter and mean flow velocity (Fig. 1*C* and *D*). While modest differences in the external iliac mean flow velocity did not change volumetric blood flows (Fig. 2*A*), uterine artery volumetric blood flow was markedly diminished at high altitude (Fig. 2*B*, $P < 0.001$). The altitude-associated reduction in uterine blood flow was similar in Europeans and Andeans. For each study participant, the sum of blood flows from both uterine arteries and both external iliac arteries was calculated and used as an index of total lower body blood flow. Total lower body volumetric blood flow was reduced at high altitude in both ancestry groups (Fig. 2*C*). While the reduction in uterine arterial

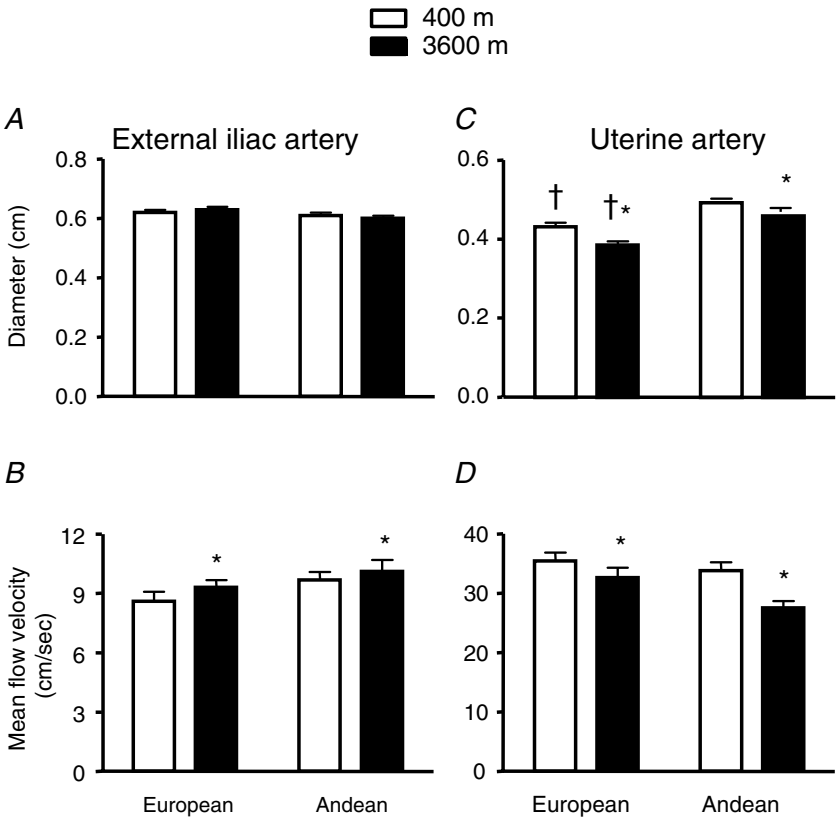

Figure 1. Diameters and mean blood flow velocities from the left and the right arteries
A, external iliac artery diameter was similar at low and high altitude and between European and Andean ancestry groups. *B*, external iliac artery mean flow velocity was increased at high altitude ($P < 0.05$) in both ancestry groups, but did not differ between the ancestry groups. *C*, uterine artery diameter was smaller in women of European ancestry, regardless of altitude (†$P < 0.0001$). Altitude reduced uterine artery diameter in both ancestry groups to a similar degree (*$P < 0.05$, −7% Andean and −11% in Europeans, $P = 0.58$). *D*, there were no differences in the mean flow velocity of blood travelling through the uterine arteries within each altitude. However, mean flow velocity was reduced at high altitude ($P < 0.01$). The reduction was similar in European (−10%) *versus* Andean women (−16%, $P = 0.24$).

volumetric blood flow was substantial (Fig. 2*B*), the fall in O_2 delivery was not as pronounced (Fig. 3*A*), because the higher O_2 content of the blood at high altitude partially compensated for reduced flow. Any differences in O_2 delivery were abolished between altitudes and ancestry groups when O_2 delivery was normalized to birth weight. Oxygen delivery (ml kg^{-1} min^{-1}) was equivalent (Fig. 3*B*) and ~5-fold in excess of the average mammalian near-term fetal O_2 consumption 6 ml kg^{-1} min^{-1} (Carter, 1989).

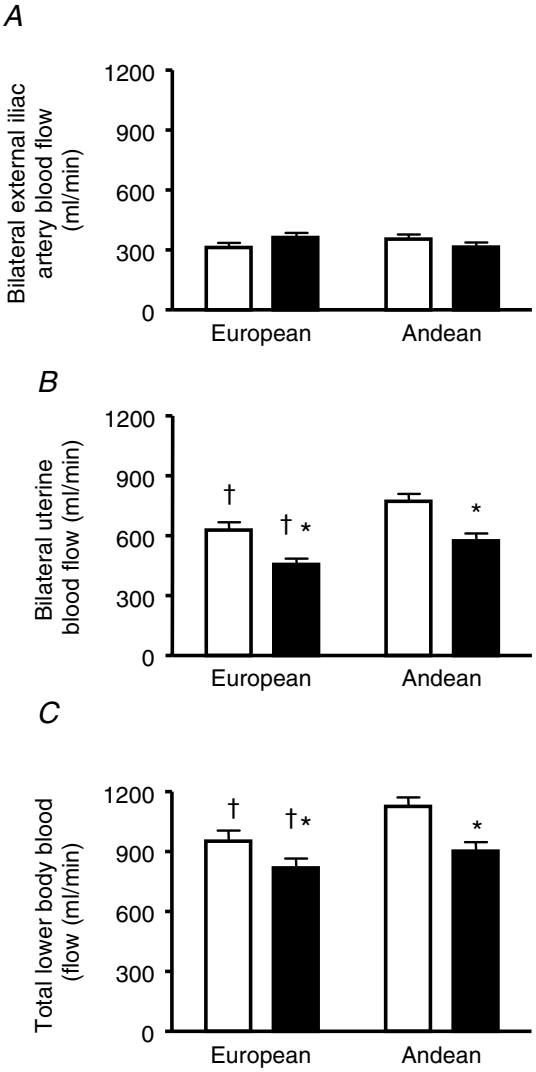

Figure 2. Bilateral arterial blood flows (left and right summed for each individual), and the estimated lower body blood flow (sums of both uterine and both external iliac arteries)
A, bilateral external iliac blood flow did not differ between altitudes or ancestry groups. *B*, bilateral uterine artery blood flow (the sum of the left and right artery within each individual) was lower in European than Andean women, regardless of ancestry (†$P < 0.005$). Bilateral uterine artery blood flow was reduced at high relative to low altitude ($P < 0.0001$) and the magnitude of the decrease was similar in European (−27%) *versus* Andean (26%) women ($P = 0.94$). *C*, total lower body blood flow (the sum of both uterine arteries and both external iliac arteries within each individual) was lower in European than Andean women (*$P < 0.01$). Total lower body blood flow was decreased at high relative to low altitude ($P < 0.001$). The decrease was similar in European (−14%) and Andean women (−20%, $P = 0.45$).

Effects of ancestry

European women were taller, younger in age and of lower parity than Andean women (Table 1), but similar in prepregnant body mass index. Birth weight was greater in Andean neonates, regardless of altitude, whether considered as the raw values or adjusted for differences in maternal age, parity, infant sex and gestational age (Table 1). Adjusted for differences in gestational age and infant sex, European babies had shorter body length, and smaller head and abdominal circumferences relative to Andean neonates (Table 1). European women had higher haemoglobin concentrations, paralleled by an increased haematocrit, and hence greater arterial O_2 content than Andean women (Table 2).

External iliac artery diameters did not differ (Fig. 1*A*), but uterine artery diameters were smaller in Europeans,

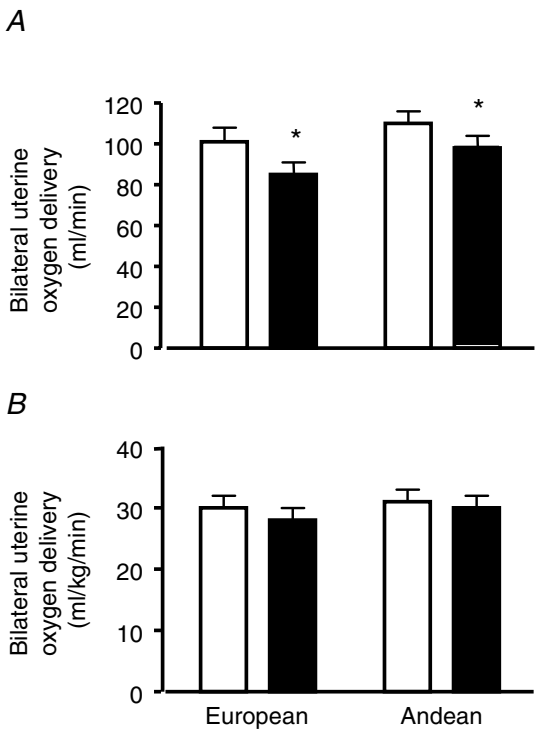

Figure 3. Total uterine arterial oxygen delivery
A, there was no difference in total (bilateral) uterine arterial oxygen delivery between ancestry groups within each altitude. Uterine arterial oxygen delivery was decreased at high altitude (*$P < 0.01$). The magnitude of decrease was similar in both ancestry groups (−16% European, −11% Andean, $P = 0.75$). *B*, when normalized for birth weight (ml kg^{-1} min^{-1}), total oxygen delivery (sum of the left and right uterine arteries for each pregnancy) was the same in all four groups.

regardless of altitude (Fig. 1*C*). As a result, European women had lower uterine artery volumetric blood flows than Andean women at both low and high altitude (Fig. 2*B*). Total lower body blood flow was also decreased in European *versus* Andean women (Fig. 2*C*), regardless of altitude. However, greater O_2 content among European women (Table 2) partially offset their reduced volumetric blood flows, such that within altitudes there was no difference in uterine O_2 delivery between the two ancestry groups (Fig. 3*A*).

Genetic admixture was unrelated to birth weight in Europeans. However as percentage European ancestry increased from 0 to 40% in Andean women (the minimal and maximal values within the group), birth weight was reduced by 48 g for each additional 5% increase in European ancestry at low altitude and by 72 g at high altitude (Fig. 4). The increased impact of European ancestry on Andean women at high altitude is consistent with the 2-fold greater magnitude of altitude-associated IUGR experienced by European women, as detailed below.

Interaction between altitude and ancestry

European women had a greater altitude-associated decrement in birth weight (–419 g) than Andean women (−236 g, $P < 0.005$), when adjusted for variation due to infant sex, gestational age, maternal age and parity. European babies had a reduced head and abdominal circumference at high altitude, whilst Andean babies preserved values similar to their low altitude controls (Table 1). Mean arterial pressure was greater in European than Andean women at 3600 m (Table 1). While both ancestry groups responded to high altitude with hyperventilation (as reflected by their lowered P_{aCO_2}) Andean women had a greater altitude-associated decrement in P_{aCO_2}, bicarbonate and base excess than European migrants to high altitude (Table 2), suggestive of greater hyperventilation.

O_2 delivery and birth outcome

Ancestry and altitude-associated differences in the mean values for blood flow and O_2 delivery corresponded to differences in the mean birth weights observed in the four groups. Blood flow, O_2 delivery and birth weight were thus greatest in Andean women at low altitude, and least in European migrants to high altitude. However, correspondence between group differences in birth weight and in blood flow and O_2 delivery does not necessarily mean that differences in blood flow or O_2 delivery cause the differences in birth weight. We therefore explored in greater detail the possible associations between birth weight and our independent variables. Uterine artery blood flow and O_2 delivery were positively associated with birth weight only among European migrants to high altitude (Table 3). For any given value for O_2 delivery there is a broad and largely overlapping distribution of birth weights for each of the four altitude/ancestry groups, ranging from 2500 to > 4000 g (Fig. 5). Andeans have greater fetal growth for any given level of blood flow or O_2 delivery as illustrated in Fig. 6. This is true for both low and high altitude, as shown in Fig. 6, where the slope, representing all individual data values, is higher in Andean than European women at their respective altitudes of residence. Figure 6 also shows that both Andean and European women at high altitude have lower birth weights for a given value of O_2 delivery than their low altitude controls. However, high-altitude Europeans are differentiated from the other three groups by having

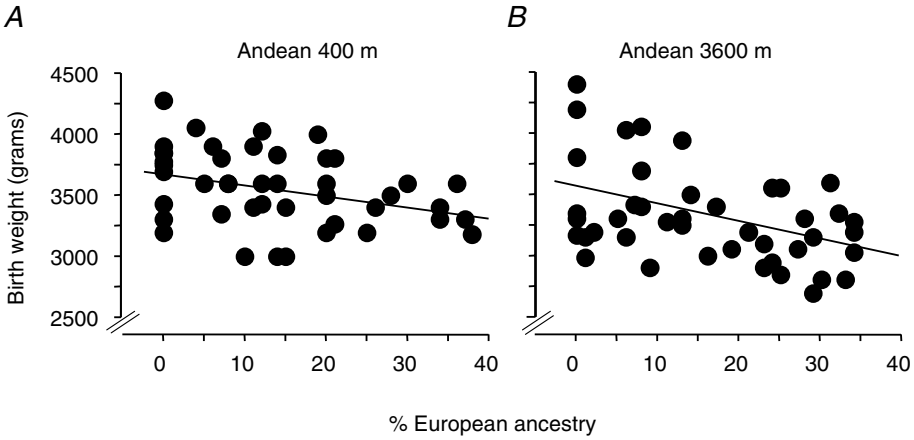

Figure 4. Percentage of European admixture present in Andean women in relation to birth weight at low (*A*) and high altitude (*B*)
The percentage of European admixture present in Andean women was negatively related to birth weight at both low (*A*) and high altitude (*B*). *A*, $y = 3698 - 9.7x$, $r^2 = 0.14$, $P < 0.05$; *B*, $y = 3544 - 14.4x$, $r^2 = 0.19$, $P < 0.01$.

Table 3. Correlation coefficients (r^2) between the independent variables and birth weight

	European ancestry		Andean ancestry	
	400 m	3600 m	400 m	3600 m
Hemoglobin concentration (gm dl^{-1})	0.02	0.01	0.01	0.01
S_{aO_2} (%)	0	0	0	0.02
P_{aO_2} (mmHg)	0	0	0.07	0.06
P_{aCO_2}	0.01	0.01	0.02	0.05
Calculated arterial O_2 content (ml l^{-1})	0	0	0.02	0.02
Bilateral uterine artery blood flow (ml min^{-1})	0	0.19*	0	0.04
Bilateral uterine artery O_2 delivery (ml min^{-1})	0	0.18*	0	0.04
Placental weight	0.30***	0.17*	0.35***	0.35***
Gestational age (days)	0.18**	0.26**	0.19**	0.22**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$.

much lower birth weight for any given value of uterine blood flow or O_2 delivery, even though the ranges for these variables nearly completely overlap (Figs 5 and 6*A* and *B*). At an O_2 delivery of 100 ml min^{-1}, for example, high *versus* low altitude European infants differ by 425 g, while Andean infants differ by 219 g (Fig. 6*B*). These data support that the ancestry-associated discrepancy in fetal growth cannot be caused by differences in O_2 delivery; the birth weight difference is virtually identical to each group's altitude-associated decrement in fetal growth at an identical O_2 delivery.

There was no relationship between birth weight and maternal hgb, P_{aO_2}, S_{aO_2}, or C_{aO_2} within or between altitudes, nor in either ancestry group (Table 3). Genetic admixture was likewise unrelated to any of the physiological variables. All of the maternal physiological variables considered together within a best-fit multiple regression analysis (heart rate, MAP, haemoglobin concentration, P_{aO_2}, S_{aO_2}, P_{aCO_2}, uterine blood blood flow or O_2 delivery) could not explain more than 15% of the variance in birth weight within any group. Inclusion of gestational age and placental weight in the multiple regression for birth weight eliminated any significance of the other physiological variables. Hence the majority of variation in birth weight (37–46%, $P < 0.001$ for each group) was explained by placental weight and gestational age alone.

Discussion

The hypothesis that Andean ancestry would increase maternal O_2 delivery and contribute to preservation of fetal growth at high altitude was not supported. Residence at high altitude decreases uterine artery diameter, blood flow and O_2 delivery, regardless of ancestry; the reduction is similar in Andean natives and European migrants. The surprising finding was that Andean ancestry is associated with greater uterine artery diameters and volumetric blood flow regardless of altitude. Thus differences attributable to ancestry appear to be maintained at high and low altitude, i.e. altitude does not interact with ancestry in terms of O_2 transport. Higher haemoglobin concentrations at low and high altitude, and hence greater arterial O_2 content in European compared with Andean women raises their uterine arterial O_2 delivery, resulting in similar O_2 delivery at their respective altitudes of residence. This similarity does not translate into equal fetal growth as Europeans experiences nearly twice the altitude-associated decrement in birth weight *versus* their Andean counterparts. Increasing levels of European admixture progressively decreased birth weights among

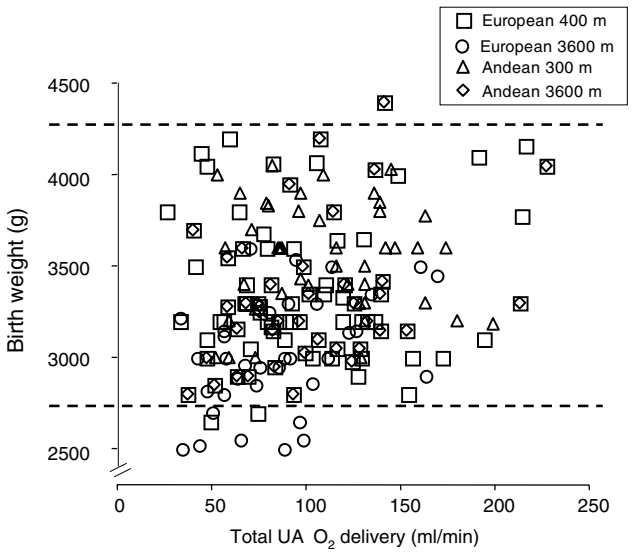

Figure 5. Individual women's bilateral uterine arterial oxygen delivery in relation to the weight of their newborns
This scatter plot of all the individual women's bilateral uterine arterial oxygen delivery in relation to the weight of their newborns shows birth weight can vary by as much as 1500 g (dashed lines) for any given level of oxygen delivery. While the relationship between birth weight and oxygen delivery is significant when all the data are considered, interindividual variation in oxygen delivery explains only 4% of the variation in birth weight ($y = 2.12x + 3120$, $P < 0.01$, $r^2 = 0.04$). This very modest correlation is entirely accounted for by the European migrants to high altitude (see Table 3).

Andean women, but the degree of admixture was unrelated to any of the other physiological variables studied here. We conclude that genetically mediated differences in maternal O_2 delivery are unlikely to explain the Andean advantage in fetal growth at 3600 m. The data support the overall conclusion that decreased maternal O_2 delivery does not cause the progressive reduction in fetal growth observed after 24 weeks of human pregnancy at high altitude (Unger *et al.* 1988; Krampl *et al.* 2000). Rather, they suggest that increased substrate delivery (greater blood flow), placental transport or the fetoplacental utilization of substrate may be more important than maternal O_2 delivery in the preservation of fetal growth at high altitude.

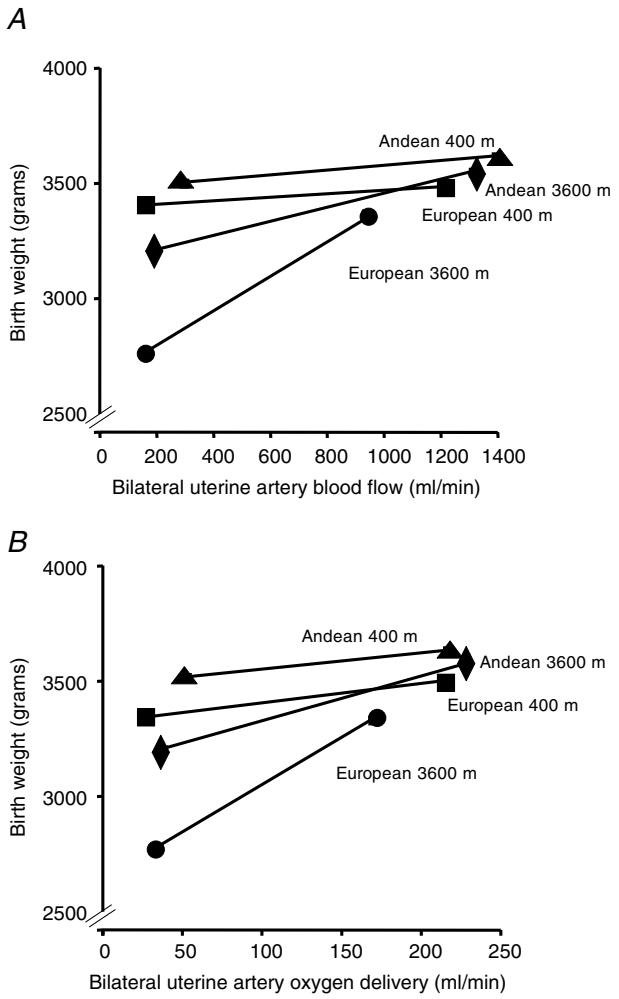

Figure 6. Bilateral uterine blood flow and oxygen delivery in relation to birth weight for each of the four altitude and ancestry groups

A, the regression lines for bilateral uterine blood flow in relation to birth weight for each of the four altitude and ancestry groups. The slopes represent the regression of all data points for each group. Each line is anchored at the beginning and end of the range of data represented on the *x* axis, in order to show the extensive overlap of the values between the 4 groups. Altitude reduces birth weight for any given value of blood flow, with the impact markedly more pronounced in the European than Andean women. Andean women at both 400 m and 3600 m show greater fetal growth for any given level of blood flow relative to the European women at the same altitude. *B*, the same relationship but using oxygen delivery as the independent variable.

Difficulties in interpreting these data relate to the potential for intersite technical variation in measurement of diameters or MFV and the degree of admixture in the European–Hispanic women. We have discussed the validity issues in measurement of blood flow and their resolution elsewhere (Palmer *et al.* 1992). Briefly, multiple methods have yielded results similar to ours, including N_2O equilibration, ^{24}Na radioactive disappearance and other studies using Doppler velocimetry. The mean value of bilateral blood flow reported here for all women residing at 400 m ($n = 88$) is 703 ml min^{-1}, similar to the mean of 725 ml min^{-1} obtained when all published studies on uterine blood flow, regardless of methodology, are averaged (Zamudio, 2007). The decrement in uterine artery diameter and consequently the 27% decrease in uterine artery blood flow at 3600 m ($n = 80$) is similar to our prior report of −33% in five subjects at 3100 m, using similar techniques (Zamudio *et al.* 1995). Agreement across a number of studies and techniques supports that the differences between high and low altitude and between ancestry groups are not technical artifacts.

Our AIMs data represent the highest level of genetic resolution pertaining to admixture that is currently available. In order to minimize the undoubted heterogeneity that genetic studies would demonstrate in populations with a 500 year history of colonialism, we excluded women who had any highland ancestors from the European group in this study. Recent studies of ancient DNA support that modern Quechua and Aymara living in the high-altitude region are descendents of the preconquest inhabitants of the highlands (Martinez-Laso *et al.* 2006; Shinoda *et al.* 2006). While the AIMs data cannot resolve origins at the tribal level, many European participants who had Native American admixture originated outside of Bolivia, supporting our conclusion that Native American admixture among the European women in this study is likely to be of lowland, not Quechua or Aymara origin. Unlike a prior report using a limited number of genes coding for specific proteins (Chakraborty *et al.* 1989), we could find no relationship between surnames and proportional admixture. Future studies should use AIMs as social concerns frequently lead to the abandonment of Native American surnames in favour of Hispanic surnames.

Decreased uterine artery diameter and blood flow, and increased mean arterial pressure in both Europeans and Andeans at 3600 m is consistent with our prior reports in smaller numbers of women living at 3100 m in North America (Zamudio *et al.* 1995; Palmer *et al.* 1999). Near-term plasma volume is reduced at high altitude in North America (Zamudio *et al.* 1993*b*). Greater mean arterial pressure combined with

lower plasma volume (as reflected by the increased haemoglobin concentration in the European women) would yield increased systematic vascular resistance and could contribute to their diminished blood flows. While both Andeans and Europeans had elevated mean arterial pressure at high altitude, the effect was magnified in the European women. Alternatively, decreased lower body blood flows in Europeans at 400 m and in both groups at high altitude may reflect similar blood volumes, but altered distribution of cardiac output.

We have demonstrated that multigenerational high-altitude natives respond to altitude in the same manner as recent migrants, i.e. with similar diminution in uterine artery diameter and blood flow characteristics. This effect appears to be environmental as we could not find any relationship between our physiological parameters of O_2 transport and ancestry. Thus based on these data, there is no obvious adaptation relative to O_2 transport among Andean women that could be considered genetic. Environmental exposures are now known to explain numerous other phenotypic differences initially hypothesized as genetic among natives to high altitude (Rupert & Hochachka, 2001; Brutsaert *et al.* 2004). In contrast, increased fetal growth in Andean women at both low and high altitude is likely to have a genetic origin; heritability of birth weight can be as high as 60% (Nance, 1976). We and others have suggested that the resistance of multigenerational natives to altitude-associated intrauterine growth restriction is related to evolutionary adaptation (Zamudio *et al.* 1993*a*; Moore, 2001). This requires careful re-examination (see Brutsaert, 2001 for a cogent critique). Across numerous environments Native Americans have increased fetal growth compared with coresident European populations (Munroe *et al.* 1984; Thomson, 1990). In the present study, across all four groups, Native American ancestry was associated with an increase in birth weight (22.5 g/5% increment in Native American ancestry, $r^2 = 0.10$, $P < 0.0001$). Tibetans and Native Americans share a northern Asian origin (Torroni *et al.* 1994; Merriwether *et al.* 1996), and both Andeans and Tibetans appear to have increased fetal growth, regardless of altitude (Haas *et al.* 1980; Tripathy & Gupta, 2005). Thus we cannot rule out that the Andean advantage at high altitude is secondary to their population origins in general, rather than a specific adaptation to high altitude.

Reduced O_2 tension (not delivery) earlier in pregnancy may influence the trajectory of fetal growth; however, the negative impact of altitude upon fetal growth is not obvious until the late second/early third trimester, in which fetal growth reaches its maximum (Unger *et al.* 1988; Krampl *et al.* 2000). If hypobaria is causally related to reduced fetal growth at high altitude, it is O_2 tension and not delivery that matters: we found a ∼5-fold excess in maternal O_2 delivery relative to the average fetal consumption of 6 ml min^{-1} per kg fetal weight (Carter, 1989). Since the uterine arteries represent ∼83% of the blood flow to the placenta, with the remainder derived from the ovarian arteries (Wehrenberg *et al.* 1977), delivery was considerably in excess of potential fetal demand. Nonetheless, when O_2 delivery was normalized to fetal weight, identical O_2 delivery was present in all four groups. This begs the question of whether reduced O_2 tension earlier in pregnancy alters the trajectory of fetal growth, or whether the amount of blood flow and O_2 delivery later in pregnancy is somehow matched to fetal growth. Even if the latter were true, our observation of decreased fetal weight relative to a given O_2 delivery at high *versus* low altitude supports that it is a substrate other than O_2 that is causally associated with altitude-associated diminution of fetal growth.

Of all the independent variables measured in this study, placental weight was the largest determinant of variation in fetal weight. We propose that the ancestry differences we have reported here ultimately have a placental origin. Given high levels of postimplantation pregnancy loss, placentae are undoubtedly subject to intense adaptive pressure or even evolutionary selection, but without necessarily inducing permanent changes in mother or offspring. It is known that placental structural adaptations permit increased O_2 diffusion at high altitude but what little data exist do not support a difference in diffusing capacity in Andean *versus* non-Andean placentae (reviewed in Zamudio, 2003). Glucose transporters are reduced by 40% in the fetal syncytial basal membranes of high altitude placentae (Zamudio *et al.* 2006), the rate limiting step in maternal to fetal glucose transport (Vardhana & Illsley, 2002). Prior studies indicate that glucose uptake is a close correlate of fetal protein synthesis (Hay, 1995; Liechty & Denne, 1998). We therefore propose an alternative hypothesis to the O_2 model of deficit in fetal growth under conditions of chronic hypoxia. Decreased placental nutrient transport, due to reduced flow, placental metabolism and/or transporter densities, may account for much of the reduction in fetal growth at high altitude, independent of O_2 delivery.

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