PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Research

Cite this article: Browne VA, Julian CG, Toledo-Jaldin L, Cioffi-Ragan D, Vargas E, Moore LG. 2015 Uterine artery blood flow, fetal hypoxia and fetal growth. *Phil. Trans. R. Soc. B* **370**: 20140068. http://dx.doi.org/10.1098/rstb.2014.0068

One contribution of 13 to a discussion meeting issue 'Human evolution: brain, birthweight and the immune system'.

Subject Areas:
physiology

Keywords:
brain sparing, Doppler velocimetry, fetal biometry, high-altitude adaptation, intrauterine growth restriction

Author for correspondence:
Lorna G. Moore
e-mail: lorna.moore@ucdenver.edu

Electronic supplementary material is available at http://dx.doi.org/10.1098/rstb.2014.0068 or via http://rstb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Uterine artery blood flow, fetal hypoxia and fetal growth

Vaughn A. Browne[1], Colleen G. Julian[2], Lillian Toledo-Jaldin[4], Darleen Cioffi-Ragan[3], Enrique Vargas[4] and Lorna G. Moore[3]

[1]Department of Emergency Medicine, [2]Department of Medicine, and [3]Department of Obstetrics and Gynecology, University of Colorado Denver, Aurora, CO 80045, USA
[4]Instituto Boliviano de Biología de Altura, La Paz, Bolivia

Evolutionary trade-offs required for bipedalism and brain expansion influence the pregnancy rise in uterine artery (UtA) blood flow and, in turn, reproductive success. We consider the importance of UtA blood flow by reviewing its determinants and presenting data from 191 normotensive (normal, $n = 125$) or hypertensive (preeclampsia (PE) or gestational hypertension (GH), $n = 29$) Andean residents of very high (4100–4300 m) or low altitude (400 m, $n = 37$). Prior studies show that UtA blood flow is reduced in pregnancies with intrauterine growth restriction (IUGR) but whether the IUGR is due to resultant fetal hypoxia is unclear. We found higher UtA blood flow and Doppler indices of fetal hypoxia in normotensive women at high versus low altitude but similar fetal growth. UtA blood flow was markedly lower in early-onset PE versus normal high-altitude women, and their fetuses more hypoxic as indicated by lower fetal heart rate, Doppler indices and greater IUGR. We concluded that, despite greater fetal hypoxia, fetal growth was well defended by higher UtA blood flows in normal Andeans at high altitude but when compounded by lower UtA blood flow in early-onset PE, exaggerated fetal hypoxia caused the fetus to respond by decreasing cardiac output and redistributing blood flow to help maintain brain development at the expense of growth elsewhere. We speculate that UtA blood flow is not only an important supply line but also a trigger for stimulating the metabolic and other processes regulating feto-placental metabolism and growth. Studies using the natural laboratory of high altitude are valuable for identifying the physiological and genetic mechanisms involved in human reproductive success.

1. Introduction

The 20-fold pregnancy rise in uterine artery (UtA) blood flow is among the greatest physiological changes experienced during the human lifespan. Considerable maternal physiological responses are required to accomplish this blood flow rise and support fetal growth. We argue here that such maternal physiological responses comprise, in addition to bipedalism and placentation, a third set of pivotal features in human evolution.

To develop this argument, we first review the literature concerning the evolutionary challenges posed by human pregnancy, the determinants of UtA blood flow and its importance in relation to fetal growth. Second, we present new data concerning UtA blood flow, fetal hypoxia and growth from very high altitude (4100–4300 m) where the hypoxia of high altitude combined with an increased incidence of preeclampsia (PE) and gestational hypertension (GH) challenge reproductive success. Specifically, we ask whether fetal hypoxia is exaggerated at high altitude, further worsened by lower UtA blood flow due to PE or GH and if so, the time course of such changes and their relationship to fetal growth. We consider that such a literature review and the new data provided will help illustrate the physiological processes contributing to reproductive success and human evolution, and point to the kinds of studies required for identifying new therapies for improving maternal and fetal health.

2. Part I: evolutionary challenges, determinants and importance of uterine artery blood flow

(a) Evolutionary challenges

Becoming bipedal and large-brained has been accompanied by evolutionary compromises that have reconfigured the *Homo sapiens* hip, lengthened gestation and altered birthing practices as described by Mick Eliot, Wenda Trevathan and Jonathan Wells [1–3]. Anthony Carter [4] and Ashley Moffett [5] address the important evolutionary changes occurring in the placenta, which shows greater morphological variation than any other mammalian organ likely due to the intense selective pressure to which it has been subject. Maternal physiological responses to pregnancy and in particular those determining uterine blood flow comprise a third set of factors influencing reproductive success and that require evolutionary trade-offs with those involved in bipedalism and placentation. For example, bipedalism and increasing tool use were accompanied by dietary changes, reduction in abdominal size and depth, and repositioning the uterus on top of the vena cava that serves, in turn, to limit third trimester venous return and lower cardiac output in all but the left-lateral supine posture [6]. Cardiac output continues to rise throughout pregnancy in quadrupeds, helping to fuel the exponential increase in UtA blood flow and fetal growth near term. We suspect that even though great apes share our deep trophoblast invasion [7,8], they are unlikely to have our limitation of cardiac output given their large abdomens and forward-tilted, knuckle-walking posture, although we know of no such direct measurements. Elsewhere, we have suggested that the lack of third trimester rise in cardiac output places a premium on vasodilation and structural remodelling of uteroplacental arteries for directing an increasing proportion of the cardiac output to the uterine circulation, and may explain the human predilection for PE relative to that of other species [9]. The evolution of the placenta is also related to maternal vascular responses as the type of placental exchange influences the amount of UtA blood flow required to maintain fetal oxygenation.

(b) Determinants of uterine artery blood flow

Blood flow to the pregnant uterus is supplied by four arteries: the right and left main UtA and the right and left uterine branches of the ovarian artery (OA). These vessels anastomose to form a bilateral arcade along each side of the uterus that, in turn, gives rise to several arcuate (*syn*. mesometrial) arteries arching over the uterus, which then branch centripetally into myometrial (*syn*. radial) arteries that penetrate the myometrium and, at the level of the decidua, terminate in approximately 200 spiral arterioles supplying blood to the intervillous space [10]. Only the contributions of the UtA to total uteroplacental blood flow have been studied to date; a recent attempt to measure simultaneous flow in the UtA and OA branches using magnetic resonance imaging concluded that the complexity of pelvic blood flow prevented its accurate assessment [11]. Estimates of total uteroplacental blood flow are therefore based on a single study in Rhesus monkeys in which it was shown that the two UtAs provided half the blood flow to the upper one-third of the uterus and all the blood flow to the lower portion, or 83% of the total, and the two OA branches the remainder [12]. While it is known that UtA blood flow on the side of placentation is approximately 18% greater than that in the contralateral UtA [13], unknown is whether placentation site also affects the relative contributions of the UtA and OA. Suggesting that it might are angiographic studies and case reports showing that the development of a collateral circulation through the OA helps overcome the limitation on cardiac output placed by vena caval compression by the pregnant uterus late in gestation [14].

The process of placentation involves fetal trophoblast cells invading the inner third of the uterus so as to decidualize it and convert the maternal spiral arterioles into large, flaccid tubes. This process, well described elsewhere in this issue, exerts important influences on maternal vascular responses to pregnancy as it effectively moves the site of vascular resistance upstream to the myometrial, arcuate and main UtA vessels. This creates a circumstance unique to pregnancy in which the limitation to blood flow is not in the end-arterioles but rather, the conduit vessels [10].[1] It is often assumed that placental influences are wholly responsible for the maternal vascular responses to pregnancy (e.g. [15]) but this is not the case given that such changes begin early and can occur outside the placental environment. Specifically, the systemic vasodilation, approximately 40% increase in blood volume and cardiac output, and doubling of UtA diameter are evident by week 6 [16,17], well before placentation is complete. Such changes are also almost certainly not flow mediated because extravillous trophoblast forms a continuous shell at the level of the decidua during the first trimester, plugs the tips of the maternal spiral arterioles and acts like a labyrinthine interface that permits the slow seepage of plasma and influx of secretions from uterine glands, but no true blood flow [18]. Further demonstrating their independence from placentation, the UtA acquires its characteristic low resistance Doppler waveform in ectopic pregnancy even though the UtAs were not supplying blood to the placenta or fetus, as they were not inside the uterus but rather implanted on the abdominal wall [19].

With removal of trophoblast plugs at the end of the first trimester, maternal blood enters the intervillous space and comes into direct contact with the fetal chorion, placentation becomes hemochorial and gas exchange begins to take place. Gas exchange in the human placenta operates as a venous equilibration system in which fetal umbilical venous blood equilibrates with maternal uterine venous, not arterial, blood. The blood in the intervillous space is stirred by the spurts of entering maternal arterial blood but also the oscillations of the fetal villi, thus creating a 'mixed pool' with a single partial pressure of oxygen (pO_2) at the level of maternal uterine venous pO_2 ([20] figure 1*a*). Supporting this are measurements obtained prior to uterine incision for Caesarean section in healthy women at sea level in which uterine venous saturation was 79% and pO_2 46 mmHg [22]. If, as in sheep, a specie with a similarly sized fetus and also a venous equilibration system of placental gas exchange, an approximately 10 mmHg O_2 gradient is required for diffusion from the uterine venous to the umbilical venous circulation 'his evidence leads to the conclusion that, in the human placenta, the maternal and umbilical circulations form a venous equilibration system that requires a higher uterine flow/O_2 uptake ratio than the countercurrent exchanger or any other exchanger that would allow the umbilical venous pO_2 to be equal to or higher than the uterine venous pO_2' [20, p. 290]. Specifically, Battaglia's and Meschia's calculations show that a threefold greater UtA blood flow is required to maintain the same uterine flow/O_2 uptake ratio as is the case in species with countercurrent systems. Applying such calculations to a human 3 kg fetus at sea level, these authors conclude that

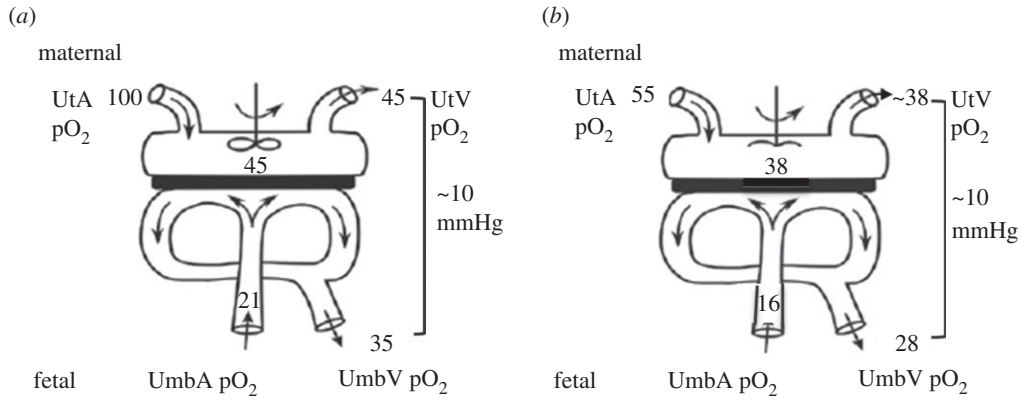

Figure 1. (*a*) At sea level, maternal UtA blood at a pO_2 of 100 mmHg enters the intervillous space where it is stirred by oscillations of fetal villi and equilibrates with UtV blood at a pO_2 of 45 mmHg. Assuming a 10 mmHg gradient, blood reaching the fetal circulation has a pO_2 of 35 mmHg and, following fetal O_2 uptake, returns to the placenta at a pO_2 of 21 mmHg. (*b*) High altitude. Using the values reported by or extrapolated from Postigo *et al.* [21] at 3600 m, maternal UtA pO_2 is 55 mmHg, UmbV pO_2 is 28 mmHg and umbilical artery (UmbA) pO_2 is 16 mmHg. Assuming a 10 mmHg UtV – UmbV gradient, the UtV pO_2 would then be 38 mmHg. (Adapted from Battaglia & Meschia [20].)

approximately 800 ml min^{-1} bilateral UtA blood flow (270 ml min^{-1} kg^{-1} newborn weight) is required [20].

(c) Importance of uterine artery blood flow

Prior to the advent of Doppler Ultrasound technology, the anatomical complexity of the uterine circulation and ethical considerations limited the ability to measure human UtA blood flow. Table 1 summarizes the still comparatively few such studies. All measured UtA blood flow as the product of vessel cross-sectional area (πr^2, where r is the UtA radius) and time-averaged flow velocity throughout the cardiac cycle. Such measurements are aided by the fact that when imaged transabdominally, the UtA appears to cross over the external iliac vessels prior to branching into uterine and cervical segments, thus providing a means for localizing the same portion in each study. Also the UtA is pulled upwards as the uterus expands and therefore velocity can be assessed at the requisite low angle of insonation. However, as is apparent in table 1, there is considerable variability among studies, which is due primarily to variation in flow velocity rather than diameter (coefficients of variation = 0.32 and 0.19, respectively, for the four studies with week-36 data).[2] Variation among studies is likely due to differences in study design and technique. For example, transvaginal versus transabdominal approaches image different portions of the UtA, which affects the level of flow. Studies also vary as to whether one or both UtA were sampled and, as flow through the two vessels varies with lateral placentation [13], total UtA blood flow cannot be assumed to be twice the unilateral value. We, but not others, have made corrections for the exaggerating effects of systole and of colour imaging on vessel diameter. Finally, as operator decisions as to where to place the cursors for measuring vessel diameter have a major influence on the flow levels obtained, comparisons between groups are best controlled by having a single operator make all measurements or limiting comparisons between groups to changes within subjects over time.

Despite such variation, several observations can be made. One is that the rise in UtA blood flow begins early and converges near term in the two studies conducted by a single operator at low altitude [13,24] at approximately 270 ml min^{-1} kg^{-1} fetal weight, the value directly measured in sheep. A second point is that UtA blood flow per kilogram fetal weight falls progressively with advancing gestation; this is due to increasing fetal size given that the absolute flows and the fraction of total cardiac output directed to the uteroplacental circulation continue to rise [13]. Third, the principal factors responsible for raising UtA blood flow differ in early versus late gestation, with a linear increase in diameter being more important early and a rising flow velocity prevailing thereafter [24,25]. This together with the early onset of maternal vascular responses to pregnancy suggests that vasodilatory and/or angiogenic effects of pregnancy hormones are likely responsible for increasing UtA diameter and that trophoblast-mediated changes in downstream vascular resistance play a major role in the progressive rise in flow velocity.

Variation in UtA blood flow has been related to fetal growth in studies at low altitude. In serial studies, Konje showed that women delivering IUGR babies had 12% and 37% lower UtA blood flows at weeks 20 and 38, respectively, and that lower flows were due primarily to smaller UtA diameters from week 20 onward [29]. Jeffreys observed strong correlations between third trimester UtA blood flow and birth or placental weights that were also primarily due to relationships with UtA diameter [30]. UtA Doppler indices indicative of increased uteroplacental vascular resistance have led to the widespread assertion that uteroplacental blood flow is low and a contributor to the IUGR frequently observed in PE/GH pregnancies, but to the best of our knowledge volumetric UtA blood flow has not actually been measured in PE/GH women at low altitude.

At high altitude, early studies in Peru by Barron and co-workers [31] in native sheep emphasized the importance of UtA blood flow, noting that the partial correlation of uterine O_2 delivery with UtA flow was much greater ($r = 0.9$) than with arterial O_2 content ($r = 0.3$) or the A-V gradient ($r = -0.5$). Infant birth weight falls at high altitude, averaging approximately 100 g per 1000 m altitude gain, due to slowed fetal growth and not shortened gestation or socioeconomic factors [32–34]. Variation in UtA blood flow is related to fetal growth at high altitude. Specifically, our studies conducted with a single operator and at the altitude of the subject's residence showed that newcomer high-altitude residents have half the rise in UtA diameter seen at low altitude and lower UtA blood flows from week 20 onward as a result [27].

As human populations have lived at high altitude for 10 000 years or more and a reduction in fetal growth increases perinatal as well as later-in-life mortality, we hypothesized

Table 1. Studies of UtA blood flow during human pregnancy. c, cross-sectional study design; l, longitudinal study design; *n*, sample size; n.d., no data.

reference	pregnancy weeks	mean UtA dia. (mm)	unilateral UtA flow (ml min^{-1})	bilateral UtA flow (ml min^{-1})	uteroplacental flow kg^{-1} fetus (ml min^{-1} kg^{-1})
[23], transvaginal,	6–8	1.9	120		
$n = 24$, l	26–28	2.8	243		
	36–39	3.7	342		
[24], transabdominal,	21	2.8	142		
$n = 5$, l	30	2.9	207		
	36	3.4	312		
	term		382		263
[25], transabdominal,	6	3.0		80	
1995, $n = 44$, l	8	3.3		120	
	10	3.7		150	
	12	4.0		270	
	14	4.3		380	
	16	4.6		600	
[13], transabdominal,	20	2.6		513	1544
$n = 57$, l	24	3.5		669	993
	28	4.0		782	618
	30	4.4		831	547
	32	4.6		875	425
	34	4.8		911	360
	36	4.8		942	317
	38	4.8		970	296
[26], transvaginal,	non-preg	n.d.	22		
$n = 10$, l	12	n.d.	150		
[27], transabdominal,	non-preg	3.4	31		
$n = 18$, l	20	5.3	463		
	30	5.0	512		
	36	5.0	613		
[27], transabdominal,	20	n.d.		200	534
$n = 59$, c	30	n.d.		330	199
	36	n.d.		472	128

some years ago that genetic attributes serving to protect long-resident groups from altitude-associated IUGR would have been selected for at high altitude [35]. Supporting this hypothesis, multigenerational, Andean or Tibetan high-altitude residents have half as much altitude-associated decline in birth weight as do shorter term (Han, European) residents of the same altitude (167 ± 36 (s.e.m.) g versus 373 ± 48 g, respectively, across a 400–3600 m gradient [36]). Not only are Andean and Tibetan birth weights greater (more normal) but Andean (and probably also Tibetan [37,38]) levels of UtA blood are also higher [39,40]. UtA blood flow at high altitude is positively correlated with infant birth weight, with a R^2-value $= 0.80$ when the full spectrum of hypertensive (PE, GH) as well as normotensive pregnancies is included [41]. In keeping with the operation of evolutionary forces, our and other's single nucleotide polymorphism-based scans of human genetic variation indicate that natural selection has operated on several gene regions in long-resident high-altitude populations [42–47]. More recently, we have shown that variation in or near the gene coding for the adenosine monophosphate kinase alpha-1 catalytic domain (*AMPKα1*) relates to UtA diameter, infant birth weight and the expression levels of several genes in the mTOR pathway in Andeans at high altitude [48], a pathway previously implicated in high-altitude IUGR [49].

Most of what is known concerning the placenta at high altitude has come from morphological studies that, in general, indicate that placental relative to fetal growth is variably affected with increases [50], decreases [51,52] or no changes [53,54] being observed. A recent, carefully conducted study at sea level and Colorado locations at 1600 and 3100 m in women of European ancestry showed that placentas weighed 30% less at 3100 m than sea level but that the reductions were proportional such that the relative amounts of stem

villi, villous interspace, intermediate/terminal villi, and chorionic and basal plates were similar at all altitudes [55]. There was no change in the harmonic mean thickness in these studies although decreased thickness has previously been observed in longer resident, Bolivian and Kirghizstani populations [53,56].

Several recent studies show that residence at high altitude affects placental metabolism; specifically, glucose consumption is increased [57] and oxidative metabolism reduced as indicted by a lower ratio of ATP to ADP levels [58], diminished mitochondrial complex protein expression and activities, and higher miR-201 expression [59]. Whether these changes relate to alterations in uteroplacental O_2 delivery and placental O_2 uptake (VO_2) is unknown. It has been suggested that they do not [57], given that 50% reductions in UtA blood flow do not change placental VO_2, but it is important to point out that such protection is only present acutely [60,61] and that chronic reductions in UtA flow decrease fetal and placental VO_2 by large amounts (50% and 75%, respectively [61,62]). As the hypoxia of high altitude and reduction in UtA flow are certainly chronic in nature, the chronic studies are more relevant to consider and, together with the metabolic observations above, suggest that placental VO_2 at high altitude may be reduced.

The effects of high altitude on fetal growth can be attributed in approximately equal measure to altitude alone, an altitude-related increase in the incidence of PE and GH, and their interactive effects [63,64]. There is little difference between multigenerational and shorter resident groups in the altitude-related increase in hypertensive complications of pregnancy [63,65] but the lack of complete vital statistic data in the Andean or Tibetan regions complicates such comparisons. Data presented below showing alterations in UtA resistance indices consistent with impaired placentation in early-onset PE, and the lack of pronounced fetal growth restriction or placental pathology in placentas from women with late-onset PE [55] suggest that impaired placentation in early-onset PE and insufficient maternal physiological responses to pregnancy in late-onset PE or GH are likely responsible for the altitudinal increase in hypertensive complications observed.

3. Part II: relationship between uterine artery blood flow, fetal hypoxia and fetal growth at high altitude

(a) Introduction

While previous studies thus support a relationship between UtA blood flow and fetal growth, the mechanisms underlying such a relationship are unclear. On the one hand, lower UtA blood flow would appear to play a causative role because differences in UtA blood flow between normal and IUGR pregnancies at low altitude and between Andean and European residents of high altitude are present by week 20, which is well before measurable slowing of fetal growth [13,66]. But on the other hand, it seems unlikely that less uteroplacental O_2 delivery is the sole cause of the growth restriction observed because (i) the small size of the fetus at week 20 means that uteroplacental O_2 delivery is very high relative to fetal demand, (ii) arterial O_2 content is well preserved by the pregnancy rise in maternal ventilation and the higher haemoglobin levels characteristic of high-altitude residents [67], and (iii) UtA blood flow even in European residents of high altitude is sufficient to preserve calculated uteroplacental O_2 delivery at low-altitude values [68].

Thus, the question arises as to the role of increased UtA blood flow during pregnancy for defending fetal O_2 supply and growth. Whether the fetus is more hypoxic at high than low altitude is contested. Newborn arterial pO_2 and O_2 saturation immediately following delivery are slightly but not significantly reduced at 3100–4300 m compared with low altitude [21,69,70], but the interpretation of these values is complicated by the influences of vaginal or cesarean delivery on the measurements obtained. Moreover, such values are not likely to be the same as those present earlier in gestation when fetal growth restriction begins. Other measures more likely reflecting chronic conditions *in utero* suggest that the fetus is more hypoxic at high than low altitude insofar as high- compared with low-altitude newborns have higher haemoglobin, haematocrit and erythropoietin levels [21,71]. Such changes are less pronounced in Andeans versus Europeans at high altitude [21,71,72], with ancestry-group differences in fetal head circumference (HC) and femur length (FL) already present by week 20 [66].

Doppler indices have been shown in recent years to be a valuable means for assessing fetal hypoxia [73,74]. Their utility stems from the fact that fetal hypoxia prompts cerebral vasodilation that, in turn, redistributes cardiac output to favour the brain and other vital organs ('brain sparing'), directs blood flow away from other organs and hence slows fetal growth. Such changes are demonstrated by an increased UmbA pulsatility index (PI), measured as the rise in blood velocity during systole weighted by the average velocity throughout the cardiac cycle and which likely indicates a rise in systemic vascular resistance; a decreased middle cerebral artery (MCA) PI, rise in MCA peak systolic velocity (PSV) and/or a greater decrease in the MCA than UmbA PI (i.e. a decreased MCA/UmbA PI ratio) that are indicative of cerebral vasodilation [73,74]. We therefore asked whether high altitude alone and/or lower UtA blood flow due to PE or GH at high altitude increased fetal hypoxia as measured by these Doppler indices and if so, the time course of such changes and their relationship to fetal growth. Our approach for answering these questions was first to compare UtA blood flow, Doppler indices of fetal hypoxia and fetal growth in healthy, normotensive Andean residents of high versus low altitude and then to compare high-altitude normal, healthy women to those with PE or GH. We considered that such studies would be useful for better understanding the mechanisms by which UtA blood flow influences fetal hypoxia and fetal growth.

(b) Subject characteristics, study techniques and statistics

This was a case-control study of 191 pregnant Andeans (Aymára or Quechua) who resided either at very high altitude (4100–4300 m, $n = 154$) or low (400 m, $n = 37$) altitudes in Bolivia. The high-altitude women were studied cross sectionally on one occasion between 18 and 38 weeks of pregnancy, and the low-altitude women serially at 20 and 36 weeks. All subjects gave written consent to study procedures that had been approved by the Colorado Multiple Institutional Review Board and the Colégio Medico, its Bolivian counterpart. Exclusion criteria were smoking, consumption of more than two alcoholic beverages weekly, active infections, or known risks for developing PE or GH (i.e. pre-existing

hypertension, cardiovascular or renal diseases, gestational diabetes and multiple gestation). The high-altitude women had lived their whole lives above more than 2500 m, whereas the low-altitude subjects had been born and raised either at low ($n = 24$) or high ($n = 13$) altitude. Subjects were subsets of those described more fully elsewhere [41,66]; new here are fetal Doppler and biometry data, and for the cross-sectionally studied women, UtA blood flows reported by gestational age.

Ancestry was confirmed using a panel of Ancestry Informative Markers (AIMs), parental and grand-parental surnames and birthplace as described previously [66]. PE and GH were defined by systolic blood pressure greater than 140 mmHg and diastolic blood pressure greater than 90 mmHg after the 20th week of pregnancy on 2+ occasions at least 6 h apart in a woman who was normotensive when non-pregnant. PE was distinguished from GH by the presence of more than or equal to 1+ proteinuria by dipstick and confirmed as more than 300 mg in a 24-h collection; women with GH were without proteinuria. Of the 154 high-altitude women, 125 remained normotensive throughout pregnancy and 29 had been diagnosed with PE or GH. Eight [13] of the women with PE were diagnosed early (less than or equal to 34 weeks, termed 'early-onset PE') and 21 with PE or GH were diagnosed late in gestation (more than 34 weeks, termed 'late-onset PE/GH').

Ultrasound studies were performed in the cross-sectional high-altitude sample using a MicroMaxx (SonoSite, Inc. Bothell, WA) equipped with a C60e transducer as previously described [41]. Women in the serial low-altitude sample were studied serially using an ATL 3000 ultrasound (Phillips) configured for obstetric use with colour imaging and Doppler with a 4-MHz curved linear array probe as previously described [66]. Briefly, the right and left UtA were imaged at the anatomic location where they appear to cross over the external iliac artery and before dividing into the uterine and cervical branches. UtA diameter was calculated as (2 × end-diastolic + peak systolic diameter)/3, corrected for the exaggerating effects of colour imaging and used together with time-averaged mean UtA flow velocity (TAM) obtained from traces with at least six consecutive cardiac cycles of good quality to calculate UtA volumetric flow as previously described [39]. Also recorded were the UtA PI ((peak systolic − end-diastolic velocity)/TAM) in each right and left main UtA as described previously. UtA blood flow is presented as the sum of values averaged from triplicate measures in the right and left vessels; other measures are the average of the values obtained from both sides.

As part of the same study, fetal heart rate (FHR), MCA and UmbA PI, MCA and UmbA PSV, head circumference, biparietal diameter (BPD), abdominal circumference (AC) and FL were measured and presented as the average of triplicate measures. Care was taken to record values during periods of fetal inactivity. The MCA or UmbA PSV was measured as close to 0° as possible, with the actual angle of insonation recorded and used to correct values to 0°. The MCA was measured at the origin of the circle of Willis and the UmbA from a representative mid-cord segment as previously described [73]. Estimated fetal weight (EFW) was calculated using the Hadlock formula [75].

All subjects delivered in hospitals where birth weights and other anthropometric measures were obtained. Infants were classified as small for gestational age (SGA) if their birth weights were below the 10th percentile of sea-level values for their gestational age and sex [76]. Gestational age was defined as weeks from the last menstrual period (LMP), confirmed by that obtained from week 20 fetal biometry values in the low-altitude subjects, and used to classify infants as preterm (less than 37 weeks) or term (more than or equal to 37 weeks). No births occurred post-term (more than 42 weeks) or were large for gestational age (more than 10th percentile for gestational age and sex).

After affirming that study variables were normally distributed using Kolmogorov and Smirnov tests, the cross-sectional data were pooled into four-week bins centred at 20, 24, 28, 32 and 36 weeks of pregnancy. Comparisons were conducted between normal subjects at low versus high altitude and between the normal women and those who developed early-onset PE or late-onset PE or GH at high altitude. Pregnancy groups were compared by two-way analysis of variance with corrections for multiple comparisons or Fisher's exact test. Linear regression was used to identify the relationship between fetal growth and the UtA, UmbA and MCA flow parameters. Actual gestational age at the time of study was included in all models given its strong association with increased UtA, UmbA and MCA blood flow. First, we introduced each factor separately to identify significance or lack thereof. Subsequently, we introduced all significant variables into a stepwise linear regression model to determine the most prominent determinants of EFW for all women, Norm and PE/GH. Results are reported in the text as the standardized beta (β) coefficient, which is the slope of the association and is bounded by −1.0 and +1.0. The R^2-values and the standardized β coefficient for all independent variables are provided in the electronic supplementary material, table S1. All analyses were conducted using Prism v. 6.0 (GraphPad Software, San Diego, CA, USA) or SPSS (Chicago, IL, USA). Comparisons between groups or across study times were considered significant when the two-tailed $p < 0.05$ and reported as trends when $0.05 < p < 0.10$. Data are reported in the text, tables and figures as the mean ± s.e.m. or as percentages with 95% confidence intervals.

(c) Results

(i) Maternal characteristics

Maternal age was similar but gravidity and parity (data not shown) were lower and body mass index (BMI) higher in the normal healthy women at low than high altitude (table 2*a*). Maternal haemoglobin levels were higher at high than low altitude in normal subjects. At high altitude, the normal women were slightly younger than the PE or GH women and had lower non-pregnant BMI, but no women were obese (BMI > 30). The normotensive and hypertensive high-altitude groups were similar in the per cent AIMs of Amerindian origin (83 ± 1% and 79 ± 2% respectively, p = n.s.) but per cent AIMs was greater in both groups than at low altitude (64 ± 3%, both $p < 0.01$). There were no differences in maternal age, gravidity or haemoglobin between the early-onset PE and the late-onset PE/GH groups (data not shown). Women with hypertensive complications more often delivered preterm than normal women at high altitude (table 2*a*) with this being especially true for the early-onset PE versus late-onset PE/GH groups (62.5% [29, 96] versus 4.8% [−4, 14], respectively, $p < 0.01$). No women with PE or GH were studied at low altitude.

Bilateral UtA volumetric blood flow was much higher in the cross-sectionally studied normal women at high altitude than in

Table 2. Characteristics of Andean mothers and babies residing at low (400 m) and high (4100–4300 m) altitude. Asterisks (*) indicate interaction between group and time, $p < 0.0001$. Sample sizes are in parentheses. AC, abdominal circumference; BMI, body mass index; BPD, biparietal diameter; dia., diameter; EFW, estimated fetal weight; FL, femur length; GH, gestational hypertension; HA, high altitude; LA, low altitude; MCA, middle cerebral artery; PE, preeclampsia; PI, pulsatility index; PSV, peak systolic velocity; SGA, small for gestational age and sex; TAM, time-averaged flow velocity; UmbA, umbilical artery; UtA, uterine artery; wk, week.

(a) general	normal low altitude	normal high altitude	all PE/GH high altitude	*p*-value
maternal age (yr)	27 ± 1 (37)	26 ± 1[b] (125)	30 ± 1[b] (29)	<0.01
gravidity, no. pregnancies	1.9 ± 0.2[c] (37)	2.4 ± 0.2 (125)	3.2 ± 0.5[c] (29)	<0.05
maternal non-pregnant BMI	25 ± 1[a] (20)	19 ± 0[a,b] (125)	21 ± 1[b] (29)	<0.0001
maternal haemoglobin (g dl^{-1})	10.3 ± 0.2[a] (37)	13.3 ± 0.2[a] (125)	12.8 ± 0.3 (29)	<0.0001
preterm (%)	17 [7, 37] (23)	5 [1, 9] (125)	19 [5, 33] (29)	<0.01
fetal demise (%)	0 (37)	2 [0, 4] (125)	10 [0, 20] (29)	n.s.
SGA (%)	14 [5, 33] (23)	14 [8, 20] (125)	48 [29, 66] (29)	<0.01
newborn birth weight (g)	3191 ± 122[c] (23)	3123 ± 50[b] (102)	2456 ± 153[b,c] (29)	<0.0001
newborn gestational age (wk)	38.8 ± 0.6[c] (23)	38.8 ± 0.2[b] (102)	36.7 ± 0.7[b,c] (29)	<0.01
newborn length (cm)	49.3 ± 0.4 (22)	49.1 ± 0.3 (84)	46.7 ± 0.9 (22)	<0.01
newborn ponderal index (kg m^{-3})	26.8 ± 0.5[c] (22)	26.3 ± 0.4[a] (84)	24.5 ± 0.7[c] (22)	n.s. (0.07)
newborn HC (cm)	35.1 ± 0.3[a,c] (37)	33.9 ± 0.2[a] (89)	33.4 ± 0.4[c] (26)	<0.01

	early-onset PE					late-PE/GH			
(*b*) Doppler/ultrasound	group	wk 20	wk 24	wk 28	wk 32	wk 36	*p*-value, time	*p*-value, normal HA versus PE/GH HA	*p*-value, normal LA versus HA
n	normal LA	37	—	—	—	37	—		—
	normal HA	11	19	31	37	27	—	—	
	PE/GH HA	—	3	2	3	21	—		
UtA dia. (cm)	normal LA	0.64 ± 0.01	—	—	—	0.65 ± 0.01	n.s.		n.s.
	normal HA	0.67 ± 0.03	0.62 ± 0.01	0.63 ± 0.02	0.61 ± 0.02	0.60 ± 0.02	n.s.	n.s.	
	PE/GH HA	—	0.61 ± 0.04	0.60 ± 0.02	0.56 ± 0.02	0.64 ± 0.02	n.s.		
UtA TAM (cm s^{-1})	normal LA	23 ± 2	—	—	—	29 ± 2	< 0.01		<0.0001
	normal HA	34 ± 3	33 ± 3	37 ± 2	42 ± 2	43 ± 2	<0.05	<0.001	
	PE/GH HA	—	16 ± 2	15 ± 4	33 ± 8	42 ± 4	<0.01		
UtA PI	normal LA	1.00 ± 0.08	—	—	—	0.82 ± 0.06	<0.05		n.s.
	normal HA	0.92 ± 0.05	0.84 ± 0.05	0.80 ± 0.03	0.73 ± 0.05	0.73 ± 0.03	<0.001	<0.0001*	
	PE/GH HA	—	1.99 ± 0.24	1.47 ± 0.86	1.43 ± 0.35	0.96 ± 0.07	<0.001		
UmbA PSV (cm s^{-1})	normal LA	—	—	—	—	—	—		—
	normal HA	34.0 ± 1.4	40.6 ± 1.6	41.9 ± 1.3	45.5 ± 1.6	50.5 ± 1.4	<0.001	<0.01	
	PE/GH HA	—	30.6 ± 4.0	37.0 ± 4.0	38.3 ± 5.3[+]	44.9 ± 2.3	<0.001		

(*Continued.*)

Table 2. (*Continued.*)

	early-onset PE					late-PE/GH			
(*b*) Doppler/ultrasound	group	wk 20	wk 24	wk 28	wk 32	wk 36	*p*-value, time	*p*-value, normal HA versus PE/GH HA	*p*-value, normal LA versus HA
UmbA PI	normal LA	1.29 ± 0.05	—	—	—	0.92 ± 0.04	<0.001		n.s. (.07)
	normal HA	1.41 ± 0.05	1.29 ± 0.04	1.14 ± 0.03	1.18 ± 0.04	0.98 ± 0.03	<0.001	<0.01*	
	PE/GH HA	—	3.12 ± 1.00	1.15 ± 0.19	1.19 ± 0.18	1.03 ± 0.05	<0.001		
MCA PSV (cm s^{-1})	normal LA	—	—	—	—	—	—		—
	normal HA	25.9 ± 1.4	26.4 ± 0.9	34.2 ± 1.7	43.4 ± 2.2	53.3 ± 2.5	<0.001	n.s.	
	PE/GH HA	—	39.2 ± 10.2	27.0 ± 4.0	55.4 ± 5.8	47.0 ± 1.9	<0.001		
MCA PI	normal LA	2.02 ± 0.15	—	—	—	1.74 ± 0.12	n.s.		<0.001
	normal HA	1.32 ± 0.04	1.95 ± 0.18	2.04 ± 0.16	1.79 ± 0.12	1.45 ± 0.08	<0.05	n.s.	
	PE/GH HA	—	1.40 ± 0.13	2.34 ± 0.30	1.22 ± 0.06	1.43 ± 0.14	n.s.		
BPD (cm)	normal LA		—	—	—				
	normal HA	4.8 ± 0.2	6.4 ± 0.1	7.3 ± 0.1	8.2 ± 0.1	8.7 ± 0.1	<0.001	n.s.	
	PE/GH HA	—	6.0 ± 0.3	7.2 ± 0.3	8.3 ± 0.2	8.9 ± 0.1	<0.001		
HC (cm)	normal LA	18.7 ± 0.5	—	—	—	31.4 ± 0.2	<0.001		n.s.
	normal HA	17.2 ± 0.8	23.4 ± 0.4	26.2 ± 0.4	30.1 ± 0.3	31.6 ± 0.4	<0.001	n.s.	
	PE/GH HA	—	22.4 ± 0.9	27.3 ± 0.5	30.9 ± 1.1	32.0 ± 0.3	<0.001		
AC (cm)	normal LA	17.1 ± 0.5	—	—	—	31.3 ± 0.2	<0.001		n.s.
	normal HA	14.9 ± 0.9	21.6 ± 0.4	25.2 ± 0.4	29.3 ± 0.5	32.4 ± 0.5	<0.001	n.s.	
	PE/GH HA	—	19.8 ± 0.8	26.8 ± 1.8	29.5 ± 1.5	32.2 ± 0.6	<0.001		
FL (cm)	normal LA	3.7 ± 0.1	—	—	—	7.0 ± 0.0	<0.001		n.s.
	normal HA	3.1 ± 0.2	4.5 ± 0.1	5.4 ± 0.1	6.2 ± 0.1	7.0 ± 0.1	<0.001	n.s.	
	PE/GH HA	—	4.1 ± 0.1	5.3 ± 0.2	6.2 ± 0.2	7.0 ± 0.1	<0.001		

[a]Normal LA versus normal HA, $p<0.05$.

[b]Normal HA versus PE/GH HA, $p < 0.05$.

[c]Normal LA versus PE/GH HA, $p < 0.05$.

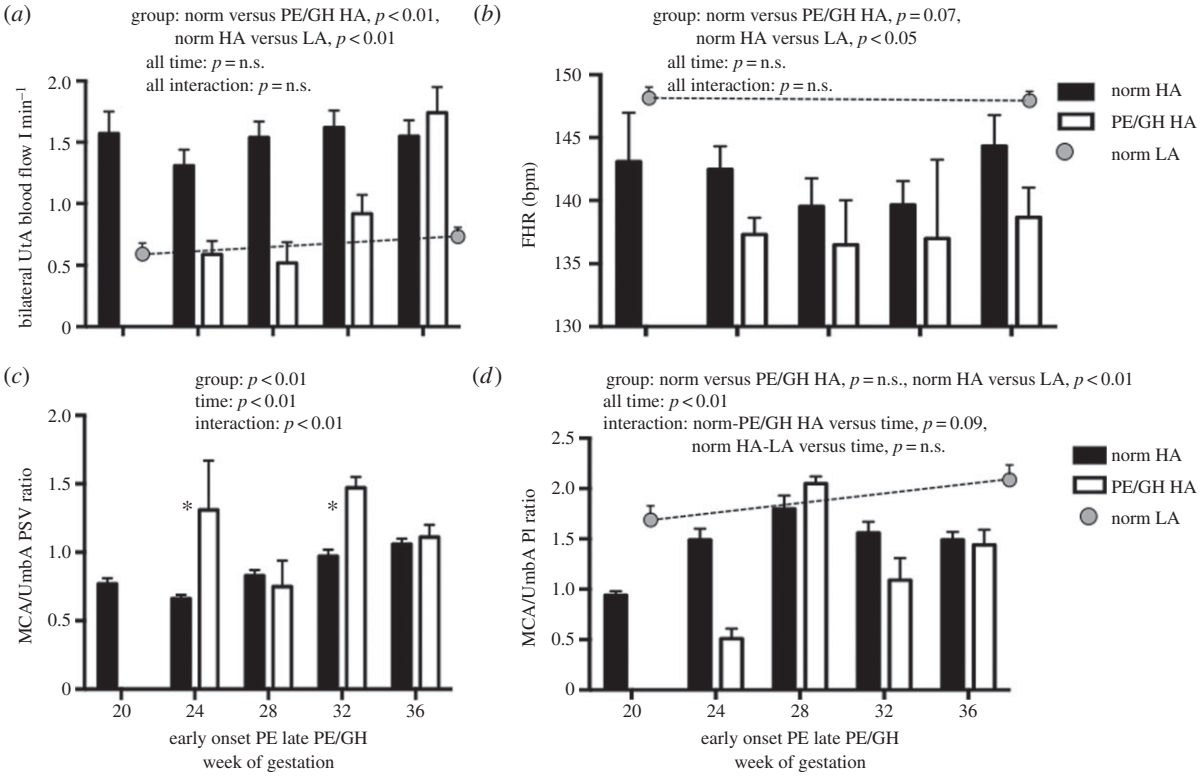

Figure 2. (*a–d*) Filled columns are mean ± s.e.m. values measured in cross-sectional samples of normal, healthy very high-altitude (4100–4300 m) pregnant Andeans at the week of gestation shown. Open columns are from cross-sectional samples of high-altitude Andeans diagnosed with PE or GH, with those diagnosed early (less than or equal to 34 weeks) or late (more than 34 weeks) shown. Grey filled circles are from 37 Andeans residing at low altitude (400 m) who were studied serially at weeks 20 and 36. (*a*) Bilateral UtA blood flow is higher in normal than early-onset PE women at high altitude, and higher in normal residents of high than low altitude. (*b*) FHR tends to be higher in normal than early- or late-onset PE/GH Andeans at high altitude and is higher in normal low- than high-altitude Andeans. (*c*) The ratio of the MCA PSV to the umbilical artery (UmbA) PSV is lower in normal than early-onset PE or late-onset PE/GH Andean residents of high altitude, especially at weeks 24 and 32 of gestation (asterisks). (*d*) The ratio of the MCA PI to the UmbA PI is lower in the normal high- than low-altitude women. The effect of advancing gestation (time) tends to differ in the normal versus PE/GH high-altitude groups, likely as the result of more variable values in the PE/GH subjects.

the serially studied women at low altitude (figure 2*a*) as the result of higher TAM velocity (table 2*b*). The cross-sectionally studied women with early-onset PE had substantially lower UtA blood flow than normal women at high altitude, due to higher UtA PI and lower TAM, whereas UtA blood flows in the late-onset PE/GH women were similar to those of healthy high-altitude women (figure 2*a* and table 2*b*).

(ii) Fetal characteristics

FHR did not change across the period of study but was lower in the cross-sectionally studied normal high- versus serially studied low-altitude samples. FHR tended to be lower still in the cross-sectionally studied, early-onset PE or late-onset PE/GH than normal pregnancies at high altitude (figure 2*b*). UmbA PSV rose with advancing gestation but was consistently lower in the early-onset PE or late-onset PE/GH versus normal high-altitude groups. UmbA PI, a measure of systemic vascular resistance, decreased with gestation in all groups but tended to be higher in the healthy high- than low-altitude pregnancies and in the hypertensive than normal groups at high altitude (table 2*b*). MCA PI was lower in normal pregnancies at high versus low altitude but neither it nor MCA PSV differed between normal and the hypertensive groups at high altitude (table 2*b*).

The MCA/UmbA PSV ratio rose during gestation at high altitude to values that were higher in the early-onset PE than normal healthy high-altitude group (figure 2*c*) due to lower UmbA PSV (table 2*b*). Advancing gestation also increased the MCA/UmbA PI ratio, with values being lower in the normal high- than low-altitude groups and with a trend towards differing effects of time in the hypertensive versus normotensive groups at high altitude (figure 2*d*).

Fetal biometry values increased with advancing gestation as expected. While HC, AC and FL were marginally lower at high than low altitude or in hypertensive than normal groups, no measures differed significantly between groups (table 2*b*). Using stepwise regression models, both advancing gestational age and greater UtA blood flow, UmbA and MCA PSV or lower UtA PI, UmbA PI and MCA PI contributed to greater EFW when all high-altitude subjects or the normal subjects alone were considered, with R^2-values for the complete models ranging from 0.78 to 0.81 (see the electronic supplementary material, table S1). While such correlations were chiefly due to the contribution of gestational age at the time of study, greater UtA blood flow and lower UtA PI contributed to the association as shown by their significant β coefficients in all the high-altitude subjects ($\beta = 0.08$ and -0.15, $p < 0.05$ and $p < 0.001$, respectively) or the normal subjects alone ($\beta = 0.09$ and -0.13, $p < 0.05$ and $p < 0.01$, respectively). Likewise, higher UmbA PSV and lower UmbA PI contributed to greater EFW in all the high-altitude groups ($\beta = 0.14$ and -0.17, respectively, both $p < 0.01$) or the normal group alone ($\beta = 0.09$ and -0.15, $p = 0.06$ and $p < 0.01$, respectively). Higher MCA PSV and lower MCA PI were also associated with greater EFW in all the high-altitude groups ($\beta = 0.17$ and -0.08, respectively, both $p < 0.01$) or the normal group alone ($\beta = 0.19$ and -0.12, both $p < 0.01$).

A higher MCA/UmbA PSV ratio was also associated with greater EFW in the normal group ($\beta = 0.11$, both $p < 0.05$). None of these flow parameters was significant when only the PE or GH groups were considered (see the electronic supplementary material, table S1), and sample sizes were too small to permit evaluating the early- and late-onset PE groups separately.

(iii) Newborn characteristics

Birth weights, gestational age, length or ponderal indices were similar in babies born to normal healthy Andeans at high versus low altitude although HC was slightly smaller (table 2*a*). Three of the five fetuses from early-onset PE pregnancies died *in utero* but the frequency of fetal demise did not differ among the groups overall. Babies born to high-altitude hypertensive women weighed less at birth, were more often SGA and tended to have lower ponderal indices than those born to normal women at low or high altitude (table 2*a*). Early- versus late-onset PE pregnancies at high altitude more often delivered preterm (62% [5 and 95% CI = 29, 96] versus 5% [−4, 14], $p < 0.01$) had greater fetal demise (38% [4, 71] versus 0%, $p < 0.05$) and lower birth weight (1555 ± 336 versus 2773 ± 126 g, $p < 0.01$) but a similar frequency of SGA infants (50% [15, 85] versus 48% [26, 69], $p =$ n.s.).

(d) Discussion

Our principal findings were twofold. First, fetal hypoxia appeared to be modestly greater at high than low altitude as indicated by a lower FHR, lower MCA PI, lower MCA/UmbA PI ratio, and trend towards higher UmbA PI throughout the period of study. However, the normal, healthy Andeans' greater UtA blood flow at high altitude appeared to compensate for the effects of hypoxia on fetal growth given the similarity in infant birth weights and ponderal indices at high and low altitude. Second, the fetuses of early-onset PE appeared to be more hypoxic than those born to normal, healthy Andean women at high altitude as indicated by their even lower FHR, lower UmbA PSV, higher UmbA PI, greater MCA/UmbA PSV ratio and greater frequency of adverse outcomes including lower birth weight, preterm delivery and fetal demise. We therefore concluded that fetal growth was well defended by higher UtA blood flows in normal Andeans at high altitude. But when the reduced O_2 availability of high altitude was compounded by early-onset PE, lower UtA blood flow exaggerated fetal hypoxia and caused the fetus to respond by decreasing cardiac output and redistributing blood flow to help maintain brain development at the expense of growth elsewhere. Reductions in UtA blood flow and alterations in Doppler indices of brain sparing detected fetal growth restriction earlier and more reliably than measures of fetal biometry in early-onset PE versus normal pregnancies at high altitude, recommending their inclusion in future prospective studies.

Our study benefited from certain strengths but also had its weaknesses. Strengths were the inclusion of normotensive as well as the full spectrum of early- and late-onset PE/GH pregnancies, which is important for gaining population and evolutionary perspectives on adaptation to high altitude. While early-onset disease has more severe outcomes, more women and babies are affected by late-onset disease making it important for both groups to be considered. These are, to the best of our knowledge, the first data in which volumetric UtA blood flow in hypertensive pregnancies has been measured in combination with Doppler indices of blood flow distribution, fetal biometry and infant birth weight at high (or low) altitude. Additional strengths were that a single operator and instrument made all the UtA blood flow measurements in the cross-sectional studies at high altitude and the same UtA protocols were followed in the Doppler ultrasound studies at low and high altitude. While the accuracy of ultrasound Doppler-derived measurements of volumetric flow is subject to limitations, our UtA flow estimates per kilogram fetal weight at low altitude agreed with directly measured values in sheep and had good reproducibility [24,27]. We also used multiple Doppler indices of brain sparing (UmbA PI, MCA PI, MCA/UmbA PI ratio), as well as a new measure (MCA/UmbA PSV) given the prior report that the MCA PSV was more predictive of adverse perinatal outcome than the MCA PI [77].

Study weaknesses included the cross-sectional design of the high-altitude studies, which allowed inclusion of a larger number of women with a range of disease severity but limited our ability to distinguish between the effects of pregnancy duration, disease progression and inter-individual variation. This is especially true in relation to hypertensive complications of pregnancy insofar as early-onset PE is often accompanied by more severe fetal growth restriction than late-onset disease, and may differ in terms of etiology [78]. Thus, we were careful to compare the early- or late-onset hypertensive groups to normal, healthy high-altitude women at the same time in gestation. We were limited in our ability to estimate gestational age as fetal biometry before week 20 is not routinely performed in Bolivia. While it is possible that some of our LMP-based values were inaccurate, none of the subjects reported doubts about the date of her LMP and LMP-derived estimates have agreed well with those determined by fetal biometry in our previous studies [66]. Another limitation was that, despite recruiting from high-risk clinics so as to increase the number with hypertensive complications, small sample sizes especially at earlier time points limited our power to detect differences between hypertensive and normal groups. While all the early-onset PE women had documented proteinuria, the lack of routine proteinuria testing in Bolivia restricted our ability to distinguish between PE and GH. In keeping with current recommendations [79], we therefore combined the late-onset women into a single PE/GH category. As other factors influence FHR and the Doppler indices considered here, future studies in larger numbers of subjects are required to confirm or refute study findings. No women with PE or GH were studied at low altitude, thus restricting our comparisons to normotensive versus hypertensive subjects at high altitude, and recommending the conduct of future studies to determine whether UtA blood flow reductions and Doppler indices of fetal hypoxia are greater in PE at high than low altitude.

The higher UtA blood flows seen in normal, healthy Andeans agreed with our and others' prior studies, as did the Andeans' protection from altitude-associated reductions in birth weight [40,66,72,80]. Lower UtA blood flow in association with IUGR in early-onset PE at high altitude also agreed with previous low-altitude studies finding lower UtA blood flow in IUGR than normal pregnancies as early as weeks 20–24 [29,30]. As noted previously [41], UtA blood flow was lower in early- but not late-onset PE or PE/GH, further underscoring likely differences in etiology between these two conditions. While abnormal UtA Doppler indices have not

Table 3. Summary of study observations in relation to those in the literature. GH, gestational hypertension; HA, high altitude; IUGR, intrauterine growth restriction; LA, low altitude; MCA, middle cerebral artery; n.d., no data; PE, preeclampsia; PI, pulsatility index; PSV, peak systolic velocity; TAM, time-averaged flow velocity; UmbA, umbilical artery; UtA, uterine artery.

Doppler index	IUGR (literature)	normal HA versus normal LA	normal versus early PE at HA	normal versus late PE/GH at HA
UtA PI	↑ [73]	=	↑	↑
UmbA PI	↑ [81]	↑ ($p = 0.07$)	↑	=
MCA PI	↓ [82]	↓	=	=
MCA/UmbA PI	↓ [83]	↓	↓	=
MCA PSV	↑ [77]	n.d.	↑	=
MCA/UmbA PSV	n.d.	n.d.	↑	=

been shown to have sufficient sensitivity for detecting IUGR (see [73] for review), no study has measured UtA volumetric flow and hence its inclusion in future studies is recommended for possibly improving detection. The higher UtA blood flows and heavier (more normal) birth weights seen in normal than early-onset PE at high altitude also agreed with previous findings of higher UtA blood flows and birth weights in normal Andean versus European pregnancies at high altitude [39,40].

Our UmbA and MCA Doppler PI findings also agreed with those at sea level (table 3) and in some, but not other, high-altitude reports. A randomized clinical trial has shown that an elevated UmbA PI indicative of greater systemic vascular resistance (such as we saw in the hypertensive versus normal high-altitude pregnancies or tended to be present in the normal high- versus low-altitude groups) predicted IUGR and other adverse outcomes in high-risk pregnancies [84]. Of interest was that we found a relationship between EFW and both the UmbA and MCA PI in all subjects or the normal high-altitude group alone, which is consistent with shunting of fetal cardiac output towards the cerebral circulation ('brain sparing') and away from other organs. A low MCA PI or a low MCA/UmbA PI ratio, both indicative of brain sparing, have also been shown to be useful surrogates for placental insufficiency and hypoxia [83], and for distinguishing fetuses with true IUGR from those who are small due to constitutional or other factors [85]. A low MCA/UmbA PI ratio is particularly useful, as poor neurodevelopmental outcomes can occur even in fetuses with normal UmbA PI but abnormal MCA/UmbA PI ratios, and has greater sensitivity than a low MCA PI for detecting IUGR and other complications because the MCA/UmbA PI ratio becomes abnormal earlier [85]. In keeping with these observations, we found that the MCA/UmbA PI ratio was lower throughout pregnancy in the normal high- versus low-altitude groups and tended to be more variable in the hypertensive than normal high-altitude women. While our MCA/UmbA PI values were not as low as those seen in severe IUGR at sea level [77], the MCA PI was lower in the normal high- than low-altitude pregnancies and marginally but not significantly lower at week 24 in PE versus normal women at high altitude. In a separate sample of high-altitude Andeans, we reported that week 36 MCA systolic/diastolic (S/D) values were below low-altitude values [66]; this agreed with the higher UmbA PI previously reported at high altitude but differed insofar as a lower MCA PI was not observed in those reports [21,86]. Such differences between studies may have been due to the use of S/D versus PI as the index of vascular resistance, to smaller sample sizes at weeks 20–24 in the Krampl report which is when we observed the greatest differences, or to the fact that the Postigo study was conducted at a single time point near term when MCA PI values at high and low altitude were more similar. We therefore concluded that the lower MCA/UmbA PI ratio seen throughout pregnancy in normal high- versus low-altitude pregnancies, the higher UmbA PI and the differing effects of gestational age on the MCA/UmbA PI ratio in the hypertensive versus normal high-altitude groups indicated that fetal hypoxia was modestly increased by high altitude alone and more markedly when the effects of high altitude and hypertensive complications were combined.

To buttress our UmbA and MCA PI observations and given Mari's report [77] that an increased MCA PSV better predicted fetal death than a low MCA PI, we also examined the MCA PSV, the UmbA PSV and their ratio. Unfortunately, MCA and UmbA PSV were not recorded in the normal pregnancies at low altitude and hence comparisons could only be made between the normal and hypertensive groups at high altitude. Consistent with the higher UmbA PI, we found a lower UmbA PSV that was not likely to be due to greater fetal haematocrit and viscosity as previously suggested [86] since anaemic fetuses also have altered PSV. Like Mari [77], we found that the MCA PSV increased more consistently with advancing gestation than did the MCA PI, but our MCA PSV values were not higher in the hypertensive than normal pregnancies, perhaps as the result of the more severe IUGR in that study insofar as birth weights ranged from 282 to 660 g, whereas our values averaged 1555 g in the early-onset PE and 2773 g in the late-onset PE/GH groups. Similar to the relationships observed with the MCA and UmbA PI, EFW was positively correlated with the UmbA PSV and the MCA PSV in the combined high-altitude groups or the normal group alone. However, the MCA/UmbA PSV ratio was more consistently higher in the PE/GH versus normal pregnancies at high altitude than was the MCA/UmbA PI ratio lower, suggesting that a high MCA/UmbA PSV ratio may be an additional useful index of impaired cerebroplacental perfusion and fetal hypoxia.

While a number of studies address the important effects of pregnancy on UtA and mesometrial artery vasodilator, vasoconstrictor and growth responses (see [10] for review) and the mechanisms by which chronic hypoxia interferes with these responses [87–89], whether and if so how such factors contribute to differences in UtA blood flow between normal and IUGR pregnancies at low altitude or between newcomer

and multigenerational high-altitude residents is unclear. Supporting the point made above that different mechanisms are likely involved in regulating UtA diameter and flow velocity, UtA diameters were similar in the normal versus PE or GH high-altitude Andeans but velocity lower in the early-onset PE women. Our data also indicate that while impaired placentation limits UtA blood flow in early-onset disease, increased flow velocity can raise UtA blood flow to normal levels even in a setting of increased UtA PI in women with late-onset PE/GH. The absence of differences in per cent Amerindian AIMs between the normal versus hypertensive groups was consistent with the likelihood that Andean ancestry helps maintain the normal pregnancy increase in UtA diameter but does not confer protection from early-onset PE or late-onset PE/GH [41]. In other words, while Andeans are protected relative to Europeans from altitude-associated reductions in fetal growth [72,90], such protection does not appear to extend to hypertensive complications of pregnancy; however, future studies with larger samples and careful documentation of early- and late-onset PE or GH and ancestry are required to confirm or refute this possibility.

The very high levels of UtA blood flow seen in the high- versus low-altitude Andeans in combination with near-normal levels of arterial O_2 content would be expected to yield greater uteroplacental O_2 delivery than at sea level [68]. Thus, the question arises as to why the high-altitude Andeans' UtA blood flow is so high. The approximately 1.5 l min^{-1} values for bilateral UtA flow in the present report are similar to what we reported in a separate sample of Andeans at 3600 m [66], but considerably greater than those reported from the same altitude by Zamudio *et al.* [40]. Higher levels of UtA blood flow in Andean than European residents of high altitude were seen in both reports and due to greater diameter in the Andeans, but flow velocities were markedly lower in the Zamudio study perhaps reflecting study differences in angle of insonation and the corrections employed for estimating velocity.

There are several possibilities as to why the Andeans' UtA blood flow levels are so high. One is that not all the UtA blood flow reaches the intervillous space given the presence of myometrial arterio-venous anastomoses [91], which may act as a reserve for protecting against intermittent hypoxia and ischemia/reperfusion injury [92]. Consistent with this are the higher anti-oxidant levels seen in Andean than European pregnant women at high altitude [93]. A second possibility is that the very high UtA blood flow is due to biomechanical factors serving to increase UtA diameter, as a large diameter permits high blood flow at slower velocity and thus protects the fragile fetal villi from the damaging effects of high velocity and villous compression [92]. Consistent with less villous compression are the higher umbilical venous blood flows reported in Andeans than Europeans at high altitude [21]. Third, high UtA flow could be vital for delivering nutrients other than O_2, namely glucose and amino acids; this is supported by the greater placental glucose consumption seen at high than low altitude although differences between Andeans and Europeans were not observed [57]. Fourth, as noted above, the human placenta operates as a venous equilibrator and thus requires higher blood flow to achieve the same O_2 delivery compared with a countercurrent system of gas exchange [20]. Using the haemoglobin and SaO_2 values we observed at high altitude [66], the umbilical venous pO_2 values reported by Postigo *et al.* [21] and assuming a 10 mmHg uterine venous-umbilical venous (UmbV) pO_2 gradient, we estimate that 62% greater UtA blood flow is required at high altitude to achieve the same level of O_2 delivery as at low altitude (figure 1*b*). Interestingly, this approximates the 74% (1422/818 ml min^{-1}) increase seen at week 36 in Andean compared with European women at 3600 m [66]. In short, while the full explanation for the Andeans' very high levels of UtA blood at high altitude is unknown, it appears to be protective against altitude-associated reductions in fetal growth given its association with less pronounced alterations in Doppler indices of fetal hypoxia and heavier (more normal) birth weight infants. However, the mechanisms by which UtA blood flow influence fetal growth are likely to be complex and, to quote Andrew Murray, be more than a simple question of supply and demand [94].

In summary, our Andean studies showed that Doppler indices of fetal hypoxia were modestly greater in normal pregnancies at high than low altitude, but UtA blood flow greater and infant birth weights similar. UtA blood flow was markedly lower in early-onset PE versus normal healthy women at high altitude, and their fetuses more hypoxic as indicated by lower FHR, lower UmbA PSV, higher MCA/UmbA PSV and more IUGR. We concluded that fetal growth was well defended by higher UtA blood flows in normal pregnancies at high altitude but when compounded by early-onset PE, lower UtA blood flow exaggerated fetal hypoxia and caused the fetus to respond by decreasing cardiac output and redistributing blood flow to help maintain brain development at the expense of growth elsewhere. Future studies are required to extend the present observations on the relationship between UtA blood flow and fetal hypoxia in early- versus late-onset PE or GH and to define the metabolic processes responsible for the fetal growth restriction observed. We speculate that UtA blood flow is not only an important supply line but also a trigger for stimulating the metabolic and other processes regulating feto-placental metabolism and growth. Consistent with metabolic changes being involved are the decreased mitochondrial complex protein and activity levels, and increased placental glucose consumption observed in high- versus low-altitude placenta from Colorado and Bolivia, respectively [57,59]. Also suggesting the involvement of metabolic factors are our genome studies that have identified the α-1 catalytic subunit of a key metabolic sensor, adenosine monophosphate kinase, as a region that has been subjected to natural selection in Andeans [42,43] and whose genetic variation is associated with birth weight and mRNA expression levels of mTOR pathway proteins [48], which is a pathway that has been previously implicated in altitude-associated IUGR [49].

In relation to the evolutionary trade-offs theme of this issue, pregnancy studies in general and under the circumstances of the reduced O_2 availability at high altitude provide a graphic example of the evolutionary trade-offs required for reproductive success. Such trade-offs are illustrated by the effects of the anatomical changes required for bipedalism and the nature of placental gas exchange on the pregnancy rise in maternal UtA blood flow and, in turn, the influences of UtA blood flow on fetal growth and well-being. Studies at high altitude offer the advantages of a relatively uniform hypoxic stimulus and human populations with multigenerational high-altitude residence in which genetic adaptation has occurred. Uncovering the specific genes involved and the biochemical and metabolic mechanisms by which they exert their effects can thus both improve our understanding of evolutionary process and lead to improved detection as well as treatment for pregnancy complications that will benefit not only the 140 million high-altitude

residents worldwide but also the countless others affected by pregnancy complications characterized by fetal hypoxia and growth restriction.

Acknowledgements. We thank the women who kindly agreed to participate and SonoSite for their generous loan of the ultrasound equipment. We also express our appreciation for the numerous persons at the Instituto Boliviano de Biología de Altura in La Paz—particularly, Armando Rodriquez, Martha Aquilar, Diva Bellido, Marcelino Guarachi and Mercedes Villena—and the hospital administrators, Ob/Gyn physicians and nurses at the Hospital de la Mujer, Hospital Materno-Infantil, Hospital Andes, Hospital Boliviano Holandes and Hospital Corea in El Alto for their help in the conduct of the high-altitude studies. Persons at the Clinica Siraní in Santa Cruz—particularly, Drs Carmelo Rodriquez, Jessica Pardo, M. Pilar Garcia, Henry Yamashiro and Julio Roca—kindly assisted with the conduct of the low-altitude studies. We also thank Drs Lindsay Reardon and Anne Ladenburger, formerly part of the mentored scholarly activity programme of the University of Colorado Denver School of Medicine, for their help in retrieving birth weights at high altitude. Dr John C. Hobbins and Prof. Giacomo Meschia provided valuable advice regarding the clinical significance of alterations in Doppler indices, placental gas exchange and the utility of Doppler measures of fetal hypoxia.

Funding statement. Grant support was provided by NIH-HLBI 079647 and TW 01188.

Endnotes

[1]Another implication is to increase the mother's vulnerability to postpartum hemorrhage when the placenta separates from the uterine vascular bed.

[2]Clearly, improvements in the accuracy of measuring UtA flow velocity are needed. Rigano [28] applied a mathematical model for calculating a spatial velocity profile, but velocity values were not presented for all subjects preventing their comparison with other studies. A. J. Micco developed a patented system for calculating the instantaneous mean of the velocity distribution throughout the cardiac cycle, which was used by Palmer [24]; its incorporation into current ultrasound units could improve the accuracy of velocity determination.

References

1. Elliot M, Crespi BJ. 2015 Genetic recapitulation of human pre-eclampsia risk during convergent evolution of reduced placental invasiveness in eutherian mammals. *Phil. Trans. R. Soc. B* **370**, 20140069. (doi:10.1098/rstb.2014.0069)
2. Trevathan W. 2015 Primate pelvic anatomy and implications for birth. *Phil. Trans. R. Soc. B* **370**, 20140065. (doi:10.1098/rstb.2014.0065)
3. Wells J. 2015 Between Scylla and Charybdis: re-negotiating resolution of the 'obstetric dilemma' in response to ecological change. *Phil. Trans. R. Soc. B* **370**, 20140067. (doi:10.1098/rstb.2014.0067)
4. Carter AM, Enders AC, Pijnenborg R. 2015 The role of invasive trophoblast in implantation and placentation of primates. *Phil. Trans. R. Soc. B* **370**, 20140070. (doi:10.1098/rstb.2014.0070)
5. Moffett A, Hiby SE, Sharkey AM. 2015 The role of the maternal immune system in the regulation of human birthweight. *Phil. Trans. R. Soc. B* **370**, 20140071. (doi:10.1098/rstb.2014.0071)
6. Ueland K, Novy MJ, Peterson EN, Metcalfe J. 1969 Maternal cardiovascular dynamics. IV. The influence of gestational age on the maternal cardiovascular response to posture and exercise. *Am. J. Obstet. Gynecol.* **104**, 856–864.
7. Pijnenborg R, Vercruysse L, Carter AM. 2011 Deep trophoblast invasion and spiral artery remodelling in the placental bed of the chimpanzee. *Placenta* **32**, 400–408. (doi:10.1016/j.placenta.2011.02.009)
8. Pijnenborg R, Vercruysse L, Carter AM. 2011 Deep trophoblast invasion and spiral artery remodelling in the placental bed of the lowland gorilla. *Placenta* **32**, 586–591. (doi:10.1016/j.placenta.2011.05.007)
9. Rockwell LC, Vargas E, Moore LG. 2003 Human physiological adaptation to pregnancy: inter- and intraspecific perspectives. *Am. J. Hum. Biol.* **15**, 330–341. (doi:10.1002/ajhb.10151)
10. Osol G, Moore LG. 2014 Maternal uterine vascular remodeling during pregnancy. *Microcirculation* **21**, 38–47. (doi:10.1111/micc.12080)
11. Pates JA, Hatab MR, McIntire DD, Cunningham FG, Twickler DM. 2010 Determining uterine blood flow in pregnancy with magnetic resonance imaging. *Magn. Reson. Imaging* **28**, 507–510. (doi:10.1016/j.mri.2009.12.009)
12. Wehrenberg WB, Chaichareon DP, Dierschke DJ, Rankin JH, Ginther OJ. 1977 Vascular dynamics of the reproductive tract in the female rhesus monkey: relative contributions of ovarian and uterine arteries. *Biol. Reprod.* **17**, 148–153. (doi:10.1095/biolreprod17.1.148)
13. Konje JC, Kaufmann P, Bell SC, Taylor DJ. 2001 A longitudinal study of quantitative uterine blood flow with the use of color power angiography in appropriate for gestational age pregnancies. *Am. J. Obstet. Gynecol.* **185**, 608–613. (doi:10.1067/mob.2001.117187)
14. Abitbol MM, Demeter E, Benarosh T. 1980 Uterine and ovarian artery blood flow in the rhesus monkey near term. *Am. J. Obstet. Gynecol.* **138**, 414–421.
15. Bamfo JE, Odibo AO. 2011 Diagnosis and management of fetal growth restriction. *J. Pregnancy* **2011**, 640715. (doi:10.1155/2011/640715)
16. Chapman AB *et al*. 1998 Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. *Kidney Int.* **54**, 2056–2063. (doi:10.1046/j.1523-1755.1998.00217.x)
17. Burchell RC. 1967 Arterial blood flow into the human intervillous space. *Am. J. Obstet. Gynecol.* **98**, 303–311.
18. Jauniaux E, Poston L, Burton GJ. 2006 Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum. Reprod. Update* **12**, 747–755. (doi:10.1093/humupd/dml016)
19. Collins SL, Grant D, Black RS, Vellayan M, Impey L. 2011 Abdominal pregnancy: a perfusion confusion? *Placenta* **32**, 793–795. (doi:10.1016/j.placenta.2011.07.032)
20. Battaglia FC, Meschia G. 2013 Review of studies in human pregnancy of uterine and umbilical blood flows. *Dev. Period Med.* **17**, 287–292.
21. Postigo L *et al*. 2009 Where the O_2 goes to: preservation of human fetal oxygen delivery and consumption at high altitude. *J. Physiol.* **587**, 693–708. (doi:10.1113/jphysiol.2008.163634)
22. Fujikura T, Yoshida J. 1996 Blood gas analysis of placental and uterine blood during cesarean delivery. *Obstet. Gynecol.* **87**, 133–136. (doi:10.1016/0029-7844(95)00300-2)
23. Thaler I, Manor D, Itskovitz J, Rottem S, Levit N, Timor-Tritsch I, Brandes JM. 1990 Changes in uterine blood flow during human pregnancy. *Am. J. Obstet. Gynecol.* **162**, 121–125. (doi:10.1016/0002-9378(90)90834-T)
24. Palmer SK, Zamudio S, Coffin C, Parker S, Stamm E, Moore LG. 1992 Quantitative estimation of human uterine artery blood flow and pelvic blood flow redistribution in pregnancy. *Obstet. Gynecol.* **80**, 1000–1006.
25. Dickey RP, Hower JF. 1995 Ultrasonographic features of uterine blood flow during the first 16 weeks of pregnancy. *Hum. Reprod.* **10**, 2448–2452. (doi:10.1093/oxfordjournals.humrep.a136317)
26. Bernstein IM, Ziegler WF, Leavitt T, Badger GJ. 2002 Uterine artery hemodynamic adaptations through the menstrual cycle into early pregnancy. *Obstet. Gynecol.* **99**, 620–624. (doi:10.1016/S0029-7844(01)01787-2)
27. Julian CG *et al*. 2008 Lower uterine artery blood flow and higher endothelin relative to nitric oxide metabolite levels are associated with reductions in birth weight at high altitude. *Am. J. Physiol.* **295**, R906–R915.
28. Rigano S, Ferrazzi E, Boito S, Pennati G, Padoan A, Galan H. 2010 Blood flow volume of uterine arteries in human pregnancies determined using 3D and bi-dimensional imaging, angio-Doppler, and fluid-dynamic modeling. *Placenta* **31**, 37–43. (doi:10.1016/j.placenta.2009.10.010)
29. Konje JC, Howarth ES, Kaufmann P, Taylor DJ. 2003 Longitudinal quantification of uterine artery blood

volume flow changes during gestation in pregnancies complicated by intrauterine growth restriction. *BJOG* **110**, 301–305. (doi:10.1046/j.1471-0528.2003.t01-1-02163.x)

30. Jeffreys RM, Stepanchak W, Lopez B, Hardis J, Clapp 3rd JF. 2006 Uterine blood flow during supine rest and exercise after 28 weeks of gestation. *BJOG* **113**, 1239–1247. (doi:10.1111/j.1471-0528.2006.01056.x)
31. Caton D, Crenshaw C, Wilcox CJ, Barron DH. 1979 O_2 delivery to the pregnant uterus: its relationship to O_2 consumption. *Am. J. Physiol.* **237**, R52–R57.
32. Lichty JA, Ting RY, Bruns PD, Dyar E. 1957 Studies of babies born at high altitudes. I. Relation of altitude to birth weight. *Am. J. Dis. Child.* **93**, 666–669. (doi:10.1001/archpedi.1957.02060040668009)
33. McCullough RE, Reeves JT, Liljegren RL. 1977 Fetal growth retardation and increased infant mortility at high altitude. *Obstet. Gynecol. Surv.* **32**, 596–598. (doi:10.1097/00006254-197709000-00012)
34. Jensen GM, Moore LG. 1997 The effect of high altitude and other risk factors on birthweight: independent or interactive effects? *Am. J. Public Health* **87**, 1003–1007. (doi:10.2105/AJPH.87.6.1003)
35. Moore LG. 2001 Human genetic adaptation to high altitude. *High Alt. Med. Biol.* **2**, 257–279. (doi:10.1089/152702901750265341)
36. Niermeyer SN, Moore LG. In press. Perinatal physiology and its impact on pulmonary hypertension and evolutionary adaptation. *Pulmon. Circ.* **5**.
37. Moore LG, Zamudio S, Zhuang J, Sun S, Droma T. 2001 Oxygen transport in Tibetan women during pregnancy at 3,658 m. *Am. J. Phys. Anthropol.* **114**, 42–53. (doi:10.1002/1096-8644(200101)114:1<42::AID-AJPA1004>3.0.CO;2-B)
38. Chen D, Zhou X, Zhu Y, Zhu T, Wang J. 2002 Comparison study on uterine and umbilical artery blood flow during pregnancy at high altitude and at low altitude. *Zhonghua Fu Chan Ke Za Zhi* **37**, 69–71.
39. Wilson MJ *et al*. 2007 Greater uterine artery blood flow during pregnancy in multigenerational (Andean) than shorter-term (European) high-altitude residents. *Am. J. Physiol.* **293**, R1313–R1324.
40. Zamudio S *et al*. 2007 Maternal oxygen delivery is not related to altitude- and ancestry-associated differences in human fetal growth. *J. Physiol.* **582**, 883–895. (doi:10.1113/jphysiol.2007.130708)
41. Browne VA *et al*. 2011 High-end arteriolar resistance limits uterine artery blood flow and restricts fetal growth in preeclampsia and gestational hypertension at high altitude. *Am. J. Physiol.* **300**, R1221–R1229.
42. Bigham A *et al*. 2010 Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLoS Genet.* **6**, e1001116. (doi:10.1371/journal.pgen.1001116)
43. Bigham AW *et al*. 2009 Identifying positive selection candidate loci for high-altitude adaptation in Andean populations. *Hum. Genomics* **4**, 79–90. (doi:10.1186/1479-7364-4-2-79)
44. Beall CM *et al*. 2010 Natural selection on EPAS1 (HIF2α) associated with low hemoglobin concentration in Tibetan highlanders. *Proc. Natl Acad. Sci. USA* **107**, 11 459–11 464. (doi:10.1073/pnas.1002443107)
45. Xu S *et al*. 2011 A genome-wide search for signals of high-altitude adaptation in Tibetans. *Mol. Biol. Evol.* **28**, 1003–1011. (doi:10.1093/molbev/msq277)
46. Peng Y *et al*. 2011 Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. *Mol. Biol. Evol.* **28**, 1075–1081. (doi:10.1093/molbev/msq290)
47. Simonson TS *et al*. 2010 Genetic evidence for high-altitude adaptation in Tibet. *Science* **329**, 72–75. (doi:10.1126/science.1189406)
48. Bigham AW *et al*. 2014 Maternal genetic variation in EDNRA and PRKAA1 is associated with infant birth weight. *Physiol. Genomics* **46**, 687–697.
49. Yung HW, Cox M, Tissot van Patot M, Burton GJ. 2012 Evidence of endoplasmic reticulum stress and protein synthesis inhibition in the placenta of non-native women at high altitude. *FASEB J.* **26**, 1970–1981. (doi:10.1096/fj.11-190082)
50. Frisancho AR. 1970 Developmental responses to high altitude hypoxia. *Am. J. Phys. Anthropol.* **32**, 401–407. (doi:10.1002/ajpa.1330320310)
51. Khalid ME, Ali ME, Ali KZ. 1997 Full-term birth weight and placental morphology at high and low altitude. *Int. J. Gynaecol. Obstet.* **57**, 259–265. (doi:10.1016/S0020-7292(97)00067-2)
52. Chabes A, Pereda J, Hyams L, Barrientos N, Perez J, Campos L, Monroe A, Mayorga A. 1968 Comparative morphometry of the human placenta at high altitude and at sea level. I. The shape of the placenta. *Obstet. Gynecol.* **31**, 178–185. (doi:10.1097/00006250-196802000-00005)
53. Reshetnikova OS, Burton GJ, Milovanov AP. 1994 Effects of hypobaric hypoxia on the fetoplacental unit: the morphometric diffusing capacity of the villous membrane at high altitude. *Am. J. Obstet. Gynecol.* **171**, 1560–1565. (doi:10.1016/0002-9378(94)90402-2)
54. Kruger H, Arias-Stella J. 1970 The placenta and the newborn infant at high altitudes. *Am. J. Obstet. Gynecol.* **106**, 586–591.
55. Tissot van Patot MC, Valdez M, Becky V, Cindrova-Davies T, Johns J, Zwerdling L, Jauniaux E, Burton GJ. 2009 Impact of pregnancy at high altitude on placental morphology in non-native women with and without preeclampsia. *Placenta* **30**, 523–528. (doi:10.1016/j.placenta.2009.04.002)
56. Mayhew TM. 1998 Thinning of the intervascular tissue layers of the human placenta is an adaptive response to passive diffusion *in vivo* and may help to predict the origins of fetal hypoxia. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **81**, 101–109. (doi:10.1016/S0301-2115(98)00154-7)
57. Zamudio S *et al*. 2010 Hypoglycemia and the origin of hypoxia-induced reduction in human fetal growth. *PLoS ONE* **5**, e8551. (doi:10.1371/journal.pone.0008551)
58. Tissot van Patot MC *et al*. 2010 Human placental metabolic adaptation to chronic hypoxia, high altitude: hypoxic preconditioning. *Am. J. Physiol.* **298**, R166–R172.
59. Colleoni F *et al*. 2013 Suppression of mitochondrial electron transport chain function in the hypoxic human placenta: a role for miRNA-210 and protein synthesis inhibition. *PLoS ONE* **8**, e55194. (doi:10.1371/journal.pone.0055194)
60. Gu W, Jones CT, Parer JT. 1985 Metabolic and cardiovascular effects on fetal sheep of sustained reduction of uterine blood flow. *J. Physiol.* **368**, 109–129.
61. Carter AM. 2000 Placental oxygen consumption. Part I: *in vivo* studies—a review. *Placenta* **21**(Suppl. A), S31–S37. (doi:10.1053/plac.1999.0513)
62. Owens JA, Falconer J, Robinson JS. 1987 Effect of restriction of placental growth on oxygen delivery to and consumption by the pregnant uterus and fetus. *J. Dev. Physiol.* **9**, 137–150.
63. Keyes LE, Armaza JF, Niermeyer S, Vargas E, Young DA, Moore LG. 2003 Intrauterine growth restriction, preeclampsia, and intrauterine mortality at high altitude in Bolivia. *Pediatr. Res.* **54**, 20–25. (doi:10.1203/01.PDR.0000069846.64389.DC)
64. Palmer SK, Moore LG, Young D, Cregger B, Berman JC, Zamudio S. 1999 Altered blood pressure course during normal pregnancy and increased preeclampsia at high altitude (3100 meters) in Colorado. *Am. J. Obstet. Gynecol.* **180**, 1161–1168. (doi:10.1016/S0002-9378(99)70611-3)
65. Miller S, Tudor C, Thorsten VR, Craig S, Le P, Wright LL, Varner MW. 2007 Maternal and neonatal outcomes of hospital vaginal deliveries in Tibet. *Int. J. Gynaecol. Obstet.* **98**, 217–221. (doi:10.1016/j.ijgo.2007.03.033)
66. Julian CG *et al*. 2009 Augmented uterine artery blood flow and oxygen delivery protect Andeans from altitude-associated reductions in fetal growth. *Am. J. Physiol.* **296**, R1564–R1575.
67. Moore LG, Jahnigen D, Rounds SS, Reeves JT, Grover RF. 1982 Maternal hyperventilation helps preserve arterial oxygenation during high-altitude pregnancy. *J. Appl. Physiol.* **52**, 690–694.
68. Moore LG. 2011 Uterine blood flow as a determinant of feto-placental development. In *The placenta and human developmental programming* (eds GJ Burton, DJP Barker, A Moffett, KL Thornburg), pp. 126–146. Cambridge, UK: Cambridge University Press.
69. Howard RC, Bruns PD, Lichty JA. 1957 Arterial oxygen saturation and hematocrit values at birth. Studies of babies born at high altitude (III). *Am. J. Dis. Child.* **93**, 674–678.
70. Sobrevilla LA, Cassinelli MT, Carcelen A, Malaga JM. 1971 Human fetal and maternal oxygen tension and acid-base status during delivery at high altitude. *Am. J. Obstet. Gynecol.* **111**, 1111–1118.
71. Ballew C, Haas JD. 1986 Hematologic evidence of fetal hypoxia among newborn infants at high

altitude in Bolivia. *Am. J. Obstet. Gynecol.* **155**, 166–169. (doi:10.1016/0002-9378(86)90104-3)

72. Soría R, Julian C, Vargas E, Moore L, Giussani D. 2013 Graduated effects of high-altitude hypoxia and highland ancestry on birth size. *Pediatr. Res.* **74**, 633–638. (doi:10.1038/pr.2013.150)
73. Society for Maternal-Fetal Medicine Publications, Berkley E, Chauhan SP, Abuhamad A. 2012 Doppler assessment of the fetus with intrauterine growth restriction. *Am. J. Obstet. Gynecol.* **206**, 300–308. (doi:10.1016/j.ajog.2012.01.022)
74. Mari G, Picconi J. 2008 Doppler vascular changes in intrauterine growth restriction. *Semin. Perinatol.* **32**, 182–189. (doi:10.1053/j.semperi.2008.02.011)
75. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. 1985 Estimation of fetal weight with the use of head, body, and femur measurements–a prospective study. *Am. J. Obstet. Gynecol.* **151**, 333–337. (doi:10.1016/0002-9378(85)90298-4)
76. Williams R, Creasy R, Cunningham G, Hawes W. 1982 Fetal growth and perinatal viability in California. *Obstet. Gynecol.* **58**, 624–632.
77. Mari G, Hanif F, Kruger M, Cosmi E, Santolaya-Forgas J, Treadwell MC. 2007 Middle cerebral artery peak systolic velocity: a new Doppler parameter in the assessment of growth-restricted fetuses. *Ultrasound Obstet. Gynecol.* **29**, 310–316. (doi:10.1002/uog.3953)
78. Redman CW, Sargent IL, Staff AC. 2014 IFPA Senior Award Lecture: making sense of pre-eclampsia—two placental causes of preeclampsia? *Placenta* **35**, S20–S25. (doi:10.1016/j.placenta.2013.12.008)
79. ACOG. 2013 Hypertension in pregnancy. *Obstet. Gynecol.* **122**, 1122–1131. (doi:10.1097/01.AOG.0000437382.03963.88)
80. Julian CG, Vargas E, Armaza JF, Wilson MJ, Niermeyer S, Moore LG. 2007 High-altitude ancestry protects against hypoxia-associated reductions in fetal growth. *Arch. Dis. Child. Fetal Neonatal Ed.* **92**, F372–F377. (doi:10.1136/adc.2006.109579)
81. Acharya G, Wilsgaard T, Berntsen GK, Maltau JM, Kiserud T. 2005 Reference ranges for serial measurements of umbilical artery Doppler indices in the second half of pregnancy. *Am. J. Obstet. Gynecol.* **192**, 937–944. (doi:10.1016/j.ajog.2004.09.019)
82. Ebbing C, Rasmussen S, Kiserud T. 2007 Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements. *Ultrasound Obstet. Gynecol.* **30**, 287–296. (doi:10.1002/uog.4088)
83. Bahado-Singh RO, Kovanci E, Jeffres A, Oz U, Deren O, Copel J, Mari G. 1999 The Doppler cerebroplacental ratio and perinatal outcome in intrauterine growth restriction. *Am. J. Obstet. Gynecol.* **180**, 750–756. (doi:10.1016/S0002-9378(99)70283-8)
84. Alfirevic Z, Stampalija T, Gyte GM. 2010 Fetal and umbilical Doppler ultrasound in high-risk pregnancies. *Cochrane Dababase Syst. Rev.* **1**, CD007529. (doi:10.1002/14651858.CD007529.pub2)
85. Cruz-Martinez R, Figueras F, Hernandez-Andrade E, Benavides-Serralde A, Gratacos E. 2011 Normal reference ranges of fetal regional cerebral blood perfusion as measured by fractional moving blood volume. *Ultrasound Obstet. Gynecol.* **37**, 196–201. (doi:10.1002/uog.7722)
86. Krampl E, Lees C, Bland JM, Espinoza Dorado J, Moscoso G, Campbell S. 2001 Fetal Doppler velocimetry at high altitude. *Ultrasound Obstet. Gynecol.* **18**, 329–334. (doi:10.1046/j.0960-7692.2001.00542.x)
87. Rockwell LC, Keyes LE, Moore LG. 2000 Chronic hypoxia diminishes pregnancy-associated DNA synthesis in guinea pig uteroplacental arteries. *Placenta* **21**, 313–319. (doi:10.1053/plac.1999.0487)
88. White MM, McCullough RE, Dyckes R, Robertson AD, Moore LG. 2000 Chronic hypoxia, pregnancy, and endothelium-mediated relaxation in guinea pig uterine and thoracic arteries. *Am. J. Physiol.* **278**, H2069–H2075.
89. Hu XQ, Xiao D, Zhu R, Huang X, Yang S, Wilson SM, Zhang L. 2012 Chronic hypoxia suppresses pregnancy-induced upregulation of large-conductance Ca_2^+-activated K^+ channel activity in uterine arteries. *Hypertension* **60**, 214–222. (doi:10.1161/HYPERTENSIONAHA.112.196097)
90. Julian CG, Hageman JL, Wilson MJ, Vargas E, Moore LG. 2011 Lowland origin women raised at high altitude are not protected against lower uteroplacental O_2 delivery during pregnancy or reduced birth weight. *Am. J. Hum. Biol.* **23**, 509–516. (doi:10.1002/ajhb.21167)
91. Schaaps JP *et al*. 2005 Shunting the intervillous space: new concepts in human uteroplacental vascularization. *Am. J. Obstet. Gynecol.* **192**, 323–332. (doi:10.1016/j.ajog.2004.06.066)
92. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. 2009 Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. *Placenta* **30**, 473–482. (doi:10.1016/j.placenta.2009.02.009)
93. Julian CG *et al*. 2012 Potential role for elevated maternal enzymatic antioxidant status in Andean protection against altitude-associated SGA. *J. Matern. Fetal Neonatal. Med.* **25**, 1233–1240. (doi:10.3109/14767058.2011.636102)
94. Murray AJ. 2012 Oxygen delivery and fetal-placental growth: beyond a question of supply and demand? *Placenta* **33**(Suppl. 2), e16–e22. (doi:10.1016/j.placenta.2012.06.006)