Haplogroup-associated differences in neonatal death and incidence of low birth weight at elevation: A preliminary assessment

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OBJECTIVE: We sought to assess reproductive fitness differences between mitochondrial deoxyribonucleic acid haplogroups at high altitude.

STUDY DESIGN: This study considers differences in outcomes of conception, birth weight, and neonatal mortality rates for 62 women classified according to haplogroups (B or non-B).

RESULTS: The number of low-weight births (<2500 g) for the non-B group was significant (P = .019). Mothers in the non-B group reported more spontaneous abortions (P = .171) and stillbirths (P = .301). The difference in conceptions per woman between groups was significant (P = .036). However, no difference in infants alive at 1 month of age was evident. Neonatal death was significant (P = .017). The odds of an unsuccessful outcome among mothers in the B group was compared with mothers in the non-B group and was significant (P = .029). The chance of an adverse outcome, that is, fetal or infant death before 1 month, for mothers in the B group was between 11.1% and 88.7% lower than for mothers in the non-B group.

CONCLUSIONS: The neonatal mortality rate for the non-B group was significantly elevated relative to the B group. The molecular basis for these observations is not clear. (Am J Obstet Gynecol 2000;182:1599-5.)

Key words: High-altitude, neonatal, infant death, birth weight, genotype

Efforts to reduce the incidence of neonatal and infant deaths in the developing world have emphasized nutrition, prenatal care, sanitation, and improved access to health care facilities. Still, in spite of significant advances and notable reductions in recent years, infant death in developing countries remains a serious health concern.[1-4]

An increased neonatal mortality rate is associated with low birth weight.[5] Furthermore, birth weight is known to be inversely related to altitude.[6-9] It is believed that high-altitude occupation is detrimental to intrauterine development, resulting in decreased birth weight and therefore contributing to increased infant mortality rates.[8, 10]

In the 17th century Father Cobo[11] noted reproductive disparities between native Andean and Spanish settlers. Modern research has demonstrated the physiologic consequences resulting from extended occupation at high elevations.[6, 12-15] Fetal development at high elevations may be compromised as a result of decreased arterial oxygenation[15] and altered umbilical blood acid-base measurements.[16] Evidence of physiologic compensation for pregnancy-related hypoxia is noted in placental abnormality as well.[12] Adaptive consequences include gaseous exchange facilitated by a thinning of villose membranes,[17] an increase in syncytial knots, and cytotrophoblastic cell formation at terminal villi caused by maternal hypoxia.[18] Additionally, fetal developmental response to a hypoxic environment includes enhanced erythropoiesis compared with that in low-altitude neonates.[19] Adaptive genetic factors have also been considered.[20]

The mitochondrial deoxyribonucleic acid (mtDNA) genome encodes enzymes responsible for cellular respiration and oxidative phosphorylation.[21] Inheritance of the 16.6-kb circular cytoplasmic genome is restricted to maternal lines, with a negligible paternal contribution. Mutations in coding regions are involved in numerous degenerative and metabolic disease processes.[22, 23] This study considers differences in outcomes of conception, birth weight, and neonatal mortality rates for mtDNA haplogroups for a high-altitude Andean population (>3800 m above sea level).

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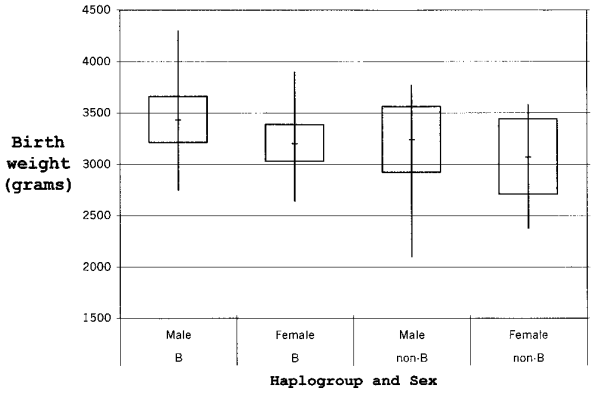

Fig 1. Mean birth weight with SD and range for B and non-B haplogroups according to sex.

Material and methods

Samples were collected with informed consent from hospitalized women (n = 62) admitted for obstetric service in Juliaca and Puno, Department of Puno, Peru. Each sample consisted of numerous scalp hairs with root. Family health background, health status, and pregnancy-related conditions, as well as reproductive histories documenting previous conceptions and outcomes, were provided by each parturient, physicians, and medical records when available. Birth weight was recorded for live births. Multiple births and stillbirths were excluded, as were premature births.

DNA was isolated as described.[24] Fragments of mtDNA were amplified by the polymerase chain reaction with primers specific for known polymorphisms. Fragments of mtDNA were amplified with primers (Appendix) flanking well-defined New World mtDNA polymorphisms.[25] Haplogroups A, C, and D contain restriction enzyme polymorphic sites in genic regions. Haplogroup B is defined by the presence of a 9–base pair intergenic deletion between the cytochrome oxidase subunit II gene and the lysine transfer ribonucleic acid gene. Haplogroup A, C, and D restriction length polymorphisms were resolved on a 2.0% NuSieve (FMC Bioproducts, Rockland, Me) agarose gel. A 4.5% Metaphor (FMC Bioproducts) agarose gel was used to screen for the haplogroup B length mutation. Positive and negative controls were run with restriction length polymorphism screening.

The predominance of the B haplogroup is well established among several South American populations.[26] Because of the predominance of the B haplogroup in this population, comparisons were made between non-B group (haplogroup A, C, and D) and B group samples.

Results

Participants. Sixty-three births (41 [65.1%] in the B group; 22 [34.9%] in the non-B group) were recorded for 62 women (40 [64.5%] in the B group; 22 [35.5%] in the non-B group). Non-B samples consisted of 3 A, 12 C, and 6 D markers, with 1 sample lacking the 9–base pair deletion classified only as non-B and not screened for A, C, or D markers. Twenty women in the B group and 7 in the non-B group were primigravid (P = .163). There were no differences in age of female participants or age at conception as determined by a t test (Table I). Of total births, 8 infants were excluded from birth weight analysis because of multiple gestation, preterm birth, or stillbirth. This information was retained and was included in subsequent considerations of reproductive history. For the B group births there were 19 female and 16 male infants. The non-B group was composed of 8 female and 12 male infants. There was no evidence of sex bias in regard to sex ratios for mothers in the B group or the non-B group (P = .918).

Table I. Participant populations and birth weight according to haplogroup

	Haplogroup		*Statistical significance*
	B	*Non-B*	
Participants (N = 62)	40	22	P = .022*
Age of parturients (y)	26.6 ± 2.2	26.5 ± 2.7	P = .966
Age at conception (y)	24.0 ± 1.26	24.7 ± 1.45	P = .478
No. of conceptions per woman	1.93 ± 0.36	2.55 ± 0.67	P = .036*
No. of births per woman	1.80 ± 0.34	2.27 ± 0.58	P = .065
No. of infants at 1 mo per woman	1.78 ± 0.34	2.00 ± 0.47	P = .213
Sex of infants			
Male	16	12	P = .304
Female	19	8	
Birth weight			
Male	3435.6 ± 225.2	3240.8 ± 322.0	P = .278
Female	3203.7 ± 183.6	3072.5 ± 366.8	P = .475
Number of low-weight births (<2500 g)	0	3	P = .019*

*Statistically significant.

Birth weight. For term infants (37-41 weeks), as determined by the attending physician, the mean birth weight for the total population by sex was 3316.4 ± 181.8 g for male infants and 3164.8 ± 156.2 g for female infants (Table I). Values according to sex and genotype indicate some difference. Results are depicted in Fig 1. The slight difference in birth weight between genotypes was analyzed by a general linear model and did not indicate statistical significance (P = .407). However, the number of low-weight births (<2500 g) for the non-B group was significant (P = .019). The minimum birth weight for the B group was 2640 g.

Reproductive histories. Reproductive histories for all female patients indicate 133 known conceptions resulting in either spontaneous abortion, a stillbirth, or a live birth. The

Table II. Outcomes of conception by haplogroup

Outcome	Haplogroup B	Haplogroup Non-B	Statistical significance
Conceptions (N = 133)	77 (57.9%)	56 (42.1%)	*P* = .069
Spontaneous abortion	1	3	*P* = .171*
Stillbirth	4	3	*P* = .301*
Live birth	72 (59.0%)	50 (41.0%)	*P* = .392
Dead within 1 month	1	6	*P* = .017†
Live at 1 month	71 (61.7%)	44 (38.2%)	*P* = .012†

*Statistically significant, Fisher exact test.
†Statistically significant.

distribution by haplogroups is represented in Table II. Seventy-seven (57.9%) total conceptions were attributed to women in the B group compared with 56 (42.1%) for women in the non-B group. Although the frequency of the B haplogroup in the population was substantially greater, these values were not significantly different from each other (*P* = .069). In the prenatal period mothers in the non-B group reported a greater proportion of spontaneous abortions (3/56) and stillbirths (3/56), as compared with mothers in the B group for the same outcomes (1/77 and 4/77, respectively). A 2 × 2 contingency table and the Fisher exact test were used to determine significance for spontaneous abortion (*P* = .171), and stillbirth (*P* = .301).

Complications. For total infant births, 6 (3 in the B group and 3 in the non-B group) were delivered by cesarean. Women in the B group reported 2 premature births (Fisher exact test, *P* = .346). Preeclampsia was noted in 3 pregnancies in the non-B group and 1 in the B group (Fisher exact test, *P* = .161). Additionally, 1 instance each of non-B monozygosity and B dizygosity was reported.

Neonatal death. Among live births, 1.4% (1/72) of infants in the B group and 12% (6/50) of infants in the non-B group had died before 1 month (*P* = .017). Overall, infants in the B group showed a 92.2% (71/77) survival rate from conception to 1 month compared with 78.6% (44/56) for infants in the non-B group (*P* = .024). Major contributors to neonatal death were recorded as failure to thrive and respiratory disease.

The number of conceptions per woman for the non-B group was 2.55 compared with 1.93 for the B group and was significant (*P* = .036). This significant difference was lost over time because no difference existed for infants alive at 1 month. The number of births per woman was 2.27 for the non-B group and 1.80 for the B group, and the number of infants alive at 1 month per woman was 2.00 for the B group and 1.78 for the non-B group (Fig 2).

Reproductive outcomes. To account for clustering caused by multiple births by the same mother, the data were analyzed by use of generalized estimating equations in the Genmod procedure of SAS (SAS Inc, Cary, NC). The odds of an unsuccessful outcome among mothers in the B group was compared with that among mothers in the non-B group, and the difference was significant (*P* = .029). The chance of an adverse outcome, that is, a fetal or infant death before 1 month, for mothers in the B group was between 11.1% and 88.7% lower than for mothers in the non-B group.

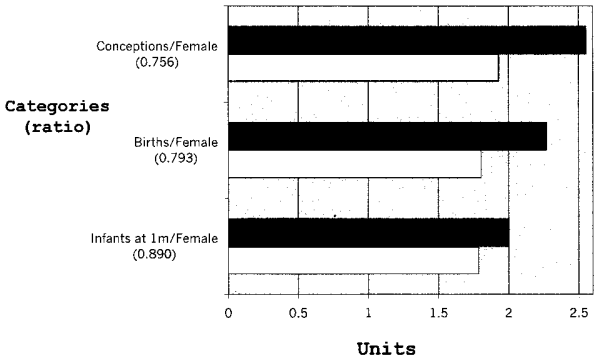

Fig 2. Reproductive estimates per woman depicting consistent increase in relative proportions of B haplogroup over time. *Black bars,* Non-B; *white bars,* B.

Risk. The extent to which conceptions in the non-B group were more likely than conceptions in the B group to result in a spontaneous abortion, stillbirth, or dead infant before 1 month was also determined. The odds ratio for reproductive events and survival in the non-B group showed the most pronounced disparity between birth and 1 month (odds ratio, 9.7). Similarly, increased risk was associated with conception to birth (odds ratio, 1.7) and conception to 1 month (odds ratio, 3.2).

Comment

Although there were nearly twice as many participants in the B group as in the non-B group, no significant difference in reported conceptions was detected. During the prenatal period participants in the non-B group reported a greater proportion of lethal events. This was reflected in a slightly elevated odds ratio. The number of infants surviving to 1 month as an outcome of conception was much higher for the B haplogroup. A significant component of this observed difference was an increased likelihood of death in the non-B group during the neonatal period (Fig 3).

An indicator associated with neonatal death is birth weight.[27] No difference in birth weight was detected between haplogroups for this study, possibly as a result of the small sample size. However, the difference in number of low-weight births was significant and may contribute to the difference seen in neonatal death for this population. Nevertheless, these observations deserve additional confirmation and subsequent investigation to understand the basis and application of these initial results.

This study found that the B haplogroup showed decreased infant mortality rates during the neonatal period.

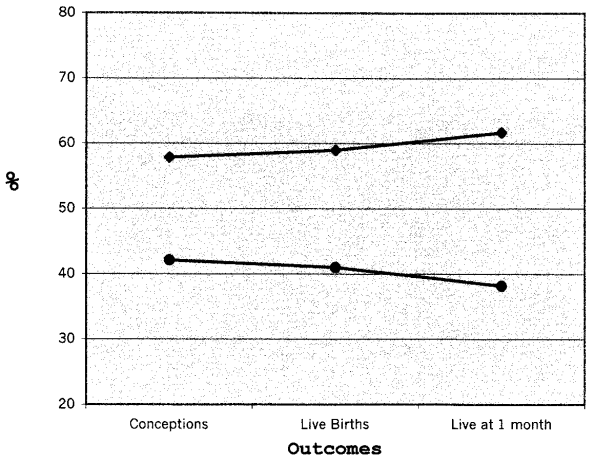

Fig 3. Haplogroup-related trends from conception to live birth at 1 month. *Triangles,* B; *circles,* non-B.

Infant mortality for the entirety of Peru was 47.9 per 1000.[28] Estimates extrapolated from the data presented here equate to 57.4 per 1000, with the largest component being 6 of 50 infants in the non-B group who died within 1 month post partum. The infant mortality estimate for neonates in the B group was significantly lower (1/72). Although poverty, social conditions, and access to health care facilities can influence these figures, no differences were noted in this sampling.

The migratory tendency of the Altiplano populations can contribute to the dynamics of genetic substructure and mortality estimates. The environmental effects produced by lowland arrivals to high elevations tended to contribute to lower infant birth weight and may possibly be related to genetic factors.[20] If the B haplogroup is more representative of the indigenous Altiplano populations, with the non-B group indicative of the migrant populations, this may correlate somewhat with the number of low-weight births in the non-B group.

Our reported infant mortality values are derived from physician-assisted births in a hospital setting, and actual infant mortality values are assumed to be greater. The cumulative effect of lethal events between conception and infants alive at 1 month indicates a significant difference between haplogroups. Accurate recall was verified against medical records when available and by health care providers. The events most subject to recall bias would be those between conception and birth, at a time when the participant might not detect a miscarriage. There was no significant difference between groups for this period (conception to birth). The significance of this study identified the disproportionate number of live-born infants who died within 1 month. A live birth and subsequent infant death would be less subject to recall bias.

With regard to the associated genetic polymorphism, further research on a larger scale is needed to confirm and explore the molecular basis of these detected differences. Although the B haplogroup frequency is elevated among indigenous Altiplano residents, it is not unique to the Peruvian Altiplano, being found in other Native American, Asian, and Hispanic populations. Because the marker used to define this haplotype is an intergenic deletion, it should not have an expressed or direct effect on selective pressure. If the genetic basis for this adaptive observation is mitochondrial encoded, it is a reproductive advantage that is perpetuated throughout generations along maternal lines. Because of the negligible mitochondrial DNA paternal contribution, any realized enhanced fitness would be strongly dependent on maternal genotype. Additionally, several nuclear alleles may influence these findings and contribute to adaptive fitness.

In conclusion, the data for this population indicate that the B mitochondrial DNA haplogroup is associated with reduced neonatal death and decreased incidence of low birth weight. Additionally, the likelihood of an adverse outcome of conception for women in the B group is significantly reduced comparatively. However, the basis for these observations is not clear.

The knowledge that certain genotypes are at a disadvantage at elevation to survive infancy makes preventative measures possible. Widespread genetic screening of pregnant women is costly and impractical in this setting. However, because population data are available that clearly show regional differences in the frequency and distribution of the genotypes in question, those populations with potential risk can be assessed on the basis of haplogroup prevalence, maternal geographic origins, and past reproductive events.

Nevertheless, understanding genotype is only one component of overall health maintenance among patient populations and is important but not crucial to health care and prevention. Adequate education and precautions that can decrease the probability and prevalence of risk factors can be implemented and practiced. Still, these data should provide some understanding of the range of factors contributing to infant death in the Peruvian Altiplano. This insight can be used as a step to inform potential mothers and health care providers to reduce the risk of infant death.

Gratitude is extended to the mothers and infants who participated in this study. We acknowledge the assistance and support of the hospitals of Juliaca and Puno, Department of Puno, Perú, and Dr G. Paredes E. for his cooperation in this research. We thank Drs I. Borecki and E. Amon for their interest and helpful review of an earlier manuscript. Statistical assistance was provided by Dr B. Scalia.

REFERENCES

1. Stidham GL. Emergencies in international child health. Curr Opin Pediatr 1997;9:254-8.

2. Biddulph J. Child health in the Third World. Med J Aust 1993;159:41-5.
3. Waterlow JC. Post-neonatal mortality in the Third World [letter]. Lancet 1988;2:1303.
4. Pinotti JA, Faundes A. Obstetric and gynecological care for Third World women. Int J Gynaecol Obstet 1984;22:449-55.
5. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med 1999;340:1234-8.
6. Jensen GM, Moore LG. The effect of high altitude and other risk factors on birthweight: independent or interactive effects? Am J Public Health 1997;87:1003-7.
7. Unger C, Weiser JK, McCullough RE, Keefer S, Moore LG. Altitude, low birth weight, and infant mortality in Colorado. JAMA 1988;259:3427-32.
8. Yip R. Altitude and birth weight. J Pediatr 1987;111:869-76.
9. McCullough RE, Reeves JT, Liljegren RL. Fetal growth retardation and increased infant mortility at high altitude. Obstet Gynecol Surv 1977;32:596-8.
10. Zamudio S, Palmer SK, Dahms TE, Berman JC, Young DA, Moore LG. Alterations in uteroplacental blood flow precede hypertension in preeclampsia at high altitude. J Appl Physiol 1995;79:15-22.
11. Cobo B. Volume 3: historia del nuevo mundo. Seville (Spain): E. Rasco; 1893.
12. Khalid ME, Ali ME, Ali KZ. Full-term birth weight and placental morphology at high and low altitude. Int J Gynaecol Obstet 1997;57:259-65.
13. Moore LG. Altitude-aggravated illness: examples from pregnancy and prenatal life. Ann Emerg Med 1987;16:965-73.
14. Moore LG, Brodeur P, Chumbe O, D'Brot J, Hofmeister S, Monge C. Maternal hypoxic ventilatory response, ventilation, and infant birth weight at 4,300 m. J Appl Physiol 1986;60: 1401-6.
15. Moore LG, Rounds SS, Jahnigen D, Grover RF, Reeves JT. Infant birth weight is related to maternal arterial oxygenation at high altitude. J Appl Physiol 1982;52:695-9.
16. Yancey MK, Moore J, Brady K, Milligan D, Strampel W. The effect of altitude on umbilical cord blood gases. Obstet Gynecol 1992;79:571-4.
17. Reshetnikova OS, Burton GJ, Milovanov AP. Effects of hypobaric hypoxia on the fetoplacental unit: the morphometric diffusing capacity of the villous membrane at high altitude. Am J Obstet Gynecol 1994;171:1560-5.
18. Ali KZ, Ali ME, Khalid ME. High altitude and spontaneous preterm birth. Int J Gynaecol Obstet 1996;54:11-5.
19. Ballew C, Haas JD. Hematologic evidence of fetal hypoxia among newborn infants at high altitude in Bolivia. Am J Obstet Gynecol 1986;155:166-9.
20. Niermeyer S, Yang P, Shanmina, Drolkar, Zhuang J, Moore LG. Arterial oxygen saturation in Tibetan and Han infants born in Lhasa, Tibet. N Engl J Med 1995;333:1248-52.
21. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457-65.
22. Johns DR. Seminars in medicine of the Beth Israel Hospital, Boston: mitochondrial DNA and disease. N Engl J Med 1995;333:638-44.
23. Wallace DC. Mitochondrial DNA mutations in diseases of energy metabolism. J Bioenerg Biomembr 1994;26:241-50.
24. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 1991;10:506-13.
25. Wallace DC, Garrison K, Knowler WC. Dramatic founder effects in Amerindian mitochondrial DNAs. Am J Phys Anthropol 1985;68:149-55.
26. Merriwether DA, Ferrell RE. The four founding lineage hypothesis for the New World: a critical reevaluation. Mol Phylogenet Evol 1996;5:241-6.
27. Spinillo A, Capuzzo E, Piazzi G, Baltaro F, Stronati M, Ometto A. Significance of low birthweight for gestational age among very preterm infants. Br J Obstet Gynaecol 1997;104:668-73.
28. Instituto Nacional de Estadistica y Informatica. Infant mortality by department. Lima: INEI; 1996.

Discussion

DR TARIQ A. SIDDIQI, Cincinnati, Ohio. Mitochondria are semiautonomous organelles found in all eukaryotic cells and are believed to have originated in ancestral eukaryotic cells through endosymbiosis of free-living bacteria capable of metabolizing oxygen, a highly reactive and potentially poisonous element in the environment. In fact, the outer mitochondrial membrane represents the original invaginated host plasma membrane, whereas the inner mitochondrial membrane represents the bacterial cell wall, the site of oxidative phosphorylation, which is the primary life-sustaining function of mitochondria.

Mitochondria contain a separate genetic system composed of mitochondrial DNA, which is capable of autonomous replication and specifies a small but essential part of the ribonucleic acid and proteins found in mitochondria. Most proteins present in mitochondria are, however, encoded by nuclear DNA because most mitochondrial genes have migrated to the nucleus over the course of evolution. Unlike nuclear DNA, most cells contain between 10^3 and 10^4 copies of mitochondrial DNA and an even higher number of copies in mature oocytes.

All mitochondrial DNA molecules in a single normal organism are identical at birth (a state known as *homoplasmy*) and contain no major gene repetitions, with the genome being extremely compressed with no introns. Mitochondrial DNA exists mainly as a closed-circular double-stranded DNA molecule of approximately 16.6 kb, encoding 13 proteins and the RNA molecules that are

Appendix. Haplogroups with defining polymorphic site

Haplogroup	*Primers (coordinates)*	*Polymorphism*	*Gene*
A	(580)-TATGTAGCTTACCTCCTCAA-(599) (704)-ATGCTTGCATGTGTAATCTTA-(684)	+*Hae*III Nucleotide 663	*12S rRNA*
B	(8196)-ACAGTTTCATGCCCATCGTCCT-(8217) (8317)-AATGTAAGTTAGCTTTACAGTG-(8295)	9 bp deletion Region V	Intergenic
C	(13160)-CTCTAACACTATGCTTAGGC-(13179) (13320)-GTGCAGGAATGCTAGGTGTG-(13301)	–*Hin*cII Nucleotide 13259	*NADH DH5*
D	(5100)-CTAACTACTACCGCATTCCT-(5119) (5231)-CAGGCCTCCTAGGGAGAGGA-(5211)	–*Alu*I Nucleotide 5176	*NADH DH2*

Primers are written as 5′→3′ with nucleotide coordinates. Coordinates are after Cambridge reference sequence (see reference 21 of article). Restriction enzymes used for screening and nucleotide positions are listed, as well as gene association.

necessary for the translocation of these proteins. The abnormal state of heteroplasmy where more than one mitochondrial DNA genotype occurs in an individual is extremely rare except in the noncoding regions. Defects caused by inherited or cumulative mutations and deletions may lead to heteroplasmy outside the noncoding regions, and this is believed to be central to many mitochondrial disease states and to apoptosis and aging.

Mitochondrial DNA is inherited maternally and does not recombine, and mutations thus accumulate sequentially through maternal lineages. This maternal mode of inheritance, high copy number, and rapid rate of mutation make mitochondrial DNA ideal for evolutionary studies. In fact, it is the study of human mitochondrial DNA variation that has led to the popularly known "African Eve" hypothesis whereby contemporary populations can be traced back to a single African ancestor who lived about 200,000 years ago. The mitochondrial DNA of most native North, Central, and South Americans has been shown to cluster into four lineages—haplogroups A, B, C, and D, which are identified by the presence or absence of characteristic restriction enzyme cleavage sites.

The stated objective of this study is to evaluate the effect of high altitude on birth weight and perinatal and neonatal mortality rates, yet the study is actually designed to retrospectively determine the reproductive performance of a unique Andean population living at >3800 m above sea level. The authors divided the study population into two groups on the basis of their laboratory-determined mitochondrial DNA haplotype. One group (non-B) comprised the combined haplogroups A, C, and D (with restriction enzyme polymorphic sites in genic regions) whereas the other group comprised haplogroup B (with a 9–base pair deletion in the small intergenic region between the cytochrome oxidase subunit II gene and the lysine transfer ribonucleic acid gene).

They then determined differences in outcomes of conception, birth weight, and neonatal death between these two groups on the basis of hospital records from 63 births for the entire population of 62 women (41 births in the B group; 22 births in the non-B group), as well as patient recall of prior normal and abnormal pregnancy outcomes. After a relatively complicated primary and secondary statistical analysis, the authors concluded that the overall perinatal and neonatal mortality rates were increased in the non-B group when compared with the B group, as were the adverse pregnancy events of infertility, spontaneous abortion, and stillbirth.

Because there was no non–high altitude control population for comparison, how do you relate any adverse pregnancy outcome to the effect of high altitude or to the makeup of mitochondrial DNA (especially because you seem to imply that high-altitude low birth weight may be related to impaired cellular respiration and oxidative phosphorylation)?

Because there is an apparent predominance of the B haplogroup, not only in the population studied but also among other South American populations, do you believe that the mitochondrial DNA intergenic deletion confers a reproductive advantage over the other haplogroups with polymorphic sites in genic regions?

Mitochondrial DNA is perpetuated through maternal lines, and it is possible that the B haplogroup is associated with other genetic advantages such that, over time, the non-B haplogroups will eventually die out. Are there any other known survival advantages associated with the B haplogroup, or are there any specific health impairments or degenerative disorders seen more frequently in the non-B haplogroups?

Although the number of low-birth-weight infants was significantly greater in the non-B group, do these data really have any meaning considering the small and unequal sample sizes and the retrospective nature of the study? Did you perform a power analysis to determine the number of mothers needed in each group to achieve 95% accuracy?

What measures did you use to ensure accurate recall of reproductive histories? Selective recall could easily have biased the data, especially in the presence of a small sample size.

DR WOODWARD (Closing). This study considered the difference between B and non-B for a high-altitude population and was intended as a preliminary assessment. On the basis of these findings we intend to expand this research to include high- and low-altitude populations where these haplogroups are present. We have subsequently initiated a much more comprehensive follow-up specifically designed to test the reproductive fitness of the B haplogroup at 4 different elevations from sea level to 13,000 feet.

There is clearly a predominance of the B haplogroup in the population studied. This initial observation led us to consider the basis for this from a variety of measures (Mark J. Rowe looked at longevity and found B individuals to live longer as well, plus the body mass index data, growth curves, and the like). We know that in this area the B lineage is the most diverse, having polymorphisms in noncoding and coding regions. Furthermore, the B lineage is the oldest lineage in this location (but not necessarily in South America). However, the B lineage is not the predominant haplogroup in South American populations and is entirely absent in numerous subjects (from Merriwether and Ferrell; see reference 26 of article). The notion of the predominant B in South America is related to the populations studied. Merriwether and Ferrell included several Aymara populations (genetically and geographically related to our population) and heavily weighted the total Bs in South America (sample sizes of ≥100 when others at the time were using ≤30). Our analysis of >6000 samples from more than 50 locations in Peru (coast, mountains, jungles) supports the fact that the predominance of the B haplogroup is a geographic and very possibly altitude-related phenomenon. We do not believe that the 9–base pair deletion confers any selective advantage; it is intergenic and not expressed. We suggest that it can be used as a marker, linked to mtDNA or nuclear polymorphisms, which may confer a reproductive advantage at elevation. This may or may not be in the electron transport chain (although Mark J. Rowe has identified ex-

pressed mutations in the B haplogroup [ND1 subunit?]). Several studies have shown adaptive consequences in high-altitude Andean populations, including hemoglobin concentration (and something else that contributes to reduced blood viscosity, clearly an advantage at high elevation).

In the absence of migration this would be true. It appears that until relatively recently the B-haplogroup frequency was even higher than current values. This decrease has most likely occurred in the last 500 years as a result of economic development and colonial/Inca population displacement. The mainland "traditional" locations retain elevated B, whereas the mainland "commercial" centers show the largest percentage of non-B. The economic pull to these centers is bringing large numbers of lowland migrants to the Altiplano. (We determined in Juliaca alone, in another article, that 94% of samples were nonnative in the previous two generations. However, in traditional centers this number is <5% and the B frequency is at or near fixation.) Because of migration and the number of non–high-altitude peoples settling in the Altiplano, we believe that if this does represent a marker for reproductive advantage, a simple personal history can help to identify those mothers at increased risk. This is something that health care providers have noticed but not understood. We believe that this finding regarding B (Altiplano/high-altitude type) and non-B (non-Altiplano type) may begin to elucidate this observation.

We readily acknowledge the limitations of a retrospective study and recognize this as a potential weakness in this paper. However, birth weight was not subject to recall bias. These values were collected at the time of birth. However, we did find that non-B individuals reported a greater number of conceptions, but this difference was not reflected in births. Furthermore, whereas the number of parturients was significant with nearly 2 times as many Bs, the number of conceptions and total births was not different. This is important, especially when a population has a ratio of B to non-B individuals of 4:1. We initially thought we would pick up more Bs in these hospital populations than we did. Instead we found that the hospital population has a lower frequency of B than the general population. We believe this may represent differential utilization of health care. The indigenous population (Bs) has a greater aversion to hospitalizations than the migrant urban population represented by the non-B. It would be very difficult, if not impossible, to randomly collect equal numbers of each haplogroup in populations where the frequency of B is 0.8.

Accurate recall was verified against medical records when available and by health care providers. We believe that the events most subject to recall bias are those between conception and birth, when a miscarriage might not even be detected by the participant. There was no significant difference between groups for this period (conception to birth). The significance of this study identified the disproportionate number of infants who were born alive but died within 1 month. We believe a live birth and subsequent infant death to be less subject to recall bias. These are all very good points that we have considered when designing our follow-up investigation.