Pregnancy at high altitude in the Andes leads to increased total vessel density in healthy newborns

Norina N. Gassmann,1,2* Hugo A. van Elteren,3* Tom G. Goos,2,3 Claudia R. Morales,4 Maria Rivera-Ch,4,5 Daniel S. Martin,6 Patricia Cabala Peralta,7 Agustín Passano del Carpio,7 Saul Aranibar Machaca,7 Luis Huicho,3,8 Irwin K. M. Reiss,2 Max Gassmann,1,8* and Rogier C. J. de Jonge2*

1Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology (ZIHP), Medical Faculty, University of Zurich, Zurich, Switzerland; 2Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children’s Hospital, University Medical Center, Rotterdam, The Netherlands; 3Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands; 4Laboratory of Adaptation to High Altitude, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru; 5Center for Research for Integral and Sustainable Development (CIDIS), UPCH, Lima, Peru; 6University College London Centre for Altitude Space and Extreme Environment Medicine, University College London Hospital (UCLH) National Institute for Health Research (NIHR) Biomedical Research Centre, Institute of Sport and Exercise Health, London, United Kingdom; 7Hospital III Puno EsSalud, Puno, Peru; and 8School of Medicine, UPCH, Lima, Peru

Submitted 21 June 2016; accepted in final form 19 July 2016

Gassmann NN, van Elteren HA, Goos TG, Morales CR, Rivera-Ch M, Martin DS, Cabala Peralta P, Passano del Carpio A, Aranibar Machaca S, Huicho L, Reiss IK, Gassmann M, de Jonge RC. Pregnancy at high altitude in the Andes leads to increased total vessel density in healthy newborns. J Appl Physiol 121: 709–715, 2016. First published July 21, 2016; doi:10.1152/japplphysiol.00561.2016.—The developing human fetus is able to cope with the physiological reduction in oxygen supply occurring in utero. However, it is not known if microvascularization of the fetus is augmented when pregnancy occurs at high altitude. Fifty-three healthy term newborns in Puno, Peru (3,840 m) were compared with sea-level controls. Pre- and postductal arterial oxygen saturation (SpO2) was determined. Cerebral and calf muscle regional tissue oxygenation was measured using near infrared spectroscopy (NIRS). Skin microcirculation was noninvasively measured using incident dark field imaging. Pre- and postductal SpO2 in Peruvian babies was 88.1 and 88.4%, respectively, which was 10.4 and 9.7% lower than in newborns at sea level (P < 0.001). Cerebral and regional oxygen saturation was significantly lower in the Peruvian newborns (cerebral: 71.0 vs. 74.9%; regional: 68.5 vs. 76.0%, P < 0.001). Transcutaneously measured total vessel density in the Peruvian newborns was 14% higher than that in newborns born at sea level (0.001). Transcutaneously measured total vessel density in the Peruvian newborns (cerebral: 71.0 vs. 74.9%; regional: 68.5 vs. 76.0%, P < 0.001). Cerebral and calf muscle regional tissue oxygenation was measured using near infrared spectroscopy (NIRS). Skin microcirculation was noninvasively measured using incident dark field imaging. Pre- and postductal SpO2 in Peruvian babies was 88.1 and 88.4%, respectively, which was 10.4 and 9.7% lower than in newborns at sea level (P < 0.001). Cerebral and regional oxygen saturation was significantly lower in the Peruvian newborns (cerebral: 71.0 vs. 74.9%; regional: 68.5 vs. 76.0%, P < 0.001). Transcutaneously measured total vessel density in the Peruvian newborns was 14% higher than that in newborns born at sea level (0.001). Cerebral and regional oxygen saturation was significantly lower in the Peruvian newborns (cerebral: 71.0 vs. 74.9%; regional: 68.5 vs. 76.0%, P < 0.001). Transcutaneously measured total vessel density in the Peruvian newborns was 14% higher than that in newborns born at sea level (P < 0.001).

Incident dark field imaging; neonates; microcirculation; oxygen profiling; near infrared spectroscopy; hypoxia

NEW & NOTEWORTHY

The natural hypoxic environment at high altitude results in reduced oxygenation, especially in the growing human fetus. Our prospective observational study on healthy term newborns in Peru (Puno at 3,840 m) that included novel noninvasive visualization of microcirculation demonstrates that vessel density is elevated by 14% in neonates born to women living at high altitude compared with babies born at sea level, most likely revealing an early adaptive mechanism to a highly hypoxic antenatal environment.

It is estimated that in the South American Andes over 30 million people, most of them belonging to the Quechua or Aymara population and termed here “Andean,” permanently live above 2,500 m (8,200 ft), defined as high altitude (2, 10). At high altitude, the environmental conditions are extreme, including dramatic temperature changes and low atmospheric pressure leading to hypobaric hypoxia. The consequences of this are often exacerbated by low socio-economic status and negatively impact the health of infants (46). Of note, people living at high altitude not only show genetic adaptation but also plasticity in development in response to hypoxia (1, 17). Despite the harsh conditions at the high altitudes of the Andes, most fetuses develop well and are delivered at term (31). For that matter, it must be understood that the intrauterine environment already represents an extreme surrounding at sea level that is exacerbated in pregnancies at high altitude. In general, proper in utero development requires adequate oxygen delivery to the fetus, which is achieved by increased maternal ventilation rate and thus increased blood oxygen saturation (SpO2) level (22, 25). Under conditions of chronic hypoxia, however, the utero-placental blood flow is lower (16) and, consequently, oxygen uptake by the fetus is reduced. This process can even be exacerbated by the presence of maternal preeclampsia (12). When pregnancy occurs at 3,100 m, however, the placenta increases antioxidant capacity (38) while the fetus is able to adapt to maternal and placental hypoxemia by increasing nitric oxide production in utero and after birth. This adaptive response might be necessary to sustain placental blood flow but may also lead to improvement of microcirculatory blood flow (28).

It was shown, decades ago, that babies born to indigenous Andean women have a higher birth weight than non-Andean neonates both born at high altitude (9). A more recent study revealed that elevated uterine artery blood flow and thus increased oxygen delivery protect Andeans from fetal growth retardation when pregnancy occurs at high altitude (16). Prenatal Doppler and ultrasound studies in Andean fetuses per-
formed at 3,600 m showed reduced umbilical blood flow, compensated for, however, by the fetuses’ elevated neonatal hemoglobin concentration and increased oxygen extraction capability (31). As a result, fetal oxygen delivery and oxygen consumption at high altitude do not differ from values measured at low altitude (31), supporting the notion that the fetus copes with the extreme in utero situation by increasing systemic blood flow and thus oxygen delivery. Note that the present study does not include the Tibetan population, which is known to maintain better neonatal oxygenation than Andeans (reviewed in Ref. 24).

Apart from vasodilation, an obvious strategy to increase blood and thus oxygen supply to the tissue is to increase microvascular density. Microcirculation studies in critically ill neonates (40) found a low microvascular density to be a predictor for mortality in sepsis (39). However, no studies have reported on the effect of antenatal hypobaric hypoxia on fetal microcirculatory development. Thus, in the present prospective observational study, the aim was to obtain microcirculatory profiles of term babies born at high altitude and compare these with the profiles of babies born at sea level. We postulated that the microvascularization of the neonate born to mothers at high altitude is elevated and that this phenomenon reflects a general adaptive mechanism.

**MATERIALS AND METHODS**

**Subjects.** This prospective observational study was performed in August 2014 at the pediatric department of the Hospital EsSalud III in Puno (Peru) located at 3,840 m above sea level. The Peruvian microcirculatory measurements were compared with those performed at sea level in the maternity ward of the Erasmus MC-Sophia Children’s Hospital in Rotterdam, The Netherlands (altitude: 0 m) where measurements were performed by the same operator using identical instrumentation. Before any measurements were taken, all parents gave their written informed consent. The study protocol was approved by the Ethics Committee of the Universidad Peruana Cayetano Heredia (UPCH 180-17-14; 62794) as well as by the local Ethics Authorities represented by the Red Asistencial Puno EsSalud and the Erasmus MC Rotterdam Ethics Committee (NL4844S.078.14). The measurements were carried out in accordance with the approved guidelines. Eligible for participation were healthy, singleton newborns of women either residing at high altitude (Puno and surroundings) or at sea level (Rotterdam and surroundings) at least during pregnancy, delivered either vaginally or by cesarean section, with Apgar scores of 8 or higher and not older than 30 h at the time of measurement. Newborns were considered healthy if born at term to apparently healthy mothers not suffering from obvious pregnancy complications (no anten- or postnatal abnormalities). Maternal data on smoking were not collected. Babies delivered by cesarean section at high altitude (n = 19), but not those at sea level, were placed in an incubator (33°C, 21% O2) until the mother recovered. The latter babies were measured at a mean of 17 h after birth (similar to the vaginal-delivered ones: 14h) and 30 min after being taken out of the incubator. The room temperature at which the babies were measured was 22-23°C. Exclusion criteria included gestational age below 37 or above 42 wk; any known congenital, hematologic, or cardiopulmonary disorder; and refusal of written parental informed consent.

We intended to assign ancestry by analyzing the babies’ parental surnames, a method that was validated by analyzing ancestry informative genetic markers (4, 45). Babies born to Andean parents acquire both parental surnames that are not changed upon marriage. Accordingly, this custom yields four parental surnames for every child. By using one and the same technical study operator present at both sites. A distinction was made into small vessels, medium and large vessels: Ø 10, 10 –20, and 20 –100 μm. Full microcirculatory profiles were obtained by the following measurements performed simultaneously: pre- and postductal arterial oxygen saturation (SpO2), regional cerebral tissue oxygen (rSO2 and crSO2), and total vessel density (TVD) using transcutaneous microcirculation imaging. All newborns were asleep or awake but remain calm during measurements. While full microcirculatory profiles were obtained in Puno, in 33 newborns from Rotterdam only the transcutaneous microcirculation profiles were obtained.

**Measurement methods.** Pre- and postductal arterial oxygen saturation (SpO2) levels were measured on the right and left wrist using two MASIMO RADICAL 7 pulse oximeters (Masimo, Irvine, CA).

Regional tissue oxygen saturation was measured by near infrared spectroscopy (NIRS) using the INVOS device (Somanetics, Troy, MI). This device uses near-infrared light at wavelengths of 730 and 810 nm to measure oxygenated and deoxygenated hemoglobin. Tissue oxygen saturation, defined as the percentage of oxygenated hemoglobin/total hemoglobin, was measured on the forehead to determine the cerebral oxygen saturation (crSO2) and on the skeletal calf muscle to determine the regional oxygen saturation (rSO2). Fractional tissue oxygen extraction (FTOE) was calculated as (preductal arterial saturation – cerebral saturation)/preductal arterial saturation [(SO2 – crSO2)/SO2] for cerebral (crFTOE) and with the rSO2 for the skeletal calf muscle measurements (rFTOE). Pulse oximetry and NIRS measurements of Peruvian newborns were compared with published reference values (13, 27, 29, 41).

Skin microcirculation was measured on the upper inner arm using incident dark field (IDF) technology (Braedius, Huizen, The Netherlands). This device (CYTOCAM) is a handheld microscope with an illumination unit (green light: 450 nm) that allows optimal absorption of deoxy- and oxyhemoglobin thereby permitting visualization of the erythrocytes (44). The transcutaneous approach was chosen because sublingual measurement in newborns is not possible and a newborn’s skin is thin enough to allow this (43). Identical instrumentation was used in Puno and Rotterdam and the measurements were performed by one and the same technical study operator present at both sites. A minimum of three video clips were recorded and those that did not meet the quality criteria according to Massey et al. (21) were excluded from further analysis. TVD was automatically analyzed using CCoTTools (Version 1.7.12, brightness 500, sensitivity level 95%). A distinction was made into small vessels, medium and large vessels: Ø ≤10, 10–20, and 20–100 μm, respectively. The automated analysis standardizes the process of analysis and thereby excludes interobserver variability (42). Following standard guidelines, a minimum of three video clips per newborn was used for automated analysis (5).

The microvascular flow index (MFI) and the heterogeneity index (HI) semiquantitatively describe the velocity of microcirculatory perfusion (5). Each video image was divided in four equally sized
Birth weight, g 3,310 (2,590–4,180)† 3,353 (2,475–4,450)†

interval.

compared for total vessel density (TVD; see Fig. 2). CI

at high altitude (Puno, Hispanic resulting in

ificant difference (the comparison between indigenous vs.

groups, adjusted for sex and gestational age, showed no sig-

surnames. The birth weight of indigenous, mixed and Hispanic

babies were classified as “indigenous,” 6 as “mixed” and 19 as

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Gender distribution was approximately even and gestational

age and birth weight were similar between Peru and Rotter-

ed. The crude association between skin microcirculation

parameters and country (Peru/Netherlands) was adjusted for sex,

gestational age, birth weight z-score, Apgar score (5 min), mode of
delivery, pregnancy (primigravida/multigravida), and rectal temper-

ture. Collinearity analysis to explore correlation between all covari-

ates using a correlation matrix was performed. A cut-off value of 0.7

was used for the exclusion of variables in the model. Residual plots

were constructed to check for normality of the distribution of the

residuals.

RESULTS

Comparison of demographic data is shown in Table 1. Gender distribution was approximately even and gestational age and birth weight were similar between Peru and Rotterdam. About one-third of the Peruvian newborns were delivered by cesarean section vs. circa 60% in Rotterdam. In Puno, 18 babies were classified as “indigenous,” 6 as “mixed” and 19 as “Hispanic.” The remaining 10 babies could not be classified by surnames. The birth weight of indigenous, mixed and Hispanic newborns was 3,374 (SD 315), 3,325 (SD 414), and 3,196 (SD 220) g, respectively. Comparison of birth weight between these groups, adjusted for sex and gestational age, showed no significant difference (the comparison between indigenous vs. Hispanic resulting in \( P = 0.1 \)). Nevertheless, this trend of higher birth weight in indigenous newborns was in accordance to recent studies (8, 15) reporting that high altitude generally
decreases birth weight but that birth weight of neonates of Andean descent was higher than that of neonates of combined origin.

Additional clinical data from the 53 healthy Peruvian newborns (3,840 m above sea level) were the following: mean heart rate 145 (SD 13) n/min, mean respiratory rate 53 (SD 5) n/min, mean hematocrit 0.57 (SD 0.06), mean hemoglobin 19.0 (SD 1.9) g/dl, mean platelet count 247 (SD 53 × 10^9) dl, and mean leukocyte count 18.6 (SD 4.1 × 10^9) dl.

Mean pre- and postductal saturation in Peruvian newborns was 88.1% (SD 4.1%) and 88.4% (SD 4.6%), respectively (Fig. 1). These values were significantly lower \(( P < 0.001)\) than reference values (13) obtained from a total of 13,714 term newborns at sea level, which are 98.5 and 98.7%, respectively. The relative difference between pre- and postductal saturation in high and low altitude born babies thus was 10.4 and 9.7%, respectively. The results of cerebral and regional NIRS measurements at high altitude are also shown in Fig. 1. These data were compared with published reference values of term infants (cerebral \( n = 339 \) and regional \( n = 72 \)), born at sea level and measured with the same NIRS device (27, 29, 41). Tissue oxygen saturation was significantly lower (cerebral 71.0 vs. 74.9%; calf muscle 68.5 vs. 76.0%, \( P < 0.001 \)). Lower arterial and tissue saturation was not associated, however, with different tissue oxygen extraction (crFTOE 0.19 vs. 0.19, \( P = 0.610 \); rFTOE 0.22 vs. 0.24, \( P = 0.199 \)).

Regarding cutaneous microcirculation data, in only two cases (one from Puno and one from Rotterdam) microcirculation data could not be analyzed due to low quality video imaging and thus both were excluded from further analysis. As for the remaining cases, the mean TVD in the Peruvian babies born was 14% higher than that in the Rotterdam babies (Fig. 2, top right). Automated morphometric analysis revealed that both small and medium sized vessels (but not large ones) were significantly longer in the Peruvian newborns (Fig. 2, bottom). To assess as whether ancestry might have an impact on increased microvascularization in newborns at high altitude,
TVD was calculated for the three groups mentioned above: indigenous, mixed, and Hispanic (n = 18, 6 and 19, respectively). No statistical differences in TVD were found between any two groups tested. Moreover, there was a remarkable difference in incidence of cesarean sections between the Rotterdam and Puno group (60.6 vs. 35.9%), but we observed no differences in TVD between the two delivery modes (Rotterdam: cesarean section vs. vaginal delivery: mean TVD 25.86 and 26.18 mm/mm², respectively, P = 0.761; Puno: cesarean section vs. vaginal delivery: mean TVD 29.55 and 29.72 mm/mm², respectively, P = 0.728).

Multivariable linear regression analysis adjusted for possible confounding variables between countries showed no collinearity between the independent variables used in the model and normal distribution of the residuals. Table 2 shows the corresponding crude and adjusted differences for microcirculatory parameters: after adjustment the difference between the Peruvian and Rotterdam groups remained significant. Moreover, both the MFI and HI were not altered in either group.

DISCUSSION

Reduced oxygenation of the placenta is linked to severe complications including intra-uterine growth retardation and preeclampsia (12, 23, 36). Of note, despite reductions in systemic oxygen supply, such as occurs at high altitude, the fetus is able to cope with this extreme but still physiologic hypoxic condition. While many studies have addressed the hypoxic placenta’s vascular remodeling and metabolic changes (reviewed in Refs. 19, 36), data on the mature fetus’s adaptation to a hypoxic environment are scarce. The present study is the first, to our knowledge, to examine microvascular density in healthy term neonates born to mothers that were living at high altitude during pregnancy (3,840 m). Our major finding was that their TVD was ~14% higher than in neonates born at sea level, pointing towards a possible adaptive fetal strategy to cope with reduced oxygenation. In addition, based on our surname assessment, we suspect that the increase in TVD was independent of the babies’ ancestry.

The microcirculation is defined as vessels equal to or smaller than 100 µm in diameter that form the capillary network (11). The above-mentioned difference in TVD was still significant when the crude data were adjusted for the following predefined, potentially confounding variables: country, gender, gestational age, birth weight, Apgar score (5 min), mode of delivery, primigravida/multigravida, and rectal temperature. Increased vascularization was observed in small (Ø: ≤10 µm)
Table 2. Crude and adjusted difference between Puno and Rotterdam for microcirculatory parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted difference (95% CI)</th>
<th>P value</th>
<th>Adjusted* difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vessel density, mm/mm²</td>
<td>3.67 (2.68–4.66)</td>
<td>&lt;0.001</td>
<td>3.57 (2.37–4.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TVD small, mm/mm²</td>
<td>1.46 (1.02–1.91)</td>
<td>&lt;0.001</td>
<td>1.14 (0.64–1.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TVD medium, mm/mm²</td>
<td>2.79 (1.91–3.66)</td>
<td>&lt;0.001</td>
<td>3.08 (2.00–4.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TVD large, mm/mm²</td>
<td>-0.58 (-1.26–0.10)</td>
<td>0.129</td>
<td>-0.64 (-1.49–0.20)</td>
<td>0.132</td>
</tr>
<tr>
<td>MFI small, AU</td>
<td>-0.02 (-0.14–0.09)</td>
<td>0.688</td>
<td>-0.08 (-0.21–0.06)</td>
<td>0.261</td>
</tr>
<tr>
<td>MFI nonsmall, AU</td>
<td>0.03 (-0.04–0.09)</td>
<td>0.381</td>
<td>0.02 (-0.06–0.09)</td>
<td>0.646</td>
</tr>
<tr>
<td>HI small, AU</td>
<td>0.001 (-0.08–0.09)</td>
<td>0.854</td>
<td>0.02 (-0.08–0.13)</td>
<td>0.640</td>
</tr>
<tr>
<td>HI nonsmall, AU</td>
<td>0.03 (-0.04–0.09)</td>
<td>0.367</td>
<td>0.04 (-0.04–0.11)</td>
<td>0.368</td>
</tr>
</tbody>
</table>

Crude and adjusted differences between microcirculatory parameters obtained from neonates born at high altitude and sea level. Crude data from the babies mentioned in Table 1 (Puno n = 52; Rotterdam n = 32) were adjusted (*) for country, sex, gestational age, birth weight z-score, Apgar score (5 min), mode of delivery, pregnancy (primigravida/multigravida) and rectal temperature as described in MATERIALS AND METHODS. Small, medium, and large vessels have Ø of <10, 10–20, and 20–100 μm, respectively. MFI, microvascular flow index; HI, heterogeneity index; CI, 95% confidence interval; AU, arbitrary units.

and medium (Ø: 10–20 μm) vessels but not in larger ones. This implies that vessel density is only increased at the level of gas exchange (i.e., capillaries and small arterioles). In a study of healthy adults with no high-altitude ancestry (20), a 10.9% increase in TVD was found in subjects first measured at sea level and thereafter at high altitude (5,300 m). Also, in preterm infants born small for gestational age, most often caused by more extreme hypoxic conditions, TVD was significantly higher soon after birth (van Elteren HA, deJonge RC, and Reiss IK unpublished observations).

Considering that blood flow in the umbilical vein is reduced at high altitude (31) and that vascularization seems to be independent of ancestry, it is plausible to speculate that enhanced microvascularization is a general adaptive mechanism that might be induced by hypoxia-driven stabilization of the α-subunits of the hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) (reviewed in Refs. 7, 37). In turn these heterodimeric regulatory transcription factors upregulate hypoxia-dependent genes including those that trigger angiogenesis such as the vascular endothelial growth factor (VEGF) (26, 36). In contrast to the Andean population, evolution has selected a blunted erythropoietic response for Tibetans as an adaptive strategy to high altitude: a missense mutation in the EGLN1 gene that encodes for the main cellular oxygen sensor results in increased HIFα degradation under hypoxic conditions (18) but this observation has been challenged recently (33). Nevertheless, it would be of interest to determine TVD in healthy babies born to Tibetan mothers at high altitude. Apart from such mutations epigenetic modifications may also support adaptation to exogenous factors such as hypoxia, which can be transmitted to next generations. As such, Julian et al. (14) recently provided evidence that unique DNA methylation patterns occur in genes known to influence vascular development and integrity in offspring of hypertensive pregnancies.

While the babies’ heart rate at high altitude (mean 145, SD 13 n/min) did not deviate from published data, levels of hematocrit (0.57 vs. 0.49–0.50) and hemoglobin concentration (19.0 vs. 16.8 – 17.1 g/dl) values in our Peruvian population were higher than those reported in a study performed at 3,600 m (31). We cannot explain this difference as the hospital in which our study was conducted was located only ~300 m higher. Nevertheless, in the present study the flow-related parameters MFI and HI did not differ between the high-altitude and sea-level groups despite a physiological higher hematocrit level in the high-altitude group. However, hematocrit values measured in arterial or venous blood differ greatly from hematocrit at a microcirculatory level. Known as tube hematocrit, it is significantly lower and highly variable in the presence of a constant systemic hematocrit (6). Systemic hematocrit is therefore not correlated to viscosity and blood flow at a microcirculatory level. Moreover, it should be noted that MFI values are often lower in disease states, especially in individuals suffering from septic shock (32).

Previously, a study on NIRS measurements in 24 children reported a significant decrease in cerebral tissue oxygen saturation on ascent from 1,610 to 3,109 m (78 to 67%, P < 0.001) (47). In another study, reporting NIRS measurement in 17 children during emergency helicopter transport, NIRS decreased from 69.2 to 66.3% in patients transported to altitudes higher than 5,000 ft (1524 m) above sea level (35). Although these two studies measured the response to acute hypoxia, these observations are in line with our results showing that exposure to high altitude significantly lowers cerebral tissue oxygenation.

Limitations. Due to unforeseen administrative delays in Peru, measurements could not be performed in the local sea-level control group that of note is mainly represented by a Hispanic population. Therefore, measurements at high altitude were compared with sea-level values either found in the literature (pulse oximeter and NIRS data) or by own data obtained from our Rotterdam cohort (determination of TVD). Although a control group of babies born at sea level in Peru is also not completely similar to the neonates in Puno, the use of a Dutch control group might have introduced additional unknown confounding factors. The number of participants in the referred studies exceeded the number of participants in our control group, thereby serving as a reliable comparison group unless ancestry plays an important role. This was assessed and despite the fact that all four parental surnames of the neonates were not always obtained, it was possible to classify a significant number as indigenous (n = 18) or Hispanic (n = 19). Although ancestry classification by surname is not as precise as genetic analysis, this strategy, first being described and validated back in 1989 (4), has been successfully applied recently (30, 34). Considering that elevated TVD was observed in all analyzed neonates who consisted of Andean and combined ancestry, we propose that comparison of our data obtained in neonates born at high altitude to sea-level neonates from the literature is sound.
The automated computer IDF technology used for microcirculatory analysis has, just like its predecessor methods (side-stream dark field imaging and orthogonal polarization spectral imaging), only been validated against its predecessor. However, given that the same method was used in both the Peruvian and the Rotterdam group, under supervision of the same experienced operator, any limitation of the software should be equally reflected in both groups. Thus the data provided are comparable within this study but cannot be extrapolated to other studies.

To conclude, in this study, microvascular vessel density measured using IDF imaging was higher in babies born at high altitude than in babies born at sea level. Neonatologists are often confronted with hypoxemia in infants due to cardiorespiratory insufficiency and prematurity. Visualizing the cutaneous microcirculation represents a new, noninvasive, and fast diagnostic tool in neonatal intensive care helping to understand the balance between macrocirculation and peripheral perfusion and tissue oxygenation in newborns.

ACKNOWLEDGMENTS

We thank to all mothers and partners for commitment to participate in this study. We are also indebted to the large community of pediatricians, obstetricians, nurses, laboratory experts, and administrative personal of the Hospital EsSalud III in Puno (Peru) who all made this work possible especially Ximena Rodrigo Machicaco, Sandra Y. Mamani Velarde, Yuliana Mamani Pilco, Paul Martines, and Jorge Anibal Osorio Terrones. We also thank Jorge Soliz and Silvia Urrutia from Quebec and La Paz, respectively, for valuable help in classifying surnames as being of Andean or Hispanic origin, as well as Sir Peter Ratcliffe from Oxford and Jorge Soliz from Quebec for valuable comments on the manuscript. We acknowledge the artwork and the professional English editing performed by Jeanne Peter (Zürich) and Ko Hagoort (Rotterdam), respectively.

GRANTS

N. N. Gassmann and M. Gassmann acknowledge the Swiss National Science Foundation, the Zurich Center for Integrative Human Physiology (ZIHP), and the Stiftung für wissenschaftliche Forschung an der Universität Zürich for valuable financial support.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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