The FASEB Journal • Research Communication

Inhibition of peroxisome proliferator-activated receptor γ: a potential link between chronic maternal hypoxia and impaired fetal growth

Colleen G. Julian,*[,1] Ivana V. Yang,[†] Vaughn A. Browne,[‡] Enrique Vargas,[||] Carmelo Rodriguez,[¶] Brent S. Pedersen,[†] Lorna G. Moore,[§] and David A. Schwartz[†]

*Division of Biomedical Informatics and Personalized Medicine and [†]Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, [‡]Department of Emergency Medicine, and [§]Department of Obstetrics and Gynecology, University of Colorado–Denver, Denver, Colorado, USA; [||]Bolivian Institute of High Altitude Biology, La Paz, Bolivia; and [¶]Clinica Siraní, Santa Cruz de la Sierra, Bolivia

ABSTRACT **Chronic exposure to hypoxia raises the risk of pregnancy disorders characterized by maternal vascular dysfunction and diminished fetal growth. In an effort to identify novel pathways for these hypoxia-related effects, we assessed gene expression profiles of peripheral blood mononuclear cells (PBMCs) obtained from 43 female, high-altitude or sea-level residents in the nonpregnant state or during pregnancy (20 or 36 wk). Hypoxia-related fetal growth restriction becomes apparent between 25 and 29 wk of gestation and continues until delivery. Our sampling strategy was designed to capture changes occurring before (20 wk) and during (36 wk) the time frame of slowed fetal growth. PBMC gene expression profiles were generated using human gene expression microarrays and compared between altitudes. Biological pathways were identified using pathway analysis. Modest transcriptional differences were observed between altitudes in the nonpregnant state. Of the genes that were differentially expressed at high altitude *vs.* sea level during pregnancy (20 wk: 59 probes mapped to 41 genes; 36 wk: 985 probes mapped to 700 genes), several are of pathological relevance for fetal growth restriction. In particular, transcriptional changes were consistent with the negative regulation of peroxisome proliferator-activated receptor γ (PPARγ) at high altitude; such effects were accompanied by reduced birth weight ($P < 0.05$) and head circumference ($P < 0.01$) at high altitude *vs.* sea level. Our findings indicate that chronic exposure to hypoxia during pregnancy alters maternal gene expression patterns in general and, in particular, expression of key genes involved in metabolic homeostasis that have been proposed to play a role in the pathophysiology of fetal growth restriction.—Julian, C. G., Yang, I. V., Browne, V. A., Vargas, E., Rodriguez, C., Pedersen, B. S., Moore, L. G., Schwartz, D. A. Inhibition of peroxisome proliferator-activated receptor γ: a potential link between chronic maternal hypoxia and impaired fetal growth. *FASEB J.* 28, 1268–1279 (2014). www.fasebj.org**

Key Words: PPARγ · intrauterine growth restriction

Chronic exposure to hypoxia due to hypoxia-related disease or high altitude (≥2500 m) residence impedes maternal vascular adaptation to pregnancy and impairs fetal growth (1–5). Exemplifying this, the incidence of preeclampsia and intrauterine growth restriction (IUGR) is ~3-fold greater at high altitude compared with sea level (6), such that high-altitude residents are the largest at-risk population for these two complications of pregnancy. IUGR and preeclampsia are predominant causes of perinatal and maternal morbidity and mortality worldwide (7–9).

The hypoxia-inducible factor (HIF) and other oxygen-sensitive pathways are thought to contribute to the maternal vascular dysfunction common to IUGR and preeclampsia, and as a means by which chronic hypoxia impairs fetal growth (5, 10). Such pathways are logical targets for inquiry. First, HIF pathways are intimately involved in physiological responses to hypoxia that serve to maintain tissue oxygenation and metabolism in the face of limited oxygen supply (11, 12). Second,

Abbreviations: ADRB2, adrenergic β2 receptor; AIM, ancestry informative marker; AMPKa1, adenosine monophosphate–activated protein kinase isoform a1; BH, Benjamini-Hochberg; EGF, epidermal growth factor; EGR1, early growth response 1; F2, coagulation factor 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; IL1B, interleukin 1β; IL8, interleukin 8; IPA, Ingenuity Pathway Analysis; IUGR, intrauterine growth restriction; NF-κB, nuclear factor of κ light polypeptide gene enhancer in B cells; PBMC, peripheral blood mononuclear cell; PPARγ, peroxisome proliferator-activator receptor γ; TREM1, triggering receptor expressed on myeloid cells 1; VEGF, vascular endothelial growth factor

[1] Correspondence: Department of Medicine, University of Colorado–Denver, 12700 E 19th Ave., Mailstop 8611, 3rd Floor Research Complex 2, Aurora, CO 80045, USA. E-mail: colleen.julian@ucdenver.edu

doi: 10.1096/fj.13-239749

This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.

 0892-6638/14/0028-1268 © FASEB

TABLE 1. *Maternal characteristics at low and high altitude*

Characteristic	Nonpregnant		Wk 20		Wk 36	
	Low	High	Low	High	Low	High
n	11	12	14	13	15	14
Time of study (pregnancy week)	–	–	21.0 ± 3.1	22.7 ± 5.1	35.9 ± 0.9	35.0 ± 2.2
Maternal age (yr)	25.7 ± 4.9	29.8 ± 4.4	25.1 ± 4.4	31.1 ± 6.0*	26.1 ± 4.2	29.5 ± 5.5#
Maternal weight (kg)	66.3 ± 12.1	56.1 ± 6.5*	67.2 ± 13.5	62.1 ± 8.0	76.6 ± 12.7	67.7 ± 9.7*
Maternal height (cm)	160.5 ± 7.2	150.1 ± 28.6	159.5 ± 4.4	155.9 ± 9.3	161.0 ± 7.0	157.4 ± 8.4
Skinfold sum (mm)	64.7 ± 17.5	53.2 ± 40.2	49.0 ± 12.5	41.7 ± 16.0	63.9 ± 18.2	–
MAP (mmHg)	77.5 ± 6.2	75.1 ± 7.3	76.0 ± 5.8	76.3 ± 6.5	76.9 ± 5.3	75.4 ± 6.8
S_aO_2 (%)	98.7 ± 0.9	93.0 ± 1.9**	99.0 ± 0.8	93.3 ± 3.1**	98.7 ± 0.7	93.8 ± 1.3**
Ancestry, AIMs						
African (%)	8.3 ± 4.7	10.0 ± 7.3	9.2 ± 4.5	9.0 ± 7.0	9.3 ± 4.3	10.6 ± 8.1
European (%)	46.5 ± 20.1	50.8 ± 28.4	40.1 ± 22.5	51.5 ± 33.7	40.1 ± 18.6	46.8 ± 34.9
Indigenous American (%)	45.2 ± 17.7	33.9 ± 29.0	50.6 ± 21.0	38.8 ± 34.5	50.5 ± 16.8	41.9 ± 33.7

Comparisons between altitudes at each time point are indicated. MAP, mean arterial pressure; S_aO_2, arterial oxygen saturation. *$P < 0.05$; **$P < 0.01$; #$0.10 > P > 0.05$.

genetic variations within oxygen-sensitive pathways purportedly influence susceptibility to IUGR and preeclampsia (13, 14). Circulating levels of oxygen-sensitive factors that mediate vascular growth, vasoreactivity, and vascular remodeling are also altered during high-altitude pregnancy and correlate with uteroplacental blood flow and birth weight (15–17). However, despite several studies that have explored the role of genes and proteins regulated, in part, by HIF or other oxygen-sensitive pathways for hypoxia-related IUGR (*e.g.*, refs. 17–21), it remains unclear whether or how such factors act to impair fetal growth under conditions of chronic maternal hypoxia.

In an effort to identity novel pathways or to derive new explanations for hypoxia-related fetal growth restriction we compared genome-wide transcriptional profiles of maternal peripheral blood mononuclear cells (PBMCs) obtained during pregnancy and in the nonpregnant state from women of similar ancestry permanently residing at high altitude compared with sea level. Our findings are novel insofar as this report is the first to indicate that environmental hypoxia alters maternal gene expression during pregnancy in general and, in particular, of key genes involved in metabolic homeostasis. Specifically, our results suggest that maternal exposure to hypoxia represses the expression of peroxisome proliferator-activated receptor γ (PPARγ), a ligand-activated nuclear receptor that is a key regulator of energy homeostasis (22), as has also been observed in IUGR and preeclampsia (reviewed in ref. 23). In light of recent evidence that hypoxia-related IUGR may be the product of fetal hypoglycemia rather than fetal oxygen deficit alone (45), we consider the possibility that alterations to PPARγ-mediated regulation of glucose homeostasis influence impaired fetal growth at high altitude.

MATERIALS AND METHODS

Subjects

Subjects consisted of 43 women residing at high altitude (n=25; 3600–4300 m; P_B=495-470 mmHg) or sea level (n=18; 300 m; P_B=725 mmHg) who were participants in a larger investigation designed to identify physiological and genetic components of hypoxia-associated IUGR (*e.g.*, refs. 24, 25). Subjects were referred by their prenatal care providers and were selected for inclusion based on the absence of hypertensive complications and the availability of high-quality RNA for microarray studies. High-altitude samples were obtained at 20 (n=13) and 36 (n=14) wk of pregnancy and in the nonpregnant state [n=12, (9 postpartum, 3 nulliparous)]. Sea-level samples were also collected at 20 (n=14) and 36 (n=15) wk and in the nonpregnant state (n=11, all postpartum). Our rationale for obtaining samples at 20 and 36 wk of pregnancy is based on the fact that the effect of high altitude to reduce fetal growth (as assessed by Doppler fetal biometry) begins between 25 and 29 wk of gestation (4). For this reason, we sought to collect samples both before and during the time frame of slowed fetal growth to increase the probability that we would observe changes that were likely to be the most temporally relevant to the phenotype of interest. Since some women provided samples at more than 1 time point, the total number of samples is greater than the number of subjects. Given the logistical difficulties associated with identifying subjects before pregnancy, we relied on postpartum and nulliparous measurements as an index of the nonpregnant state. High-altitude studies were conducted at the Bolivian Institute of High Altitude Biology, the Southern Clinic, and 5 obstetrical hospitals in La Paz-El Alto, Bolivia. Sea-level studies were conducted at the Siraní Clinic (Santa Cruz de la Sierra, Bolivia).

For inclusion, women were required to be receiving prenatal care, having a singleton pregnancy, and to be at no known risk for pregnancy complications (*e.g.*, diabetes, obesity). We excluded smokers and women who developed hypertension during pregnancy. All subjects gave their written informed consent to all study procedures, which were approved by the human subject review committees of the Colorado Multiple Institutional Review Board of the University of Colorado Denver and the Colegio Médico, its Bolivian equivalent.

Using a panel of 100 ancestry informative markers (AIMs; ref. 26) we confirmed that the proportion of each woman's genetic background attributed to African, European, or Indigenous American origin was similar between altitudes for pregnant and nonpregnant subjects (**Table 1**). Details, including allele frequencies in all parental populations, DNA sequences, exact positions of single-nucleotide polymorphisms

(SNPs), PCR primers, and the amplification conditions used, are available from the dbSNP database (U.S. National Center for Biotechnology Information, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/SNP) under the submitter handle PSU-ANTH.

Study procedures

On the first visit, each woman completed a questionnaire in her spoken language to determine her altitude of birth, childhood and current residence, body weight before pregnancy, socioeconomic status, and medical/reproductive history. Subsequent visits consisted of a general clinical exam, followed by a blood draw.

During the clinical exam, we measured resting heart rate, bilateral upper extremity blood pressures, height and weight; estimated adiposity by the sum of biceps, triceps, and subscapular skin-fold thicknesses using Lange calipers (Beta Technology, Santa Cruz, CA, USA); collected urine samples to screen for infection and proteinuria; and drew venous blood for the storage of serum, plasma, and the isolation of PBMCs for microarray studies. Gestational age was based on the date of last menstrual period and confirmed by fetal biometry at wk 20 or clinical assessment at delivery. Birth weights, newborn characteristics, and the occurrence of perinatal or maternal complications were obtained from medical records and postnatal follow-up interviews.

Maternal and newborn characteristics

Comparisons of maternal and newborn characteristics between altitude groups at each study time were made using Student's t tests for continuous variables and χ^2 tests for nominal variables in SPSS 19.0 (IBM SPSS, Chicago, IL, USA). Newborn characteristics were adjusted for gestational age and maternal height, based on the known relationship of these variables to fetal size (27, 28). We did not correct for prepregnancy weight (or weight gain during pregnancy) since maternal weight (nonpregnant, 20 or 36 wk) was not associated with birth weight [R^2=0.31, R^2=0.04, and R^2=0.14, respectively; all nonsignificant (NS)] in our data set and we did not have nonpregnancy weights for all subjects. Data are expressed as means ± SEM. A value of $P < 0.05$ (2-tailed) was considered the threshold for significant differences between groups. Values of $0.05 < P < 0.10$ were considered to indicate trends.

Sample collection and processing

Peripheral blood samples (8 ml) were collected from an antecubital vein using standard phlebotomy and placed into a BD Vacutainer CPT cell preparation tube (BD Biosciences, San Jose, CA, USA) containing sodium citrate and Ficoll-Hypaque density fluid. PBMCs were isolated according to the manufacturer's guidelines, resuspended in RNAlater (Ambion, Austin, TX, USA) solution, and stored at −80°C until analysis. Total mRNA was isolated using an AllPrep DNA/RNA Mini Kit (Qiagen, Germantown, MD, USA) and subsequently tested for quality and concentration using Agilent's 2100 bioanalyzer and RNA 6000 Nano LabChip (Agilent Technologies, Santa Clara, CA, USA). cDNA synthesis and amplification were performed using the TransPlex Complete Whole Transcriptome Amplification (WTA) Kit (Sigma-Aldrich, St. Louis, MO, USA).

Assessment of gene expression

cDNA samples were hybridized to the Roche NimbleGen Human Gene Expression 12 × 135K Array (version 5.1; Roche, Madison, WI, USA) as indicated by the manufacturer and scanned using the NimbleGen MS 200 scanner. Gene expression profiles were extracted with NimbleScan 2.6 software. Raw chip files were background corrected, $\log_2$ transformed, and normalized using robust multiarray average (RMA) in the Affymetrix Expression Console (Affymetrix, Santa Clara, CA, USA; ref. 29). To generate a matrix, including an expression value for each probe, a linear model was then fit to the normalized data.

Gene-expression profiles were first compared between altitudes at each study point (*e.g.*, 36 wk) using the Limma and Surrogate Variable Analysis packages in R (30, 31), a freeware program designed for statistical analysis and graphics, with ancestry (%Indigenous American AIMs) and gene-chip processing date included as covariates. The resulting P values were adjusted for multiple comparisons using the Benjamini-Hochberg (BH) procedure (32). Genes with a $\log_2$ fold change > 0.8 between altitudes and a BH-adjusted value of $P < 0.05$ were considered to be differentially expressed.

Validation of gene expression array data was performed using RT-PCR. cDNA was generated using random primers with Superscript III First-Strand Synthesis System SuperMix (Invitrogen, Carlsbad, CA, USA). PCR reactions were prepared using TaqMan Fast Advanced Master Mix and TaqMan Gene Expression Assays (Applied Biosystems, Carlsbad, CA, USA) as specified by the manufacturer. RT-PCR was performed and analyzed on the ViiA 7 Real-Time PCR System with ViiA 7 1.22 software (Applied Biosystems). The quantification of target gene transcription relative to that of a housekeeping gene [human glyceraldehyde 3-phosphate dehydrogenase (GAPDH)] was assessed using the $2^{-\Delta\Delta CT}$ method (33). Unpaired Student's t tests were used to identify differential expression between high-altitude and sea-level samples using a significance threshold of $P < 0.05$. Data are expressed as means ± SD.

To determine whether altitude influenced transcriptional patterns across time, we performed a secondary set of analyses that was limited to women with either a 20-wk (n=6 at high altitude, n=10 at sea level) or a 36-wk sample (n=7 at high altitude, n=8 at sea level) and a nonpregnant (postpartum) sample. Longitudinal analyses were performed using the Lme4 and Car packages in R, and included altitude × gestational week as an interaction term to detect variations between altitudes across time.

Biological pathway analysis

To identify biological pathways of potential importance, lists of differentially expressed genes were introduced to Ingenuity Pathway Analysis (IPA; Ingenuity Systems, Inc., Redwood, CA, USA), with the top 5 enriched canonical pathways meeting the IPA default threshold significance level ($-\log P > 1.3$) being reported. Subsequently, we used the upstream regulator function within IPA to detect possible mediators of the transcriptional patterns identified as being unique to high-altitude pregnancy. This procedure uses 2 statistical measures, an overlap P value and an activation z score that express the degree of commonality between known and observed targets of each upstream regulator, and the level of agreement between anticipated and observed expression changes of gene targets for each upstream regulator, respectively. The predicted activation state is based on the z score (activated $z > 2$) or suppressed ($z < -2$).

RESULTS

Maternal and newborn characteristics

Maternal characteristics are provided in Table 1. Highland women tended to be older and weigh less than their lowland counterparts when nonpregnant or at 36 wk but were of similar height and had equivalent skinfolds and mean arterial blood pressure during pregnancy (Table 1). Arterial oxygen saturation was ~5% lower at high altitude regardless of pregnancy status.

Birth weight and head circumference at the time of delivery were reduced at high altitude compared with sea level [2974±127 *vs.* 3354±106 ($P<0.05$) and 33.7± 0.4 *vs.* 35.4±0.3 ($P<0.01$), respectively]. Birth length and ponderal index were equivalent at high altitude *vs.* sea level [49.0±0.4 *vs.* 49.7±0.3 (P =NS) and 25.3±0.9 *vs.* 27.2±0.7 (P =NS), respectively].

Effect of chronic maternal hypoxia on gene expression

First, we compared nonpregnant gene-expression profiles and identified 24 down-regulated and 19 up-regulated transcripts at high altitude *vs.* sea level (**Fig. 1*A*** and Supplemental Table S1); these probes mapped to 36 known genes. **Table 2** shows the top 20 transcripts ranked by $\log_2$ fold change. To narrow subsequent analyses to those genes that differed only between altitudes during pregnancy, we excluded genes that were differentially expressed in the nonpregnant state from 20- and 36-wk gene lists.

A larger number of genes were differentially expressed between altitudes during pregnancy than in the nonpregnant state. At 20 wk, 59 probes mapped to 41 genes were differentially expressed at high altitude *vs.* sea level (Fig. 1*B* and Supplemental Table S2). At 36 wk, 985 probes differed in expression between altitudes (BH corrected $P<0.05$), including 360 that were down-regulated and 625 that were up-regulated (Fig. 1*C* and Supplemental Table S3); these probes mapped to 700 known genes.

As shown in Table 2, there was substantial overlap at 20 and 36 wk between transcripts with the greatest fold change at high altitude *vs.* sea level. Specifically, G_0/G_1switch 2 (GOS2), amphiregulin (AREG), interleukin 8 (IL8), regulator of G-protein signaling 1 (RGS1), nuclear receptor subfamily 4, group A, member (NR4A2), heparin-binding EGF-like growth factor (HBEGF), and similar to amphiregulin precursor (LOC653193) expression were reduced at high altitude at both pregnancy times. Expanded annotated gene lists for the nonpregnant state, 20 wk, and 36 wk are included in Supplemental Tables S1–S3, respectively.

Enriched biological pathways during high-altitude pregnancy

Biological pathways of potential relevance for maternal physiological responses during high-altitude pregnancy were identified using the canonical pathway analysis function in IPA. No canonical pathway reached statistical significance in the nonpregnant state when comparing high altitude *vs.* sea level. Pathways identified at 20 wk were related to PPARγ signaling, triggering receptor expressed on myeloid cells 1 (TREM1) signaling; macrophage migration inhibitory factor (MIF) regulation of innate immunity, glucocorticoid receptor signaling; and IL17A in arthritis. Canonical pathways enriched by genes that were differentially expressed between altitudes at 36 wk included TREM1 signaling, coagulation systems, cAMP-mediated signaling, atherosclerosis signaling, and hepatic stellate cell activation pathways. Genes belonging to each pathway and corresponding $\log_2$ fold changes are shown in **Fig. 2**.

Upstream regulators governing differential gene expression in high-altitude *vs.* sea-level pregnancy

Using the upstream regulator function in IPA, we identified genes that may govern unique transcriptional patterns during high-altitude pregnancy. The activation state of each transcriptional regulator was based on a z-score, which reflects the level of agreement between anticipated and observed expression changes for gene targets. Several of the top upstream regulators were common to 20 and 36 wk, including interleukin 1β (IL1B), TREM1, epidermal growth factor (EGF),

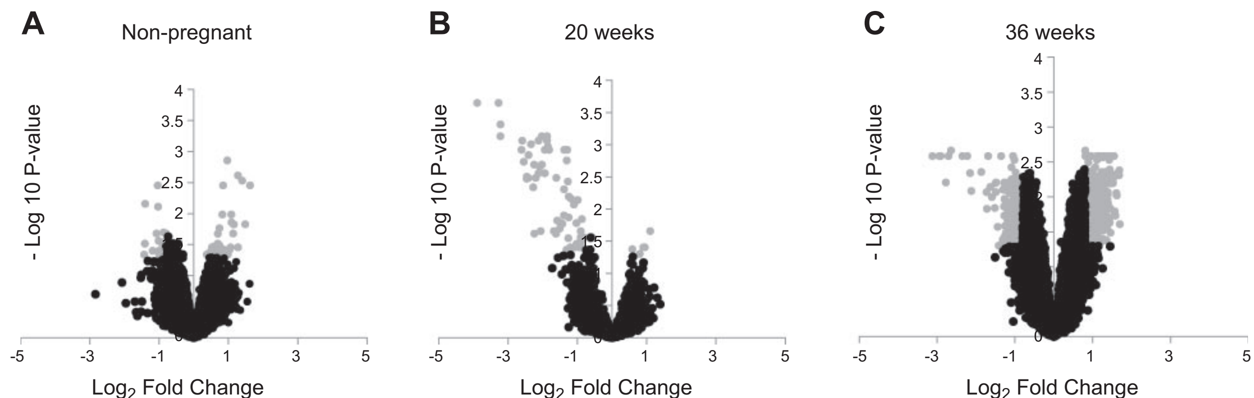

Figure 1. Differentially expressed genes between altitudes in the nonpregnant state (*A*), at 20 wk of pregnancy (*B*), and at 36 wk of pregnancy (*C*). Solid gray circles indicate genes that differed between altitudes with a $\log_2$ fold change ≥ 0.8 and Benjamini-Hochberg adjusted $P<0.05$. Black circles indicate genes that were not differentially expressed between altitudes using the specified criteria.

TABLE 2. *Top 20 differentially expressed genes (probes) ranked by log_2 fold change; high altitude vs. sea level*

Gene	Overlap	Log_2 FC	Adj. *P*
Nonpregnant			
ZNF2		1.62	0.004
GPM6B		1.48	0.015
CES1		−1.44	0.046
SOD2		−1.41	0.030
DSC2		−1.40	0.007
FAM43A		1.40	0.003
PER2		1.27	0.035
LOC115648		1.27	0.002
SOCS3		−1.19	0.040
TRGV7		1.15	0.015
SAR1A		1.14	0.021
CXorf52		1.09	0.014
N/A		1.08	0.010
N/A		−1.08	0.021
ZNF568		1.06	0.035
GAS6		−1.04	0.004
GAS6		−1.03	0.008
RFP2		1.03	0.038
STEAP4		−1.01	0.027
AP1S1		−1.01	0.040
20 wk			
IL8	×	−3.89	
GOS2	×	−3.28	
AREG	×	−3.22	0.001
LOC653193	×	−3.22	
NR4A2	×	−2.61	0.001
EGR1		−2.59	0.001
CCL3L1		−2.55	0.002
CCL3		−2.46	0.003
NR4A2	×	−2.46	0.003
CCL3L1		−2.41	0.003
NR4A2	×	−2.41	0.001
PTGS2		−2.34	0.001
RGS1	×	−2.27	0.005
HBEGF	×	−2.25	0.002
FOSB		−2.24	0.024
CCL3L3		−2.20	0.003
IL1B		−2.12	0.003
DUSP2		−2.12	0.001
CCL3		−2.09	0.002
RGS1		−2.06	0.022
36 wk			
GOS2	×	−3.12	0.003
LOC653193	×	−2.94	0.003
AREG	×	−2.91	0.003
IL8	×	−2.78	0.006
RGS1	×	−2.71	0.003
RGS1	×	−2.64	0.002
NR4A2	×	−2.35	0.003
NR4A2	×	−2.23	0.003
NR4A2	×	−2.15	0.005
HBEGF	×	−2.12	0.008
OSM		−1.87	0.004
NR4A3		−1.73	0.009
FOS		−1.72	0.015
N/A		1.70	0.025
SNAI1		−1.69	0.003
RXFP4		1.68	0.004
C12orf33		1.68	0.005
A2M		1.64	0.005
RNASE4		−1.63	0.007
N/A		1.62	0.010

Overlap indicates genes that are common to 20- and 36-wk lists. A2M, α-2-macroglobulin; Adj. *P*, B-H-adjusted *P* ; AP1S1, adaptor-related protein complex 1, σ1 subunit; AREG, amphiregulin; C12orf33, chromosome 12 open reading frame 33; CCL3, chemokine ligand 3; CCL3L1, chemokine ligand 3-like 1; CCL3L3, chemokine ligand 3-like 3; CES1, carboxylesterase 1; CXorf52, chromosome X open reading frame 52; DSC2, desmocollin 2; DUSP2, dual specificity phosphatase 2; EGR1, early growth response 1; FAM43A, family with sequence similarity 43 A; FC, fold change; FOS, v-fos FBJ murine osteosarcoma viral oncogene homolog; FOSB, FBJ murine osteosarcoma viral oncogene homolog B; GAS6, growth arrest-specific 6; GOS2, G_0/G_1 switch 2; GPM6B, glycoprotein M6B; HBEGF, heparin-binding EGF-like growth factor; IL1B, interleukin 1β; IL8, interleukin 8; LOC115648, similar to hypothetical protein FLJ13659; LOC653193, similar to amphiregulin precursor; NR4A2, nuclear receptor subfamily 4, group A, member 2; NR4A3, nuclear receptor subfamily 4, group A, member 3; OSM, oncostatin M; PER2, period homolog 2; PTGS2, prostaglandin-endoperoxide synthase 2; RFP2, ret finger protein 2; RGS1, regulator of G-protein signaling 1; RNASE4, RNase A family, 4; RXFP4, relaxin family peptide receptor 4; SAR1A, SAR1 gene homolog A; SNAI1, snail homolog 1; SOCS3, suppressor of cytokine signaling 3; SOD2, superoxide dismutase 2; STEAP4, STEAP family member 4; TRGV7, T-cell receptor γ variable 7; ZNF2, zinc finger protein 2; ZNF568, zinc finger protein 568.

platelet-derived growth factor β polypeptide (PDGF BB), nuclear factor of κ light polypeptide gene enhancer in B cells (NF-κB), coagulation factor II (F2), and cAMP-responsive element binding protein 1 (CREB1) (**Table 3**). To further mine our data, we explored common target genes for these upstream regulators. PPARγ was a target gene for each upstream regulator that was shared between 20 and 36 wk with the exception of F2. Moreover, at 36 wk, 13 of the top 15 upstream regulators identified were effectors of PPARγ. Strengthening the potential importance of this observation, all downstream targets of PPARγ in our data were modified in a direction consistent with PPARγ inhibition (36 wk; **Fig. 3**). Other notable upstream regulators were HIF1α and vascular endothelial growth factor A (VEGFA), each predicted to be downregulated at high altitude *vs.* sea level during pregnancy.

We reviewed each stage of our analysis for genes with two qualities: consistency (*i.e.*, differential expression between altitudes, and appearances in both canonical pathway and upstream regulator analyses); and biological plausibility with respect to the maternal physiological changes and impaired fetal growth characteristic of high-altitude pregnancy. PPARγ emerged as the most prominent theme in our data. **Figure 4** shows genes within the PPARγ pathway that differed between altitudes during pregnancy. Specifically, we noted that canonical pathways were enriched by genes related to PPARγ signaling during pregnancy at high altitude and that transcriptional changes of upstream regulators and downstream targets were consistent with reduced PPARγ expression at high altitude *vs.* sea level. Indicating that such alterations were confined to PPARγ and unique to

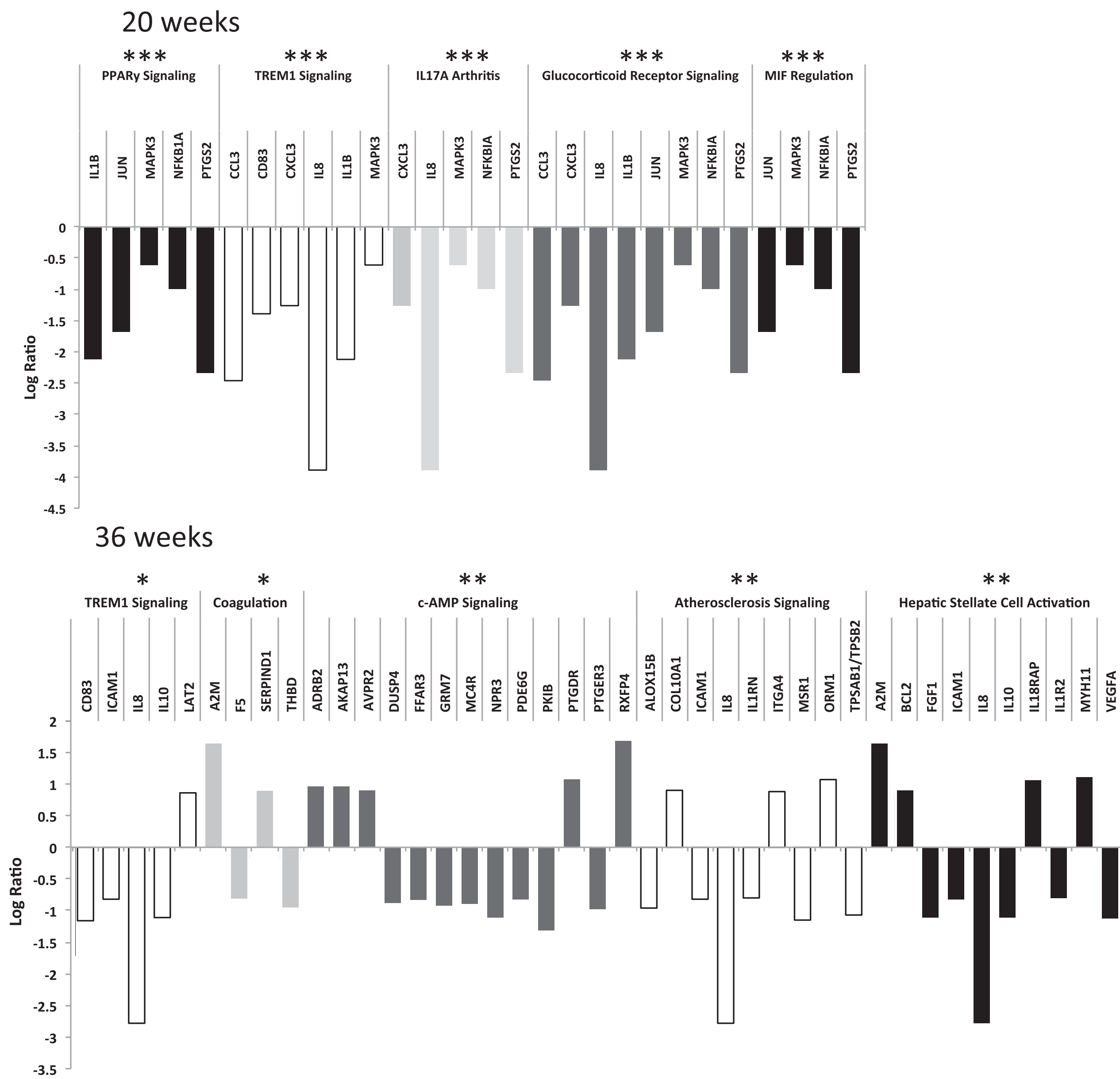

Figure 2. Top IPA canonical pathways at 20 wk (top) and 36 wk (bottom) of pregnancy. Canonical pathways are labeled on the *x* axis. Genes belonging to each canonical pathway are listed vertically beneath; the bars indicate the $\log_2$ fold change. Significance values for each pathway are marked. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

pregnancy, no other PPAR family members were differentially expressed between altitudes at any single study time, and PPARγ expression was altitude independent in the nonpregnant state.

Next, we sought to identify whether the expression of genes belonging to the PPAR family or PPARγ pathway varied across time and, if so, whether such patterns were affected by altitude. To meet this aim, we restricted our analyses to women with a 20 or 36 wk and a postpartum sample, and conducted longitudinal analyses including an interaction term (altitude×pregnancy) that allowed us to detect whether changes across time were similar between altitudes. Although PPARγ expression varied between the nonpregnant state and 20 or 36 wk and tended to differ between altitudes at 36 wk, the pattern of change across time was similar at high altitude and sea level (**Table 4**). Given the observed effects of pregnancy and altitude at 36 wk, the inability to detect an interaction effect for PPARγ specifically is likely the result of the restricted sample size included for longitudinal analyses. Compared with the nonpregnant state, pregnancy affected other members of the PPAR family that belong to adipocytokine or insulin-signaling pathways, including PPAR binding protein (PPARBP; 20 wk), PPARα (36 wk), PPARγ coactivator 1, α (PPARγC1α; 20 wk), and PPARγC1β (20 and 36 wk). The expression of numerous PPARγ targets also differed between altitudes or with pregnancy (Table 4), including 4 genes that were affected by altitude in both 20- and 36-wk comparisons [*i.e.,*

TABLE 3. *Top 20 transcriptional regulators ranked by activation z score*

TR	Log ratio	PPARγ	Overlap	*z*	*P*
20 wk					
TNF				−4.46	6.45E-23
IL1B	−2.1		×	−4.18	1.17E-27
TREM1			×	−3.79	4.64E-20
EGF			×	−3.75	4.39E-18
PDGF BB			×	−3.71	3.43E-17
CSF2				−3.50	2.63E-13
NFkB			×	−3.49	6.99E-18
Cg		×		−3.42	2.93E-17
Leuko D4		×		−3.40	1.32E-22
TGFB1				−3.25	7.18E-18
IL3				−3.24	1.51E-15
RAF1				−3.24	5.26E-13
IFNG				−3.24	5.40E-16
F2			×	−3.22	3.62E-12
CREB1			×	−3.17	9.85E-19
IL5				−3.12	1.41E-10
Tretinoin				−3.11	2.16E-06
TLR9				−3.11	6.54E-14
HGF				−3.09	3.68E-09
CREM	−1.3			−2.65	4.75E-15
36 wk					
PDGF BB		×	×	−3.52	6.62E-09
IL1B		×	×	−3.31	3.00E-11
EGF		×	×	−3.11	3.11E-05
LDL		×		−3.09	1.39E-09
CREB1		×	×	−3.03	5.79E-07
IL4		×		−2.95	3.05E-05
CHUK				−2.94	1.18E-02
P38 MAPK		×		−2.92	3.16E-06
NFkB		×	×	−2.84	3.40E-06
CD40LG				−2.82	3.02E-08
TREM1		×	×	−2.81	1.10E-07
EGR1	−1.2	×		−2.77	1.40E-03
PPARy	−1.0			−2.75	3.46E-01
F2			×	−2.69	5.82E-04
VEGF				−2.68	1.29E-07
Insulin		×		−2.68	1.01E-03
TLR2				−2.60	3.80E-03
EPAS1		×		−2.59	2.27E-04
CCL5				−2.58	6.91E-04
SRC				−2.57	8.84E-03

Log ratio indicates transcriptional regulator (TR) expression difference between altitudes, if significant. PPARy indicates TRs that affect PPARy. Overlap indicates TRs that are shared between 20- and 36-wk lists. Negative and positive *z* scores reflect inhibition and activation, respectively. CCL5, chemokine ligand 5; CD40LG, CD40 ligand; CHUK, conserved helix-loop-helix ubiquitous kinase; CREB1, cAMP responsive element binding protein 1; CREM, cAMP responsive element modulator; CSF2, colony stimulating factor 2; EGF, epidermal growth factor; EGR1, early growth response 1; EPAS1, endothelial pass domain protein1; F2, coagulation factor 2; IFNG, interferon γ; IL3, interleukin 3; IL4, interleukin 4; LDL, low-density lipoprotein; NFkB, nuclear factor of κ light polypeptide gene enhancer in B cells; P38 MAPK, mitogen-activated protein kinase *p38* α; PDGF BB, platelet-derived growth factor β polypeptide; RAF1, v-raf-1 murine leukemia viral oncogene homolog 1; TGFB1, transforming growth factor β1; TLR2, Toll-like receptor 2; TNF, tumor necrosis factor; TR, transcriptional regulator; TREM1, triggering receptor expressed on myeloid cells 1; VEGF, vascular endothelial growth factor.

adrenergic β2 receptor (ADRB2), IL8, nuclear receptor subfamily 4, group A, 1 (NR4A1), and oxidized low-density lipoprotein receptor 1 (OLR1)].

Validation of gene expression by RT-PCR

We validated the top genes based on $\log_2$ fold change in the nonpregnant state [zinc finger protein 2 (ZNF2)] and at 20 wk (IL8) by RT-PCR. For 36 wk we selected PPARγ, as the PPARγ signaling pathway was one of the most prominent pathways altered during pregnancy at high altitude compared with sea level. In accordance with our microarray data ZNF2 expression was 1.7-fold greater (nonpregnant, $P < 0.005$), IL8 expression 11.0-fold lower (20 wk, $P < 0.01$), and PPARγ expression 1.4-fold lower (36 wk, $P < 0.05$) at high altitude compared with sea level.

DISCUSSION

Our findings demonstrate that chronic exposure to hypoxia during pregnancy alters maternal gene-expression patterns compared with normoxic pregnancy and that such effects are accompanied by reduced birth weight and head circumference. Our report is the first to indicate that environmental hypoxia alters genome-wide transcriptional patterns during pregnancy. That only modest transcriptional differences were apparent between altitudes in the nonpregnant state compared

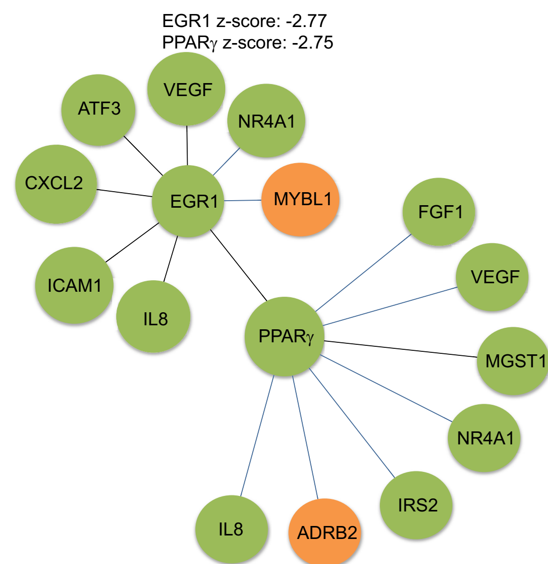

Figure 3. Relationship between early growth response protein 1 (EGR1), PPARγ, and downstream PPARγ targets. Transcriptional regulators (EGR1 and PPARγ) are shown in the center of each circle, and target or otherwise affected genes comprise the outer ring. Down- and up-regulated genes are highlighted in green and orange, respectively. Solid lines connecting the circles indicate agreement between predicted and observed expression. EGR1 expression was inhibited at high altitude *vs.* sea level at 36 wk. As shown on the left side of the cluster, all EGR1 target genes, including PPARγ, were expressed in a direction consistent with EGR1 inhibition. Likewise, the right side of the cluster shows that all PPARγ-target genes were expressed in a direction consistent with the negative regulation of PPARγ. Based on the *z* score, genes were considered to be activated ($z > 2$) or suppressed ($z < -2$).

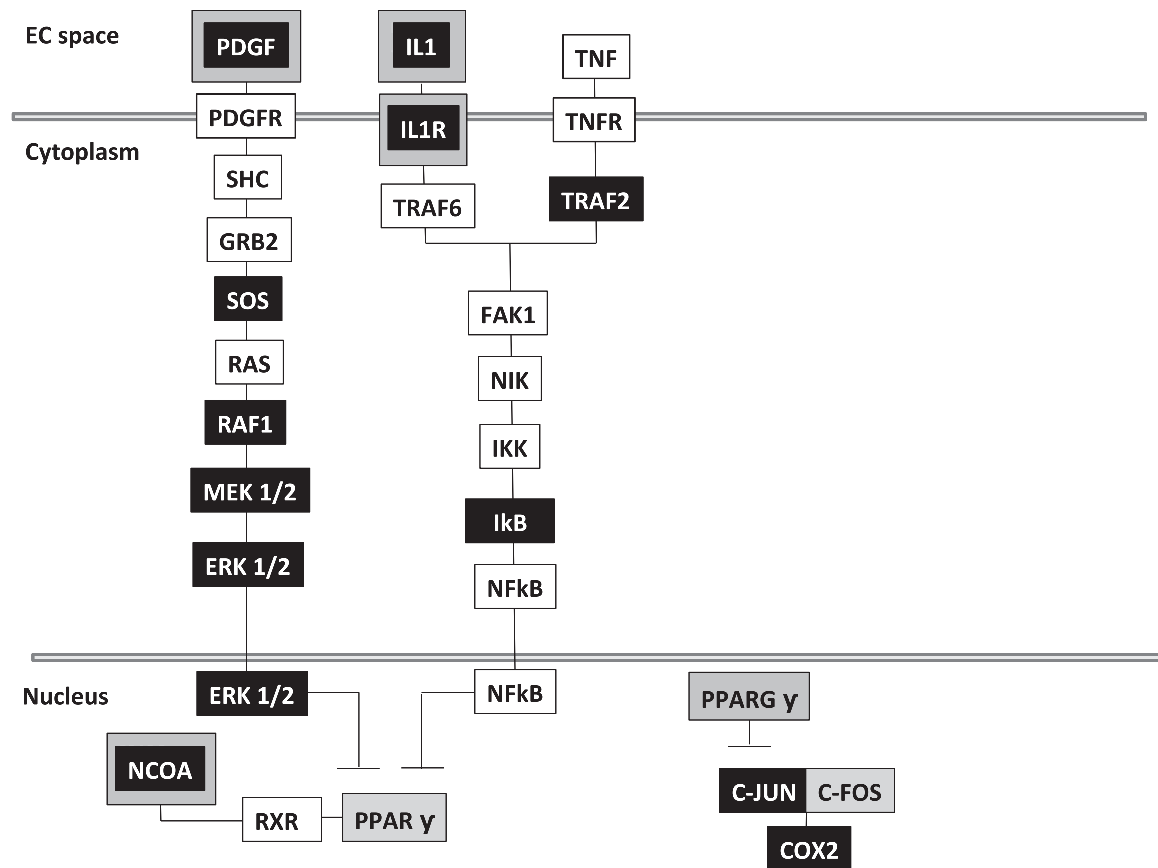

Figure 4. PPARγ signaling pathway genes that were differentially expressed during high-altitude *vs.* sea-level pregnancy at 20 wk (black), 36 wk (gray), or both (black with a gray outline). EC, extracellular space.

with 36 wk of pregnancy is notable given that the onset of altitude-associated fetal growth restriction as assessed by ultrasound biometry begins between 25 to 29 wk of gestation and persists until delivery (4). Also unique is our finding that PPARγ expression is reduced during high-altitude pregnancy and that several PPARγ transcriptional regulators and downstream targets were modified in a direction consistent with this observation. Taken together, these findings suggest that chronic maternal hypoxia interrupts transcriptional processes associated with normal pregnancy. We propose that PPARγ expression may be relevant to physiological and clinical outcomes associated with high-altitude pregnancy, including impaired fetal growth.

Methodological considerations

The interpretation of our data should take into account the nature of the population and sample chosen for analysis. Only normotensive women were included so as to avoid the influence of hypertensive pregnancy on gene transcription during high-altitude pregnancy. Even with the exclusion of hypertensive pregnancy, birth weight declined 381 g at high altitude *vs.* sea level, a magnitude that is consistent with previous reports (1, 6) and therefore sufficient to meet our objectives. However, given that hypoxia-associated preeclampsia accounts for half of the birth weight reduction at high altitude (6), one limitation of this approach is that our results are not applicable to IUGR coexisting with preeclampsia or gestational hypertension, which frequently occurs at elevations >2500 m.

One challenge to the effective use of expression profiling to study human disease is the acquisition of the most relevant cells or tissue in adequate quantity and purity. Although our use of maternal PBMCs does not allow us to infer tissue-specific responses of importance for fetal growth and pregnancy outcome, we reasoned that this cell type would allow us to gain insight into systemic mechanisms underlying altitude-related fetal growth restriction. PBMCs are the most transcriptionallyactive cells in the blood and mediate inflammatory and other processes that are likely central drivers of maternal vascular adaptation to pregnancy and, in turn, fetal growth. Further supporting the use of this cell type, transcriptional changes (including that of several genes within the PPARγ pathway) have been detected in maternal PBMCs with advancing gestation (34) and PBMCs have effectively been used as a surrogate sample type to identify markers of other vascular diseases (*e.g.*, pulmonary vascular disease ref. 35).

Finally, given the logistic challenge of recruiting subjects before pregnancy we were limited to nulliparous or postpartum (≥3 mo) for an index of the nonpregnant state. If pregnancy-related differences in gene expression persist postpartum, as is the case with select cardiovascular parameters (36), our findings

TABLE 4. *Effect of altitude and pregnancy on PPAR genes and PPARγ targets in subjects with serial samples*

Gene	P Alt	P Preg	P Alt × Preg	Probes
Nonpregnant to 20 wk				
PPAR family				
PPAR α	0.426	0.440	0.086#	2
PPARBP	0.001*	0.045*	0.938	1
PPARD	0.208	0.324	0.078#	1
PPAR γ	0.799	0.027*	0.519	2
PPAR γC1 α	0.645	0.360	0.012*	2
PPAR γC1 β	0.528	0.018*	0.908	2
PPARγ target				
IL8	0.003*	0.180	0.067#	1
IRS2	0.834	0.909	0.062#	1
NR4A1	0.027*	0.795	0.168	1
OLR1	0.034*	0.179	0.347	2
Nonpregnant to 36 wk				
PPAR family				
PPAR α	0.171	0.029*	0.374	2
PPAR γ	0.050#	0.000*	0.319	3
PPAR γC1 β	0.796	0.000*	0.874	2
PPARγ target				
ADRB2	0.007*	0.028*	0.213	3
FGF11	0.072#	0.541	0.997	1
IL8	0.053#	0.850	0.243	1
IL8RA	0.004*	0.396	0.926	2
NR4A1	0.357	0.924	0.076#	1
OLR1	0.004*	0.000*	0.883	2
VEGF	0.038*	0.079#	0.020*	1

Alt, altitude; Preg, pregnancy; Alt × Preg, interaction of altitude and pregnancy. Probes indicates number of probes for each gene showing similar patterns of change and significance. Nominal *P* values are shown. ADRB2, adrenergic β2 receptor; FGF11, fibroblast growth factor 11; IL8RA, interleukin 8 (receptor α); IRS2, insulin receptor substrate 2; NR4A1, nuclear receptor subfamily 4, group A, 1; OLR1, oxidized low density lipoprotein receptor 1; PPARγC1α, PPARγ coactivator 1, α; PPARγC1β, PPARγ coactivator 1, β; PPARBP, PPAR binding protein; PPARD, PPARδ; $*P < 0.05$. $\#0.10 > P > 0.05$.

would be expected to underestimate changes associated with pregnancy.

Potential role of PPARγ signaling for impaired fetal growth at high altitude

The PPAR family is a group of ligand-activated nuclear receptors that mediate the transcription of numerous genes that are involved in pivotal processes for normal pregnancy including placentation (37), inflammatory response (38), energy homeostasis (22), and vascular function (39). Strengthening the hypothesis that PPARγ may be related to clinical outcomes associated with high-altitude pregnancy, it is repressed in preeclampsia and IUGR (reviewed in ref. 23) and in response to hypoxia (40).

Both experimental animal models and human studies implicate PPARγ in preeclampsia and IUGR. Administering a PPARγ antagonist to pregnant rats impairs fetal growth and induces a constellation of phenotypic changes characteristic of preeclampsia in humans, including hypertension, proteinuria, and endothelial dysfunction (41). Subsequent administration of rosiglitazone, a PPARγ agonist, reverses hypertension and endothelial dysfunction in pregnant animals (42). In humans, PPARγ expression is directly related to fetal and placental weight, being 2-fold lower in IUGR compared with control placentas (43). PPARγ activators in maternal serum are also suppressed in early onset preeclampsia *vs.* normal pregnancy (44). In contrast, others report increased or unchanged placental PPARγ expression and greater PPARγ binding activity in IUGR, preeclampsia, or coexisting IUGR-preeclampsia (45, 46). Despite somewhat inconsistent findings, evidence supporting a role for PPARγ in pregnancy-related disease is strong enough that it has been proposed as a therapeutic target for preeclampsia, IUGR, gestational diabetes and prematurity (23).

PPARγ functions of potential relevance for high-altitude pregnancy

We consider that there are four possibilities by which PPARγ may be involved in high-altitude pregnancy and hypoxia-related IUGR (**Fig. 5**). The first of these relates to its fundamental role in placentation, and specifically the maturation and differentiation of trophoblast lineages that permit remodeling of the spiral arteries, which are maternal vessels supplying the endometrium (47). During early human pregnancy, extravillous cytotrophopblasts invade the decidualized endometrium and initiate extensive vascular remodeling of the maternal spiral arteries (48). The loss of vascular endothelial and smooth muscle cells that occurs during remodeling reduces vessel contractility and creates permanently dilated channels that serve to reduce uteroplacental vascular resistance and facilitate uninterrupted oxygen and nutrient transport to the developing fetus. Incomplete trophoblast invasion reduces spiral artery remodeling and uteroplacental perfusion in preeclampsia, IUGR (49), and high-altitude pregnancy (50). In this regard, the relative PPARγ deficiency we observed may be relevant for impaired fetal growth at high altitude.

A second possibility relates to the role of PPARγ as an inflammatory mediator (Fig. 5). PPARγ negatively regulates proinflammatory transcription factors [*e.g.*, NF-κB, adaptor-related complex 1 (AP-1)], thereby attenuating cytokine production (*e.g.*, IL1B and IL8) (38, 51). However, given that IL1B and IL8 expression was reduced at high altitude *vs.* sea level in this study, our data suggest that inhibition of PPARγ expression does not act to increase the transcription of proinflammatory factors during high-altitude pregnancy. Although gene transcription generally shows a positive correlation with protein expression, it is an imperfect predictor of circulating protein levels (52). For this reason, we consider it possible that circulating cytokine levels were altered by altitude or the inhibition of PPARγ expression, but we were unable to capture such changes at the level of gene transcription.

A third possibility pertains to the ability of PPARγ activation to reverse endothelial dysfunction, lower blood pressure in hypertensive disease, and improve endothelium-dependent vasodilation (reviewed in ref. 42). Given that reduced uterine artery diameter and uteroplacental oxygen delivery have been implicated as

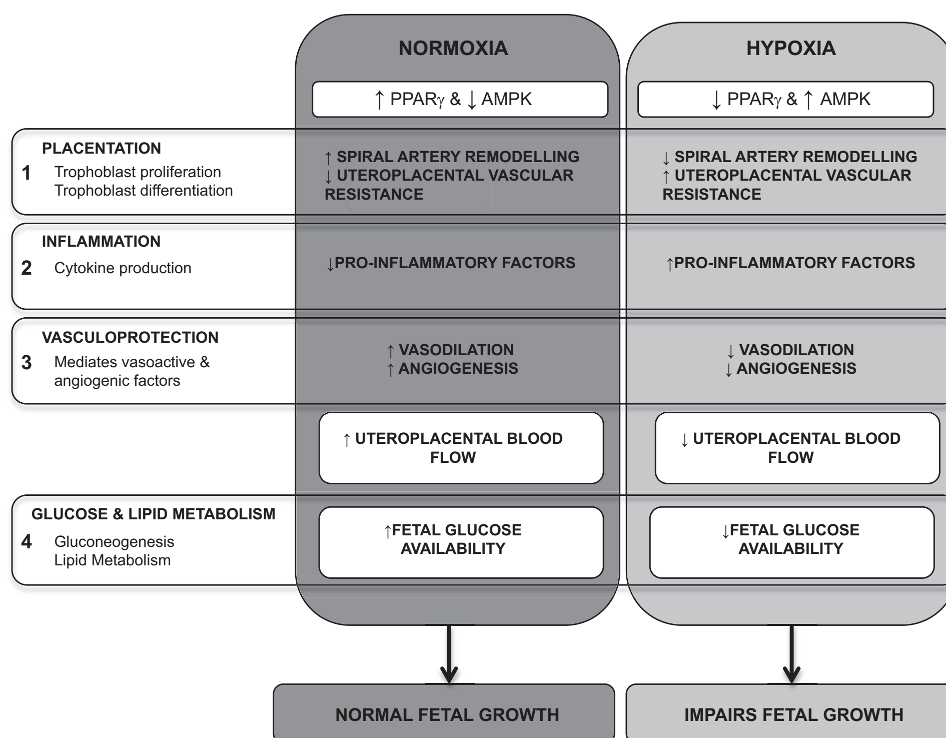

Figure 5. Four mechanisms by which inhibition of PPARγ during high-altitude pregnancy may influence fetal growth: *1*) alterations to placentation; *2*) alterations to inflammatory processes; *3*) alterations to vascular function; and *4*) alterations to glucose homeostasis. Inhibition of PPARγ due to hypoxia (right panel) could impede uteroplacental blood flow by impairing spiral artery remodeling and increasing uteroplacental resistance, increasing the production of proinflammatory factors, or reducing vasodilaton and angiogenesis *vs.* normoxic pregnancy (left panel). PPARγ-mediated regulation of glucose homeostasis may also influence fetal growth at high altitude.

contributors to hypoxia-related IUGR (5, 25), the vasoprotective effects of PPARγ may be relevant for successful pregnancy at high altitude. PPARγ exerts its vasoprotective properties by inhibiting vasoconstrictors (*e.g.*, endothelin 1) and antiangiogenic (*e.g.*, sFlt-1) factors, increasing VEGF production (39), and modulating nitric oxide bioavailability (23). However, with the exception of reduced VEGF mRNA expression at high altitude *vs.* sea level, there were no prominent transcriptional changes of genes encoding vasoactive or angiogenic factors between altitudes. In agreement with this observation, circulating levels of endothelin 1 or sFlt-1 were similar between altitudes (data not shown) as well as during pregnancy at 3100 m relative to lower altitude (1609 m) (17). However, as was the case for cytokines, our observation that vasoactive or angiogenic factor gene transcription was unaffected by altitude may be related to the fact that mRNA transcription is not equivalent to protein expression (52).

Fourth and finally, a recent study proposing that hypoxia-related IUGR is the product of fetal hypoglycemia rather than fetal oxygen deficit (53) led us to consider the possibility that PPARγ-mediated regulation of glucose homeostasis influences fetal growth at high altitude. PPARγ acts in coordination with adenosine monophosphate-activated protein kinase isoform a1 (AMPKa1) to sense and regulate cellular energy homeostasis (54). Specifically, PPARγ agonists enhance AMPK-dependent metabolic activity to repress gluconeogenesis (55) and enhance glucose uptake (56) independent of PPARγ activity. Activated AMPK inhibits PPARγ expression (57). This suggests that the comparative down-regulation of PPARγ at high altitude we observed may be due to enhanced AMPK activity, which, in turn, would be expected to increase maternal blood glucose uptake and reduce fetal glucose availability, as has been observed during pregnancy at high altitude relative to sea level (58). Further supporting the potential importance of metabolic pathways for impaired fetal growth, we recently reported that maternal genetic variation within *AMPKa1* is associated with birth weight; specifically women homozygous for the T allele at *AMPKa1* locus rs1345778 (TT) delivered infants of greater birth weight relative to heterozygotes (21).

CONCLUSIONS

Our findings demonstrate that chronic maternal hypoxia during pregnancy alters gene expression patterns and supports the possibility that inhibition of PPARγ may influence maternal physiological responses to pregnancy and fetal growth at high altitude. Further study using experimental animal models to manipulate PPARγ activation during hypoxic pregnancy will be useful for clarifying the role of PPARγ during hypoxic pregnancy and to determine its direct relevance, if any, to hypoxia-associated IUGR. Such studies could also be used to further understand the interaction between AMPK and PPARγ for metabolic homeostasis during gestation. Understanding the mechanisms by which chronic hypoxia impairs fetal growth has tremendous clinical and public health implications given that fetal growth raises the risk of morbidity and mortality not only during the perinatal period but also increases susceptibility to metabolic, cardiovascular and pulmonary disease in later life (59). FJ

The authors acknowledge all the women who participated in this project. Their appreciation is also extended to physicians and technical staff at the Bolivian Institute of High Altitude Biology and Clinica Sirani for assistance with

the conduct of this project. Finally, the authors thank Dr. Megan Wilson, Corrine Hennessy, and Beth Davidson for assistance with data acquisition and analysis. C.G.J. is supported by a U.S. National Institutes of Health (NIH) Building Interdisciplinary Research Careers Women's Health grant (5 K12 HD057022-07). Support for data collection and analysis was obtained from grants NIH-HL079647 (L.G.M.), NIH-HL095393 (D.A.S.), NIH-HL101715 (D.A.S.), and NIH-ES18181 (D.A.S.).

REFERENCES

1. Julian, C. G., Vargas, E., Armaza, J. F., Wilson, M. J., Niermeyer, S., Villena, M., and Moore, L. G. (2007) High-altitude ancestry protects against hypoxia-associated reductions in fetal growth. *Arch. Dis. Child Fetal Neonatal Ed.* **92,** F372–F377
2. Palmer, S. K., Moore, L. G., Young, D. A., Cregger, B., Berman, J. C., and 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
3. Krampl, E., Espinoza-Dorado, J., Lees, C. C., Moscoso, G., and Bland, J. M. (2001) Maternal uterine artery Doppler studies at high altitude and sea level. *Ultrasound Obstet. Gynecol.* **18,** 578–582
4. Krampl, E., Lees, C., Bland, J. M., Dorado, J. E., Gonzalo, M., and Campbell, S. (2000) Fetal biometry at 4300 m compared with sea level in Peru. *Ultrasound Obstet. Gynecol.* **16,** 9–18
5. Moore, L., Shriver, M., Bemis, L., Hickler, B., Wilson, M., Brutsaert, T., Parra, E., and Vargas, E. (2004) Maternal adaptation to high-altitude pregnancy: an experiment of nature. *Placenta* **25**(Suppl.), S60–S71
6. Keyes, L. E., Armaza, J. F., Niermeyer, S., Vargas, E., Young, D., Villena, M., and Moore, L. G. (2003) Intrauterine growth restriction, preeclampsia and intrauterine mortality at high altitude in Bolivia. *Pediatr. Res.* **54,** 20–25
7. Villar, K., Say, L., Ĝulmenzoglu, A. M., Merialdi, M., Lindheimer, M. D., Betran, A. P., and Piaggio, G. (2003) Eclampsia and pre-eclampsia: a health problem for 2000 years. In *Pre-eclampsia* (Critchley, H. M. A., Poston, L., and Walker, J. J., eds) pp. 189–207, RCOG Press, London, UK
8. Walker, J. J. (2000) Pre-eclampsia. *Lancet* **356,** 1260–1265
9. Gilbert, W., and Danielsen, B. (2003) Pregnancy outcomes associated with intrauterine growth restriction. *Am. J. Obstet. Gynecol.* **188,** 1596–1599; discussion 1599–1601
10. Caniggia, I., and Winter, J. L. (2002) Hypoxia inducible factor-1: oxygen regulation of trophoblast differentiation in normal and pre-eclamptic pregnancies–a review. *Placenta* **23,** S47–S57
11. Wheaton, W. W., and Chandel, N. S. (2011) Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am. J. Physiol. Cell Physiol.* **300,** C385–C393
12. Semenza, G. L. (2007) Life with oxygen. *Science* **318,** 62–64
13. Arngrimsson, R., Hayward, C., Nadaud, S., Baldursdottir, A., Walker, J. J., Liston, W. A., Bjarnadottir, R. I., Brock, D. J. H., Geirsson, R. T., Connor, J. M., and Soubrier, F. (1997) Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region. *Am. J. Hum. Genet.* **61,** 354–362
14. Savvidou, M. D., Valance, P. J. T., Nicolaides, K. H., and Hingorani, A. D. (2001) Endothelial nitric oxide synthase gene polymorphism and maternal vascular adaptation to pregnancy. *Hypertension* **38,** 1289–1293
15. Davila, R. D., Julian, C. G., Wilson, M. J., Browne, V. A., Rodriguez, C., Bigham, A. W., Shriver, M. D., Vargas, E., and Moore, L. G. (2010) Do cytokines contribute to the Andean-associated protection from reduced fetal growth at high altitude? *Reprod. Sci.* **18,** 79–87
16. Davila, R. D., Julian, C. G., Wilson, M. J., Browne, V. A., Rodriguez, C., Bigham, A. W., Shriver, M. D., Vargas, E., and Moore, L. G. (2010) Do anti-angiogenic or angiogenic factors contribute to the protection of birth weight at high altitude afforded by Andean ancestry? *Reprod. Sci.* **17,** 861–870
17. Julian, C. G., Galan, H. L., Wilson, M. J., Desilva, W., Cioffi-Ragan, D., Schwartz, J., and Moore, L. G. (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
18. Zamudio, S., Baumann, M. U., and Illsley, N. P. (2006) Effects of chronic hypoxia in vivo on the expression of human placental glucose transporters. *Placenta* **27,** 49–55
19. Zamudio, S., Wu, Y., Ietta, F., Rolfo, A., Cross, A., Wheeler, T., Post, M., Illsley, N. P., and Caniggia, I. (2007) Human placental hypoxia-inducible factor-1alpha expression correlates with clinical outcomes in chronic hypoxia in vivo. *Am. J. Pathol.* **170,** 2171–2179
20. Baumann, M. U., Zamudio, S., and Illsley, N. P. (2007) Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1. *Am. J. Physiol. Cell Physiol.* **293,** C477–C485
21. Bigham, A. W., Wilson, M. J., Browne, V. A., Julian, C. G., Vargas, E., Rodriquez, C., Shriver, M. D., and Moore, L. G. (2012) Maternal genetic variation near PRKAA1 and EDNRA is associated with birth weight among residents of high altitude. Abstract 3290, American Society of Human Genetics Annual Meeting, San Francisco, CA, USA
22. Escher, P., and Wahli, W. (2000) Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat. Res.* **448,** 121–138
23. McCarthy, F. P., Delany, A. C., Kenny, L. C., and Walsh, S. K. (2013) PPAR-gamma – a possible drug target for complicated pregnancies. *Br. J. Pharmacol.* **168,** 1074–1085
24. Bigham, A., Bauchet, M., Pinto, D., Mao, X., Akey, J. M., Mei, R., Scherer, S. W., Julian, C. G., Wilson, M. J., Lopez Herraez, D., Brutsaert, T., Parra, E. J., Moore, L. G., and Shriver, M. D. (2010) Identifying signatures of natural selection in tibetan and andean populations using dense genome scan data. *PLoS Genet.* **6**
25. Julian, C. G., Wilson, M. J., Lopez, M., Yamashiro, H., Tellez, W., Rodriguez, A., Bigham, A. W., Shriver, M. D., Rodriguez, C., Vargas, E., and Moore, L. G. (2009) Augmented uterine artery blood flow and oxygen delivery protect Andeans from altitude-associated reductions in fetal growth. *Am. J. Physiol.* **296,** R1564–1575
26. Shriver, M. D., Mei, R., Parra, E. J., Sonpar, V., Halder, I., Tishkoff, S. A., Schurr, T. G., Zhadanov, S. I., Osipova, L. P., Brutsaert, T. D., Friedlaender, J., Jorde, L. B., Watkins, G., Fernandez, J. R., Akey, J. M., and Jones, K. W. (2005) Large-scale SNP analysis reveals clustered and continuous patterns of human genetic variation. *Hum. Genomics* **2,** 81–89
27. Witter, F. R., and Luke, B. (1991) The effect of maternal height on birth weight and birth length. *Early Hum. Dev.* **25,** 181–186
28. Oken, E., Kleinman, K. P., Rich-Edwards, J., and Gillman, M. W. (2003) A nearly continuous measure of birth weight for gestational age using a United States national reference. *BMC Pediatrics* **3,** 6
29. Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U., and Speed, T. P. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4,** 249–264
30. Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E., and Storey, J. D. (2012) The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* **28,** 882–883
31. Smyth, G. K. (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **3,** Article 3
32. Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Royal Stat. Soc. Series B* **57,** 289–300
33. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2[-delta delta C(T)] method. *Methods* **25,** 402–408
34. Weix, J., Forger, F., Haupl, T., Surbek, D., Ostensen, M., and Villiger, P. M. (2012) Influence of pregnancy on the adipocytokine and peroxisome proliferator-activated receptor pathways in peripheral blood mononuclear cells from healthy donors and rheumatoid arthritis patients. *Arthritis Rheum.* **64,** 2095–2103
35. Bull, T. M., Coldren, C. D., Geraci, M. W., and Voelkel, N. F. (2007) Gene expression profiling in pulmonary hypertension. *Proc. Am. Thorac. Soc.* **4,** 117–120

36. Capeless, E., and Clapp, J. (1991) When do cardiovascular parameters return to their preconception values? *Am. J. Obstet. Gynecol.* **165,** 883–886
37. Parast, M. M., Yu, H., Ciric, A., Salata, M. W., Davis, V., and Milstone, D. S. (2009) PPARgamma regulates trophoblast proliferation and promotes labyrinthine trilineage differentiation. *PLoS One* **4,** e8055
38. Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* **391,** 79–82
39. Jozkowicz, A., Dulak, J., Piatkowska, E., Placha, W., and Dembinska-Kiec, A. (2000) Ligands of peroxisome proliferator-activated receptor-gamma increase the generation of vascular endothelial growth factor in vascular smooth muscle cells and in macrophages. *Acta Biochim. Pol.* **47,** 1147–1157
40. Li, X., Kimura, H., Hirota, K., Sugimoto, H., Kimura, N., Takahashi, N., Fujii, H., and Yoshida, H. (2007) Hypoxia reduces the expression and anti-inflammatory effects of peroxisome proliferator-activated receptor-gamma in human proximal renal tubular cells. *Nephrol. Dial. Transplant.* **22,** 1041–1051
41. McCarthy, F. P., Drewlo, S., English, F. A., Kingdom, J., Johns, E. J., Kenny, L. C., and Walsh, S. K. (2011) Evidence implicating peroxisome proliferator-activated receptor-gamma in the pathogenesis of preeclampsia. *Hypertension* **58,** 882–887
42. McCarthy, F. P., Drewlo, S., Kingdom, J., Johns, E. J., Walsh, S. K., and Kenny, L. C. (2011) Peroxisome proliferator-activated receptor-gamma as a potential therapeutic target in the treatment of preeclampsia. *Hypertension* **58,** 280–286
43. Diaz, M., Bassols, J., Lopez-Bermejo, A., Gomez-Roig, M. D., de Zegher, F., and Ibanez, L. (2012) Placental expression of peroxisome proliferator-activated receptor gamma (PPAR-gamma): relation to placental and fetal growth. *J. Clin. Endocrinol. Metab.* **97,** E1468–1472
44. Waite, L. L., Louie, R. E., and Taylor, R. N. (2005) Circulating activators of peroxisome proliferator-activated receptors are reduced in preeclamptic pregnancy. *J. Clin. Endocrinol. Metab.* **90,** 620–626
45. Rodie, V. A., Young, A., Jordan, F., Sattar, N., Greer, I. A., and Freeman, D. J. (2005) Human placental peroxisome proliferator-activated receptor delta and gamma expression in healthy pregnancy and in preeclampsia and intrauterine growth restriction. *J. Soc. Gynecol. Investig.* **12,** 320–329
46. Holdsworth-Carson, S. J., Lim, R., Mitton, A., Whitehead, C., Rice, G. E., Permezel, M., and Lappas, M. (2010) Peroxisome proliferator-activated receptors are altered in pathologies of the human placenta: gestational diabetes mellitus, intrauterine growth restriction and preeclampsia. *Placenta* **31,** 222–229
47. Schaiff, W. T., Carlson, M. G., Smith, S. D., Levy, R., Nelson, D. M., and Sadovsky, Y. (2000) Peroxisome proliferator-activated receptor-gamma modulates differentiation of human trophoblast in a ligand-specific manner. *J. Clin. Endocrinol. Metab.* **85,** 3874–3881
48. Pijnenborg, R., Dixon, G., Robertson, W. B., and Brosens, I. (1980) Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. *Placenta* **1,** 3–19
49. Khong, T. Y., De Wolf, F., Robertson, W. B., and Brosens, I. (1986) Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br. J. Obstet. Gynaecol.* **93,** 1049–1059
50. Tissot Van Patot, M., Grilli, A., Chapman, P., Broad, E., Tyson, W., Heller, D. S., Zwerdlinger, L., and Zamudio, S. (2003) Remodeling of uteroplacental arteries is decreased in high altitude placentae. *Placenta* **24,** 326–335
51. Jiang, C., Ting, A. T., and Seed, B. (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* **391,** 82–86
52. Guo, Y., Xiao, P., Lei, S., Deng, F., Xiao, G. G., Liu, Y., Chen, X., Li, L., Wu, S., Chen, Y., Jiang, H., Tan, L., Xie, J., Zhu, X., Liang, S., and Deng, H. (2008) How is mRNA expression predictive for protein expression? A correlation study on human circulating monocytes. *Acta Biochim. Biophys. Sinica* **40,** 426–436
53. Zamudio, S., Torricos, T., Fik, E., Oyala, M., Echalar, L., Pullockaran, J., Tutino, E., Martin, B., Belliappa, S., Balanza, E., and Illsley, N. P. (2010) Hypoglycemia and the origin of hypoxia-induced reduction in human fetal growth. *PLoS One* **5,** e8551
54. Towler, M. C., and Hardie, D. G. (2007) AMP-activated protein kinase in metabolic control and insulin signaling. *Circ. Res.* **100,** 328–341
55. Zhang, B. B., Zhou, G., and Li, C. (2009) AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab.* **9,** 407–416
56. Bugianesi, E., McCullough, A. J., and Marchesini, G. (2005) Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* **42,** 987–1000
57. Sozio, M. S., Lu, C., Zeng, Y., Liangpunsakul, S., and Crabb, D. W. (2011) Activated AMPK inhibits PPAR-α and PPAR-γ transcriptional activity in hepatoma cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **301,** G739–G747
58. Krampl, E., Kametas, N. A., Cacho-Zegarra, A. M., Roden, M., and Nicolaides, K. H. (2001) Maternal plasma glucose at high altitude. *Br. J. Obstet. Gynaecol.* **108,** 254–257
59. Barker, D. J. (1999) Fetal origins of cardiovascular disease. *Ann. Med.* **31** 3–6

Received for publication August 8, 2013.
Accepted for publication November 18, 2013.