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Hypoxia and Lactate Production in Trophoblast Cells

H.H. Kay*, S. Zhu, S. Tsoi

Department of Obstetrics and Gynecology, College of Medicine, University of Arkansas for Medical Sciences, 4301 West Markham Street, #518, Little Rock, AR 72205, USA

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Abstract

The etiology of preeclampsia is unknown but is thought to be related to hypoxia in the placenta. We previously reported that the enzyme lactate dehydrogenase (LDH) has increased activity and gene expression in placentas from preeclamptic pregnancies [Tsoi SCM, Zheng J, Xu F, Kay HH. Differential expression of lactate dehydrogenase isozymes (LDH) in human placenta with high expression of LDH-A_4 isozyme in the endothelial cells of pre-eclampsia villi. Placenta 2001;22:317–22]. LDH is responsible for pyruvate conversion to lactate through glycolysis. In this study, we further investigated the role of hypoxia in primary trophoblast cells and a cultured cell line, JEG3 cells, to obtain a better understanding of how it affects the activities of lactate dehydrogenase, lactate production and regulatory genes, as a possible model for preeclampsia. Primary trophoblast cells and JEG3 cells were cultured under 1% oxygen. At 6, 12 and 24 h, cells were analyzed for LDHA and LDHB isozyme activities, mRNA and protein expression compared to standard culture conditions. Lactate was measured from cell medium. The hypoxia inducible transcription factor (HIF-1α) protein expression was confirmed by western blot. Two lactate transporters (MCT1 and MCT4) mRNA and protein expression were also studied under hypoxia. Finally, lactate was measured in plasma obtained from patients with severe preeclampsia. Under hypoxic conditions, LDHA mRNA is increased in primary trophoblast cells and JEG3 cells. The HIF-1α protein expression is higher in hypoxia-treated JEG3 cells than control. LDHA isozyme activity and its protein expression are increased most significantly at 24 h of culture under hypoxia. However, LDHB protein is unchanged while its mRNA is decreased. Lactate secretion from JEG3 cells under hypoxia is increased, as is the lactate levels in the plasma from preeclampsia patients. Of the two lactate transporters studied, MCT4 mRNA and protein level are increased under hypoxia. Our findings support the role of hypoxia in inducing HIF-1α activity in trophoblasts and increasing LDH transcription as well as its activity. Higher levels of lactate are produced and secreted which may contribute to the higher lactate levels in plasma of preeclamptic patients. These mechanisms may be important in the pathophysiology of preeclampsia.

Keywords: Trophoblasts; Preeclampsia; Hypoxia; Lactate dehydrogenase; Lactate

1. Introduction

While the etiology of preeclampsia is unknown, the pathophysiology is thought to be related to hypoxia in the placenta [1,2]. There are several clinical conditions to support this theory: preeclampsia is more frequent in women with chronic hypertension and other medical conditions leading to poor placental perfusion including multiple gestations, diabetic and molar pregnancies.

In normal tissues, hypoxia increases metabolic pathways, such as glycolysis. High glucose consumption and lactate production is normally present in the human placenta and it has been accepted that glycolysis is its baseline, major energy pathway [3–6]. Hypoxia, if encountered in preeclampsia, will further enhance glycolysis and increase the activity of lactate dehydrogenase which converts pyruvate to lactate. We have previously shown that lactate dehydrogenase activity and gene expression are higher in placentas from preeclamptic pregnancies compared to normal pregnancies [7]. We believe

* Corresponding author. Tel.: +1 501 686 5380 (office); fax: +1 501 603 1617.
E-mail address: hhkay@uams.edu (H.H. Kay).

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this observation supports the theory that hypoxia takes place within the placenta in preeclampsia.

The biological consequences of increased lactate levels within the placenta resulting from increased lactate dehydrogenase activity in preeclampsia are unknown. Lactate could serve as a signaling compound to coordinate cell and systemic function. For example, it could serve as fuel for the fetus within the hypoxic environment through glucose generation [8,9]. In tumors, lactate could facilitate invasion through acidification of the microenvironment which destroys adjacent normal tissues and also through acid-induced degradation of the extracellular matrix and promotion of angiogenesis [10]. In leukemic cells, there may be a similar destructive process leading to direct cell death [11]. In the human testis, in contrast, lactate dose-dependently inhibited germ cell death, suggesting an anti-apoptotic role for lactate [12]. We undertook this investigation to define the relationship between hypoxia, LDH isozyme activity and lactate production in trophoblasts, cells directly sensing hypoxia within the maternal circulation. We tested the hypothesis that hypoxia induces LDH isozyme activity in trophoblasts resulting in higher lactate production. A better understanding of these relationships may enhance our understanding of the pathophysiology of preeclampsia.

2. Materials and methods

JEG3 cells were purchased from ATCC (Manassas, VA). The reagents and chemicals for general use were brought from Sigma Chemical Co. (St. Louis, MO) if not indicated specifically.

2.1. Cell culture

2.1.1. Primary trophoblast

Placentas were obtained with consent at the time of delivery and trophoblast cells were isolated by digestion with Dispase (Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA) and purified by Percoll density gradient centrifugation as previously described [13].

2.1.2. JEG3 cells

To perform molecular biology studies of LDH behavior in a cell model, we chose the JEG3 choriocarcinoma cell line to minimize heterogeneity.

2.1.3. Primary trophoblasts and JEG3 cells

Both primary trophoblasts and JEG3 cells were maintained in DMEM (Dulbecco's modified Eagle's medium; Gibco BRL), supplemented with 2% (v/v) Fetal Bovine serum sterile filtered (FBS; Equitech-Bio, Kerrville, TX) for primary trophoblasts and 10% for JEG3 cells, 2 mM L-glutamine (Gibco BRL), 100 U/ml penicillin (Gibco BRL), and 0.1 mg/ml streptomycin (Gibco BRL). Cells were grown to ~80% confluence and then passaged three times in either 60-mm dishes or T75 flasks before freezing in liquid nitrogen in 10% DMSO. In subsequent experiments, cells were recovered and grown in T75 flasks to 80% confluence and subcultured (passage 4). Cell count and viability tests were performed before the cells were evenly distributed into a 6-well plate. Usually, a density of $\sim 1-5 \times 10^5$ cells per well in 6-well plates (Sarstedt, Newton, NC) were maintained at 37 °C in standard culture conditions, 5% CO_2, 95% air (control) at saturation humidity overnight for cell attachment. Fresh degassed medium was replaced immediately the next morning before hypoxia exposure. (The medium was bubbled through nitrogen gas for 30 min before use.) During hypoxia studies, the cultures were flushed independently with a gas mixture of 1% O_2, 5% CO_2, 94% N_2 in a multiple gas control incubator model MCO-18M, with a high-precision zirconia electrolyte oxygen sensor (SANYO Scientific, Bensenville, IL), for various time points (6, 12 or 24 h). Oxygen tension was measured in the cell culture medium by Blood Gases Rapidlab® 850 System (Bayer HealthCare, Tarrytown, NY) after 24 h of hypoxia and ranged from 31 to 48 mm Hg.

2.2. Cell counting and viability staining

Total cell and viability counts were carried out, on all samples before and after treatment, in a hemocytometer. Cell viability counts were carried out after diluting the suspended cell sample 1:1 with trypan blue (Sigma, T-8154).

2.3. Plasma collection

Blood sample collection was approved by the Institutional Review Board. Blood samples were obtained on admission from women with normal pregnancies ($n = 29$) and severe preeclampsia ($n = 9$) defined as blood pressure above 160/110 on two or more occasions, at least 6 h apart, and with 3 or 4+ proteinuria on dipstick or ≥5.0 g proteinuria in 24 h. Blood samples were centrifuged at 2700 rpm for 10 min, to obtain plasma for storage at −80 °C. Patients with severe preeclampsia, instead of mild preeclampsia, were selected to optimize detection of any possible difference.

2.4. Lactate measurement

A Boehringer Mannheim (BM) Accusport/Accutrend handheld lactate meter was used to measure lactate levels in plasma and cell culture media according to the company's manual. Calibration and detection of lactate with this system is described elsewhere [14].

2.5. Relative LDH isozyme activity

Standard conditions and hypoxia-treated cells from each well were collected into 1.5 ml tubes and the culture media removed by centrifugation at 10,000× *g* for 30 min at 4 °C. Cells were lysed with 0.2 ml ice cold LD buffer (Beckman Coulter, Brea, CA) after sonification for 30 s. Detailed description of electrophoresis and detection are as previously described [7]. Isozymes were quantified with a Bio-Rad Molecular Imager ChemiDoc XRS System and expressed as fold change in intensity compared to control.

2.6. Protein lysates and western blot analysis

For preparation of protein lysates for western blot analysis, JEG3 cells were lysed by adding CelLytic M cell lysis reagent according to the manufacturer's protocol (Sigma). After 30 min on ice with shaking, the lysates were centrifuged at 15,000× *g* for 10 min at 4 °C to obtain supernatant. Prior to western blot analysis, protein concentrations were determined using the BCA Protein Assay (Pierce Biotechnology, Inc., Rockford, IL). Protease inhibitor cocktail (Sigma) was added to the supernatant and stored at −80 °C. A 50 µg quantity of each protein sample was resolved by SDS-PAGE in 8% Tris–Glycine gels at 100 V for 1 h using a Bio-Rad MiniProtean II tank, transferred to Immun-Blot PVDF membrane (Bio-Rad, Hercules, CA) at constant voltage (100 V) for 70 min in a Bio-Rad Mini Trans-Blot Cell at 4 °C. Non-specific protein binding by the blots was blocked in 5% non-fat dry milk in phosphate-buffered saline (PBS) overnight at 4 °C. Membrane was then washed and incubated with primary (1°) and later secondary (2°) antibodies for 1 h at room temperature (Table 1). After incubation, the membrane was washed in PBS–1% Tween 20 and treated with enhanced chemiluminescence (ECL) reagent (Amersham). Blots were exposed to Bio-Rad Molecular Imager ChemiDoc XRS System and set at Chemi Hi Sensitivity. The image was analyzed by Quantity One software (Bio-Rad, Hercules, CA).

2.7. Reverse transcription-polymerase chain reaction (RT-PCR) assays

Total RNA samples were extracted from JEG3 cells treated with standard conditions and hypoxia at different time points using Invitrogen's

Table 1
Antibody titers used for western blotting

Protein	MW (kDa)	1° antibody source	Dilution factor	2° antibody (type[a], dilution)
LDHA	35.6	Abcam, ab-9002	1:1000	B, 1:2000
LDHB	36	Sigma, L-7016	1:500	C, 1:1500
β-Actin	42	Sigma, A-5441	1:500	C, 1:2000
MCT1	60	IMGENEX, IMG-3419	1:500	A, 1:4000
MCT4(E16)	54	Santa Cruz, sc-14932	1:500	A, 1:4000
HIF-1α	120	BD Biosciences, 610958	1:200	C, 1:2000

[a] Secondary antibodies listed in the table are as follows: A, donkey anti-goat IgG–HRP (Santa Cruz Biotechnology, Santa Cruz, CA; Cat # sc-2033); B, Rabbit anti-sheep IgG (H + L) (Rockland Immunochemicals Inc., Gilbertsville, PA; Cat # 613-4328); C, goat anti-mouse IgG (H + L)–HRP Conjugate (Bio-Rad Laboratories, Hercules, CA; Cat # 170-6516).

Total RNA purification system and 1 μg of DNA-free RNA (TURBO DNA-free kit; Ambion, Austin, TX) was transcribed into first cDNA using Transcriptor first strand cDNA Synthesis Kit (Roche, Indianapolis, IN). To determine the expression of candidate genes, PCR was performed using GoTaq DNA polymerase (Promega, Madison, WI). Ten picomoles of each primer pairs (Table 2) was combined with cDNA and reagents in a total volume of 25 μl. PCR conditions were 1 min at 95 °C, followed by 23 cycles of 95 °C for 15 s, 55 °C–60 °C (see Table 2 for specific annealing temperatures) for 30 s, 72 °C for 30 s. Products were electrophoresed on 2% agarose gel along with a 100-bp ladder for size markers (Invitrogen, Carisbad, CA). Gels were exposed to Bio-Rad Molecular Imager ChemiDoc XRS System and analyzed with Quantity One software.

2.8. Statistical analysis

Statistical analysis was performed by the unpaired *t*-test using SigmaStat (version 1.0). All data are presented as mean ± standard error (SE). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Hypoxic responses of primary trophoblast LDH isozymes

Primary human trophoblast cells demonstrated an increase in LDHA mRNA when exposed to hypoxic conditions confirming LDHA isozyme presence in placental trophoblasts and their response to hypoxia (Fig. 1A).

3.2. Hypoxic responses of JEG3 LDH isozymes

To determine if hypoxia could induce JEG3 cells to express an increase in LDHA4 isozyme activity as previously reported in preeclamptic placentas [7], cells were cultured under hypoxia. All five isozymes of LDH are detected. In mammals, two genes code for LDHA and LDHB subunits that give rise to five tetrameric LDH isozymes (LDHA4, LDHA3B, LDHA2B2, LDHAB3, LDHB4) [15]. Increased LDHA4 isozyme activity is increased in the presence of hypoxia, most significantly at 12 and 24 h. At 24 h, there is an approximate 3-fold increase in LDHA4 activity in JEG3 cells exposed to hypoxia compared to control (Fig. 1B). This behavior suggests that our previous findings of increased LDHA gene expression and activity in placentas from preeclampsia patients are due to hypoxia and that the trophoblast cells could be sensitive to that stress. This finding also supports the use of JEG3 cells to serve as a model to study trophoblast behavior.

Further confirmation that hypoxia induces LDHA activity is shown in the RT-PCR analysis in Fig. 1C. In JEG3 cells, hypoxia leads to significantly increased LDHA mRNA expression, relative to β-Actin, whereas mRNA for LDHB is decreased.

Figure 1D shows the time course for this change. At 12 and 24 h, there is a significant increase compared to control. More specifically, at 24 h, there is an approximate 2-fold increase in LDHA mRNA expression in hypoxia cells compared to control cells. Western blot analysis at 24 h, the time at which there was the most significant increase in mRNA, shows increased levels of protein for LDHA compared to control. LDHB protein is unchanged as is expected since LDHA is the isozyme induced by hypoxia (Fig. 1E). Hypoxia inducible factor (HIF1α) protein was additionally measured to confirm that the cells experienced hypoxia and was identified by western blot analysis only in the hypoxia exposed cells. Therefore, this protein marker indicates that the cells under these laboratory conditions were hypoxic.

3.3. Response of lactate production and lactate transporters during hypoxia

To confirm that the increased LDHA activity observed in the JEG3 cells exposed to hypoxia result in higher lactate

Table 2
Sequences of oligonucleotide primers used in PCR

Target cDNA	Primer name	Primer sequence (5′–3′)	Temperature[a] (°C)	PCR product sizes (bp)
β-Actin	ACTBF	ACGTTGCTATCCAGGCTGTGCTAT	60.1	240
	ACTBR	TTAATGTCACGCACGATTTCCCGC	60.4	
LDHA	LDHA932F	CACCATGATTAAGGGTCTTTACG	59.8	319
	LDHA1220R	TTCAGGAGTTGATGTTTTTCCC	60.3	
LDHB	LDHB780F	GGTGGTTGAAAGTGCCTATG	58.1	261
	LDHB1021R	GCACTTTTCTTGAGCTGAGC	58.0	
MCT1	MCT1F1	TTCTTTGGATTTGCCTTCGGGTGG	60.0	371
	MCT1R1	TCCGGAGATTCTGCTGCTTTGGTA	60.1	
MCT4	SLC16A3F	TCTTCTTTGGCATCTCCTACGGCA	60.1	320
	SLC16A3R	TGTGGCTCTTTGGGCTTCTTCCTA	60.2	

[a] Annealing temperatures.

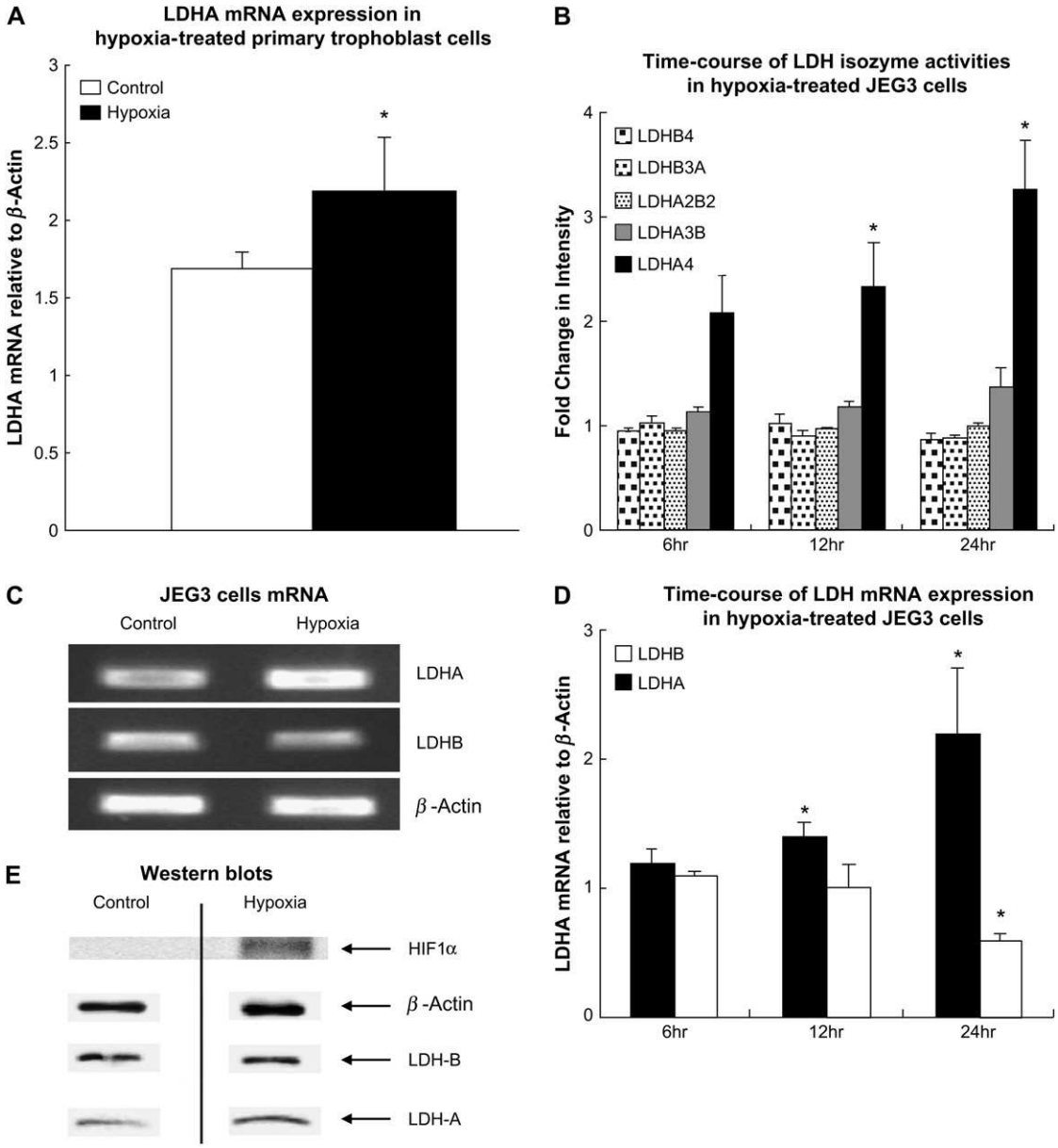

Fig. 1. (A) LDHA mRNA levels isolated from cultured primary trophoblasts after 48 h of hypoxia. β-Actin mRNA serves as a reference. (B) Time course of LDH isozyme activities in JEG3 cells exposed to hypoxia. LDHA4 is significantly higher after 12 and 24 h of hypoxia compared to other LDH isozymes (*) ($P < 0.01$). (C) LDH mRNA levels isolated from cultured JEG3 after 24 h of hypoxia are increased for LDHA, but decreased for LDHB, compared to control oxygenation. β-Actin mRNA serves as a reference. (D) Time course study of effects of hypoxia on LDHA mRNA. Data are expressed relative to β-Actin mRNA and presented as mean ± SE. At 12 and 24 h, mRNA for LDHA are statistically increased compared to control (*) ($P < 0.01$). Concomitantly, LDHB transcripts are significantly less at 24 h of hypoxia compared to control ($P < 0.01$). (E) Western blotting was carried out with antibodies to HIF1α, LDHA, LDHB and β-Actin. Under hypoxia, JEG3 cells express higher levels of HIF1α and LDHA protein.

production, media from JEG3 cells cultured under control and hypoxia were measured for lactate and results are shown in Fig. 2A. At 24 h, there is an approximate 2-fold increase in lactate within the cell culture media of JEG3 cells under hypoxia. This supports the expectation that increased LDHA activity increases conversion of pyruvate to lactate.

To further confirm that the increased lactate in the media is produced and actively secreted from the JEG3 cells under hypoxia, two lactate transporter proteins were measured by RT-PCR and western blot. MCT1 and MCT4 are such lactate transporters [16]. They are present in JEG3 cells but only MCT4 mRNA is increased in hypoxia compared to control (Fig. 2B). Increased protein of the MCT4 gene is also present under hypoxia by western blot analysis (Fig. 2C).

3.4. Lactate production from blood samples

Translation of these laboratory findings to the human subject was undertaken by determining if lactate levels are also higher in plasma of preeclamptic women. Lactate levels

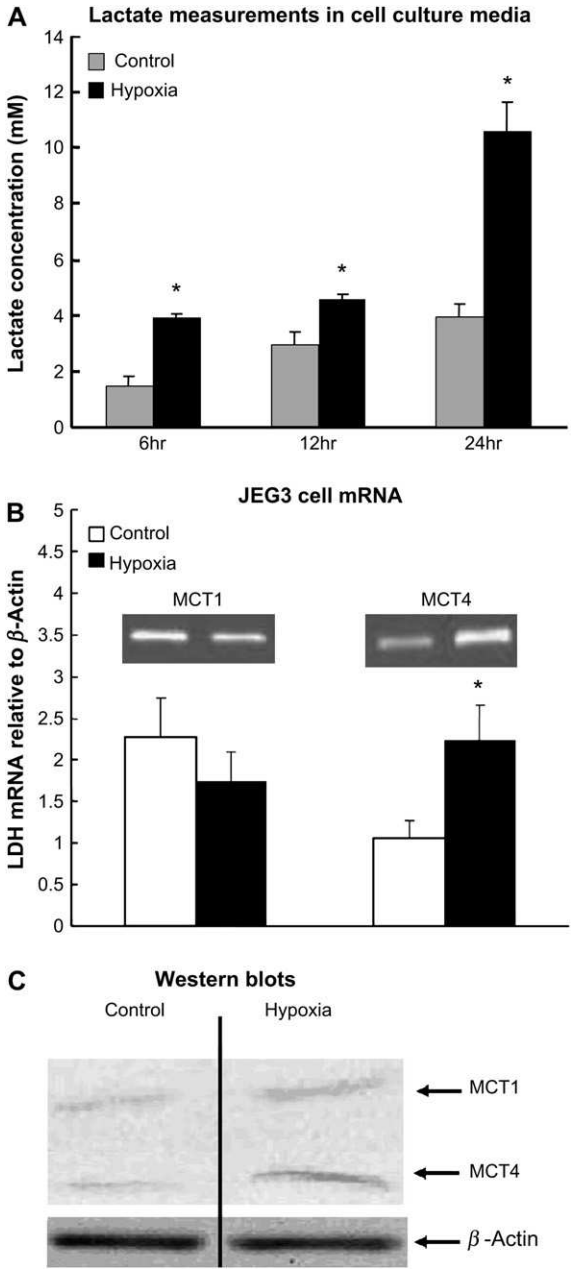

Fig. 2. (A) Lactate production from JEG3 cells under control and hypoxia. Higher levels of lactate are detected in culture supernatant at all time points under hypoxia than control. Results are expressed as mean mM ± SE ($P < 0.05$). (B) mRNA levels for MCT1 and MCT4 were determined from hypoxia-treated JEG3 cells after 24 h compared to control. Data are expressed relative to β-Actin mRNA in the same samples and are presented as mean of fold change ± SE. Only the effect of hypoxia on MCT4 mRNA is statistically significant ($P < 0.05$). (C) Western blotting was carried out with different titers of antibodies to MCT1 and MCT4. MCT4 is significantly increased under hypoxia.

were significantly higher in women with severe preeclampsia ($n = 9$) (mean 6.1 ± 1.3 mM) compared to normal pregnant women ($n = 29$) (mean 2.9 ± 0.3 mM) approximately 2-fold (Fig. 3). This rise is statistically significant ($P < 0.05$). The gestational age at delivery for the severe preeclamptic patients was 32.2 ± 2.1 weeks and for the control patients it was 38.7 ± 1.4 weeks.

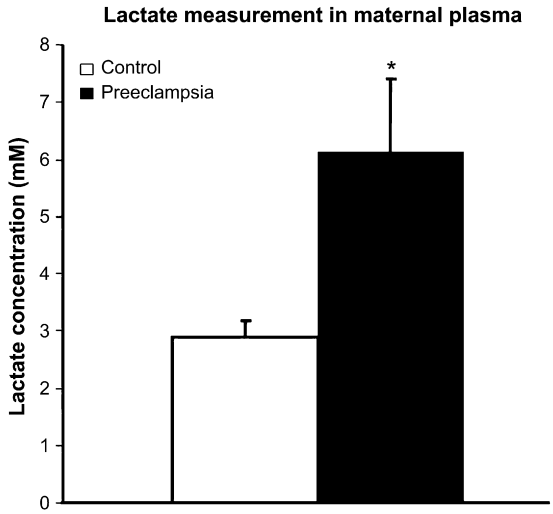

Fig. 3. Plasma lactate concentrations from women with severe preeclampsia ($n = 9$) compared to women with normal pregnancy ($n = 29$). The results represent the mean ± SE. Levels are significantly higher in women with severe preeclampsia ($P < 0.05$).

4. Comments

In our previous study [7], we reported that placentas from women with preeclampsia exhibited an increased level of LDHA4 isozyme activity and mRNA expression compared to placentas from normal pregnancies. LDHA4 is the isozyme most responsive to hypoxia because the LDHA gene is transcriptionally regulated by HIF1α [17]. In this current study, we demonstrate that LDHA gene expression is increased within primary trophoblast cells cultured under hypoxia. This suggests that, in vivo, hypoxia could play an important role in preeclampsia through LDH activity in the trophoblasts.

We report that LDHA4 isozyme activity, LDHA gene and LDHA protein expression are increased as a result of hypoxia. HIF1α, a transcription factor that binds to the LDH gene, is also increased in gene expression, possibly through the induction of oxidative stress since reactive oxygen species have been implicated in the stabilization and action of HIF1α [18]. LDHA transcription and translation activities are synergistically increased at 24 h of hypoxia suggesting a coordinated cellular response to hypoxia. However, LDHB transcription does not appear to be co-regulated as its mRNA levels decrease while the LDHB protein level remains constant. This is the first report, in any human cell, that LDHB gene is down-regulated during hypoxia. Since the half-life of the LDHB protein is not known, it is possible that LDHB protein half-life is longer than 24 h and the effect of hypoxia on translation could not be observed under the current conditions.

LDHA4 and B4 isozymes are key players in glycolysis, the pathway in which the final step is conversion of pyruvate to lactate. We confirmed increased lactate secretion in the JEG3 cells cultured under hypoxia. It is not certain why tissues have increased glycolysis during hypoxia because it is an inefficient phenotype as far as cellular energy is concerned since only two ATP molecules are produced for each molecule of

glucose metabolized. However, there is more recent evidence from muscle physiology studies that the lactate produced during hypoxic glycolysis, in a cell-to-cell ''shuttle,'' is produced for several specific cellular metabolic functions [19,20]. It does not appear to be a mere by-product of glycolysis. Lactate may serve as fuel for aerobic metabolism by the fetus [8]. It has additional properties leading to cellular functions such as inhibition of apoptosis [12]. Lactate has also been shown to increase the production of collagen in fibroblast culture [21]. Lactate can also induce VEGF production and secretion in several cell types including macrophages, human umbilical vein endothelial cells (HUVEC) and placenta trophoblast cells [20,22–24]. Because of this finding, lactate has been implicated as an essential metabolite for wound healing [20]. In tumors, glycolysis may promote survival due to a diminished need for oxygen while the increased acidity from lactate may promote metastases [20,25]. Here we suggest that anaerobic or aerobic glycolysis may be an adaptive metabolic pathway in the placenta because the increased lactate may serve several other important physiologic functions.

Lactate's ability to induce VEGF production is particularly interesting because it has been reported that circulating VEGF levels are increased in serum of women with preeclampsia, suggesting that there may be a link between lactate and VEGF in the pathophysiology of preeclampsia. Investigators have pursued the identity of a placenta factor that would activate endothelial cells and induce adhesion and coagulation, i.e. substances such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and soluble Flt-1 (sFlt-1) [26–29]. An imbalance in these angiogenic factors has been proposed as a cause for preeclampsia [30] and screening the urine of pregnant women for placental growth factor has been proposed to identify those at risk for early development of preeclampsia [31]. To our knowledge, lactate has never been studied in the role of endothelial cell activation.

We were able to strengthen the clinical significance of our in vitro findings because we found that patients with severe preeclampsia indeed had elevated plasma lactate levels. The clinical effects of elevated plasma lactate in preeclampsia have not been reported or studied in detail. This is a very interesting finding correlating with the increased expression of LDH in the placenta of preeclampsia patients as we have reported [7], a result we believe to be in response to hypoxia. Further investigations should confirm this finding in larger numbers of patients and the clinical effects of elevated lactate levels in the pathophysiology of preeclampsia should be determined.

Monocarboxylate transporters (MCTs) are transmembrane proteins that facilitate lactate transport in and out of cells [16]. MCT1 and MCT4 are two isoforms most commonly found in locomotory and cardiac muscle cells. Very little is known about these lactate transporters in trophoblast cells but MCT4 is thought to serve primarily in lactate extrusion [32]. In the current experiments, MCT4, but not MCT1, was elevated in cells cultured under hypoxia compared to normoxia. Since plasma levels of lactate are elevated in women with preeclampsia in our current study, it suggests that the placenta of these women experience hypoxia *in utero*, LDH enzyme activity is enhanced, more intracellular lactate is produced and MCT4 is increased to transport lactate to the extracellular space. Settle et al. showed that MCT4 was more abundant on the maternal-facing membranes of term human placental villi [33]. In HeLa cells, MCT4 is upregulated by hypoxia through the hypoxia binding elements located at the 5′ non-coding region of the MCT4 gene [34]. Therefore, hypoxia may also upregulate MCT4 expression in trophoblast cells through the transcriptional binding activity of HIF1α.

One potential criticism of this study is that we studied a choriocarcinoma cell line. However, primary trophoblast cultures tend to be highly heterogeneous in cell populations containing cytotrophoblasts, syncytiotrophoblasts and other non-trophoblast cells such as fibroblasts and because of this, other investigators have also chosen to study cell lines [35]. Additionally, primary trophoblasts cultured ex vivo are also not truly representative of normal trophoblast behavior *in utero*. Our use of JEG3 cells enabled us to define molecular pathways of cell metabolism in the most consistent manner. Although many other investigators utilized BeWo cells instead of JEG3, we found that JEG3 cells are more suitable for glycolysis and hypoxia studies than BeWo cells because they exhibited a more enhanced response in LDHA gene expression in response to hypoxia compared to BeWo cells (our observations).

In summary, using a JEG3 cell line model and measuring lactate in maternal plasma, we were able to establish that hypoxia induces LDHA4 isozyme activity, gene transcription and translation in trophoblasts. We propose a possible role for hypoxia in the pathophysiology of preeclampsia in the placenta, which is, that extracellular hypoxia encountered within the placenta leads to increased activity of HIF1α in trophoblasts, possibly through oxidative stress, which in turn increases glycolysis through an increase in LDH activity and increased lactate secretion. The lactate secreted extracellularly through the action of the MCT4 transporter may lead to a change in pH which promotes VEGF, an endothelial-specific mitogen, to be released from trophoblasts and circulate in the maternal vascular compartment leading to endothelial cell activation which results in the clinical condition we recognize as preeclampsia. This model offers insights into a possible pivotal role for lactate because of its production in response to hypoxia and the subsequent events on the endothelium. Future studies should define the interaction between lactate and endothelial cells and mechanisms by which the endothelial cells are activated in the pathophysiology of preeclampsia.

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