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ORIGINAL CONTRIBUTION

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Biotin supply affects rates of cell proliferation, biotinylation of carboxylases and histones, and expression of the gene encoding the sodium-dependent multivitamin transporter in JAr choriocarcinoma cells

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Summary *Background* Placental transfer of nutrients and secretion of hormones is essential for normal fetal development. *Aim of the study* To determine whether biotin supply affects biotin homeostasis, proliferation rates, and progesterone secretion in placenta cells. *Methods* JAr choriocarcinoma cells were cultured in media containing deficient (25 pmol/L), physiological (250 pmol/L), or pharmacological concentrations (10,000 pmol/L) of biotin for three weeks; markers for biotin homeostasis, proliferation, and hormone secretion were quantified. *Results* Biotin concentrations in culture media correlated negatively with expression of the biotin transporter SMVT, as judged by cellular transport rates of biotin, abundance of SMVT protein, and transcriptional activity of SMVT reporter-gene constructs. Notwithstanding this homeostatic mechanism, biotin concentrations in media correlated positively with activities of biotin-dependent propionyl-CoA carboxylase, abundance of biotinylated carboxylases, and with biotinylation of histones. Biotin deficiency was associated with decreased rates of thymidine uptake into JAr cells [pmol thymidine/(10^6 cells x 24 h)]: 1.6 ± 0.1 (25 pmol/L biotin) versus 2.3 ± 0.2 (250 pmol/L biotin) versus 3.7 ± 0.4 (10,000 pmol/L biotin), suggesting that cell proliferation depends on biotin. Secretion of progesterone was reduced in biotin-deficient cells; this effect was caused by reduced generation of new cells in deficient media rather than by an immediate effect of biotin on progesterone secretion at the single-cell-level. *Conclusions* This study provides evidence that choriocarcinoma cells cannot maintain normal activities of biotin-dependent metabolic pathways if biotin concentrations in culture media are low. It is uncertain whether activities of biotin-dependent pathways in placenta affect fetal development in vivo.

Key words biotin – carboxylases – histones – human – JAr cells – placenta – sodium-dependent multivitamin transporter

Abbreviations

PCC propionyl-CoA carboxylase
SMVT sodium-dependent multivitamin transporter

Introduction

In mammals, biotin serves as a covalently bound coenzyme for acetyl-CoA carboxylase (E. C. 6.4.1.2), pyruvate carboxylase (E. C. 6.4.1.1), propionyl-CoA carboxylase (PCC[3], E. C. 6.4.1.3), and 3-methylcrotonyl-CoA car-

boxylase (E. C. 6.4.1.4) [1]. These carboxylases catalyze essential steps in the metabolism of glucose, amino acids, and fatty acids. Degradation of holocarboxylases leads to the formation of biotinylated peptides, including biocytin (biotinyl-ε-lysine). These peptides are further degraded by biotinidase (E. C. 3.5.1.12) to release free biotin, which can then be used for the synthesis of new holocarboxylases [2]. Eukaryotic cells can also conjugate biotin to histones (DNA-binding proteins) in an enzyme-mediated reaction [3, 4]; biotinylation of histones might play a role in processes such as cell proliferation and DNA repair [4, 5]. Finally, evidence has been provided that biotin affects expression of various genes by yet unknown mechanisms [6, 7].

Biotin deficiency causes fetal malformations in animals [8, 9] and, perhaps, humans [10]. It has been proposed that increased biotin requirements of rapidly proliferating cells (such as fetal cells) may precipitate biotin deficiency and abnormal fetal development [10]. Consistent with this hypothesis, human mononuclear cells respond to proliferation with increased biotin uptake [11], mediating increased biotinylation of carboxylases [12] and histones [4] compared to quiescent cells.

Placental transfer of nutrients is essential for normal fetal development. For example, a single protein responsible for the transport of biotin, pantothenic acid, and lipoic acid across the human placenta has been characterized [13], cloned, and functionally expressed [14]; this transporter has been named sodium-dependent multivitamin transporter (SMVT). Placental transporters maintain a fetal-to-maternal gradient for many water-soluble vitamins. A recent study of fetal and maternal plasma concentrations of biotin at 18 to 24 weeks gestation of normal human pregnancies reported a fetal-to-maternal biotin ratio ranging from 3 to 17:1 [15].

Placental cells also secrete hormones that affect maternal and fetal metabolism, e. g., human chorionic gonadotropin, progesterone, estrogens, and human chorionic somatomammotropin [16]. These hormones play roles in processes such as the maintenance of the corpus luteum, the inhibition of uterine contractions, and in the stimulation of growth of the myometrium.

Theoretically, placental cells may respond to variations in biotin supply with homeostatic mechanisms that reduce fluctuations in cellular and fetal biotin concentrations in order to maintain normal fetal development. Here a human choriocarcinoma cell line (JAr cells) was used to model effects of biotin supply on hormone secretion and biotin homeostasis in human placenta. Specifically, we determined whether biotin supply affects biotinylation of carboxylases and histones, expression of the gene encoding SMVT, recycling of biotin (from biocytin), rates of cell proliferation, and secretion of progesterone in JAr cells.

Materials and methods

Cell culture

JAr (human choriocarcinoma) cells were purchased from American Type Culture Collection (Manassas, VA). Media for cell culturing contained the following components: 0.9 L of customized RPMI-1640 (Atlanta Biologicals, Norcross, GA) without biotin; 0.1 L of fetal bovine serum without biotin (see below); and 100,000 I. U./L penicillin and 100 mg/L streptomycin (final concentrations). Before media preparation, biotin was removed from fetal bovine serum by using avidin chromatography as described in our previous studies; absence of biotin was confirmed by avidin-binding assay [17]. Finally, biotin concentrations in culture media were adjusted to 25 pmol/L of biotin (denoted "deficient"), 250 pmol/L of biotin (denoted "physiological"), and 10,000 pmol/L of biotin (denoted "pharmacological") as described [17].

Biotin concentrations were chosen based on multiple reasons. First, 250 pmol/L of biotin equals the physiological concentration of biotin in plasma from healthy adults [18]. Second, 25 pmol/L of biotin is greater than two standard deviations below the mean physiological concentration in normal plasma [18]; thus 25 pmol/L equals a deficient concentration of biotin. Third, ingestion of a typical biotin supplement providing 25 times the Adequate Intake of biotin for adults [19] is associated with plasma concentrations of approximately 10,000 pmol/L of biotin in healthy adults [20]; thus, this concentration represents a pharmacological concentration of biotin in plasma.

JAr cells were cultured in biotin-defined media (25, 250, or 10,000 pmol/L) for 3 wk before analyses. Previous studies have provided evidence that culturing human cell lines in biotin-deficient medium for 3 wk provides for sufficient time to establish new steady-state biotin concentrations [17]. Culture medium was replaced with fresh medium every 24 to 48 h; cultures were trypsinized and split when cells reached confluence (typically two times per wk). For the assays described below, cell pellets and cell-free medium supernatants were collected at 60 % to 70 % confluence.

Biotin transport

Rates of biotin transport into JAr cells were determined using [^{3}H]biotin at a physiological concentration (475 pmol/L) as described previously [21]. The abundance of biotin transporter (SMVT) protein in cells was quantified by Western blotting, using a polyclonal antibody to human SMVT as described previously [22].

Riboflavin transport

Rates of riboflavin transport were quantified to determine whether biotin supply specifically affects rates of biotin transport or whether biotin supply globally affects rates of nutrient transport, e. g., riboflavin. Previous studies have shown that uptake of riboflavin and biotin into human cells is mediated by distinct transporters [21, 23]. Rates of riboflavin transport into JAr cells were determined using a physiological concentration (10 nmol/L) of [^{3}H]riboflavin as described previously [23].

Biotinidase activity

In human cells, biotinidase mediates both recycling of biotin from breakdown products (e. g., biocytin) of holocarboxylases and covalent binding of biotin to histones [2, 3]. Biotinidase activity in JAr cells was measured as the hydrolysis rate of N-*D*-biotinyl-*p*-aminobenzoic acid as described by Knappe [24] and Backman-Gullers [25] and modified in our laboratory [4]. One unit of biotinidase activity is defined as the amount of enzyme that releases 1 pmol of *p*-aminobenzoic acid per minute.

Propionyl-CoA carboxylase activity

This assay quantifies the binding rate of radioactive bicarbonate to propionyl-CoA, catalyzed by PCC in samples of lysed cells. After trypsination, aliquots of cell suspension were centrifuged for 10 min at 250 *g* to collect cell pellets (approximately 2 mg of protein). PCC activity was quantified as described previously [26] with minor modifications [17]. Briefly, lysed JAr cells were incubated with propionyl-CoA, [^{14}C]bicarbonate, and cofactors to allow for covalent binding of [^{14}C]bicarbonate to propionyl-CoA. After incubation, unbound [^{14}C]bicarbonate was volatilized by addition of perchloric acid and samples were dried. Finally, samples were suspended in scintillation fluid and the bound [^{14}C]bicarbonate was quantified by liquid scintillation counting.

Biotinylation of biotin-dependent carboxylases

Holocarboxylases (as opposed to apocarboxylases) contain covalently bound biotin. Biotin in holocarboxylases was quantified by Western blot analysis, using streptavidin peroxidase as probe for biotin as described in our previous studies [17]; gel densitometry was used to confirm that equal amounts of protein were loaded per lane [17].

Biotinylation of histones

Human cells contain five classes of histones: H1, H2A, H2B, H3, and H4. Histones were extracted from JAr cell nuclei by using hydrochloric acid as described previously [4]. Equal amounts of histones (as judged by gel densitometry after staining with coomassie blue) were electrophoresed using 16 % Tris glycine gels (Invitrogen, Carlsbad, CA); biotin in histones was probed using streptavidin peroxidase as described previously [4].

Reporter-gene constructs

The following constructs were used to determine whether biotin supply affects transcriptional activity of genes encoding SMVT and biotinidase.

(*i*) SMVT: Two distinct regulatory regions (P1 and P2) have been identified in the SMVT gene [27]. These regulatory regions (5'-flanking regions) are located within approximately 5884 bases upstream relative to the translation initiation codon of the SMVT gene; the minimal region required for basal activity of the SMVT promoter is encoded by a sequence between –5846 and –5313 for P1 and between –4417 and –4244 for P2 relative to the translation initiation codon. The following regulatory regions of SMVT were ligated into a luciferase reporter gene vector (pGL3): P1 (basepairs –5884 to –4400), P2 (basepairs –4417 to –3640), and a construct containing both P1 and P2 (basepairs –5884 to –3640); these constructs were denoted pGL3-P1, pGL3-P2, and pGL3-P1P2, respectively. The synthesis of these constructs has been described previously [27].

(*ii*) Biotinidase: The regulatory elements of the biotinidase gene are located within 560 bases upstream of the first (of two) translation initiation codons [28]. Genomic DNA from human lymphocytes was isolated using the DNeasy tissue kit (Qiagen, Valencia, CA). PCR primers were designed to add KpnI/HindIII restriction sites to the regulatory region of the biotinidase gene (GenBank accession number AF018630): 5'-ACT GGT ACC CCC ATC GCC CAT TTC TAC TCG-3' and 5'-ACT AAG CTT CTG AAT ATG CGC ATG CGC CAT-3' (Integrated DNA Technologies, Inc.). The PCR product (spanning the full regulatory sequence of the biotinidase gene and the first 21 basepairs of the coding region) was cloned by using the Acceptor Vector kit and the pST Blue-1 vector (Novagen, Madison, WI). The biotinidase insert in pST Blue-1 was sequenced four times by the DNA sequencing core facility at the University of Nebraska-Lincoln; the sequence was identical with the published sequence with the following exception. An A-to-G substitution was found in position –183 compared to the published sequence [28]; this substitution was also observed after repeated cloning of the human DNA and, thus, is likely to represent a variation in the regula-

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tory region of the biotinidase gene. The KpnI/HindIII fragment was subcloned into the pGL3-basic vector (Promega, Madison, WI) according to the manufacturer's instructions; the resulting construct was denoted BTD-pGL3. Plasmid was extracted by using the QIAprep Spin Midiprep kit (Qiagen).

(*iii*) Transfection control: A construct of the RSV promoter linked to β-galactosidase gene (denoted "RSV βgal") was used as control for transfection efficiency.

For transfections with reporter-gene constructs, cells were cultured in biotin-defined media for 3 wk. Subsequently, 1.2×10^6 cells were seeded in a 50-mL culture flask and cultured for 24 h; cells were co-transfected with luciferase constructs (either pGL3-P1, pGL3-P2, pGL3-P1P2, or BTD-pGL3) and control (RSV βgal) by using PolyFect (Qiagen) according to the manufacturer's instructions. Twenty-four hours after transfection, cells were detached by treatment with trypsin and EDTA. Luciferase activity in cells was assayed by LucLite Plus (Packard, Boston, MA) according to the manufacturer's instructions, using a Top Count NXT (Packard). β-Galactosidase activity was assayed by using a commercial assay kit (Promega, Madison, WI) and an Emax Microwell Plate Reader (Molecular Devices, Sunnyvale, CA). Luciferase activities were normalized for transfection efficiency, as judged by β-galactosidase activity in response to transfection with RSV β-gal.

Secretion of progesterone

After 3 wk of culturing in biotin-defined media, 7 x 10^5 cells (2×10^5 cells/mL medium) were seeded in 50-mL culture flasks (t = 0 h); medium was replaced with fresh medium at t = 24 h; cell-free media supernatants and cell pellets were collected at t = 48 h. Progesterone in media was quantified as follows. Progesterone was extracted from 500 μL of media with 5 mL of organic solvent (mixture of 0.1 L n-hexane and 1 L diethyl ether). The organic phase was dried and redissolved in 250 μL of "Calibrator A" provided with the "Coat-a-Count" radioimmunoassay kit (Diagnostic Product Corporation, Los Angeles, CA); progesterone was quantified according to the manufacturer's instructions. Protein in cell pellets (as a marker for cellular mass) was quantified using the bicinchoninic acid method (Pierce, Rockford, IL).

Cell proliferation

Cellular uptake of purine or pyrimidine bases (e. g., thymidine), or analogs thereof (e. g., 5-bromo-2-deoxyuridine) increases in response to cell proliferation due to synthesis of new DNA. In this study, rates of JAr cell proliferation were determined by measuring the cellular uptake of [^{3}H]thymidine (specific radioactivity 1.3 TBq/mmol; ICN; Irvine, CA); [^{3}H]thymidine uptake was measured as described previously with minor modifications [11].

Statistics

Homogeneity of variances among groups was tested using Bartlett's test [29]. Whenever variances were heterogeneous, data were log transformed before further statistical testing. Significance of differences among groups was tested by one-way ANOVA. Fisher's Protected Least Significant Difference procedure was used for posthoc testing [29]. StatView 5.0.1 (SAS Institute; Cary, NC) was used to perform all calculations. Differences were considered significant $P < 0.05$. Data are expressed as mean ± SD.

Results

Cellular biotin transport

Transport rates of biotin were quantified in JAr cells that had been cultured for 3 wk in media containing either deficient, physiological, or pharmacological concentrations of biotin. When cells were cultured in medium containing a pharmacological concentration of biotin, transport rates of biotin decreased to 47 ± 9 % of physiological controls (Fig. 1A). When cells were cultured in biotin-deficient medium, transport rates of biotin were not significantly different compared to physiological controls. These data are consistent with the hypothesis that JAr cells respond to biotin supplementation with decreased rates of biotin uptake.

Previous studies have suggested that biotin uptake into JAr cells is mediated by SMVT [14]. In the present study, abundance of SMVT protein in JAr cells was quantified by Western blotting using an antibody to human SMVT. Abundance of SMVT protein was greater in cells that were cultured in biotin-deficient medium compared to other treatment groups (Fig. 1B). This finding is consistent with our hypothesis that expression of biotin transporters in JAr cells correlates negatively with biotin supply. It is uncertain why the abundance of SMVT protein was similar in cells cultured in media containing physiological and pharmacological concentrations of biotin, whereas rates of biotin uptake were lower in cells cultured in pharmacological medium compared to physiological medium. Potential explanations for these findings are offered in the Discussion section. The size of the protein probed with antibody to SMVT corresponded to the size predicted for SMVT protein (69 kDa) [22]; no additional bands were observed in extracts from JAr cells.

Finally, transcriptional activity of the gene encoding

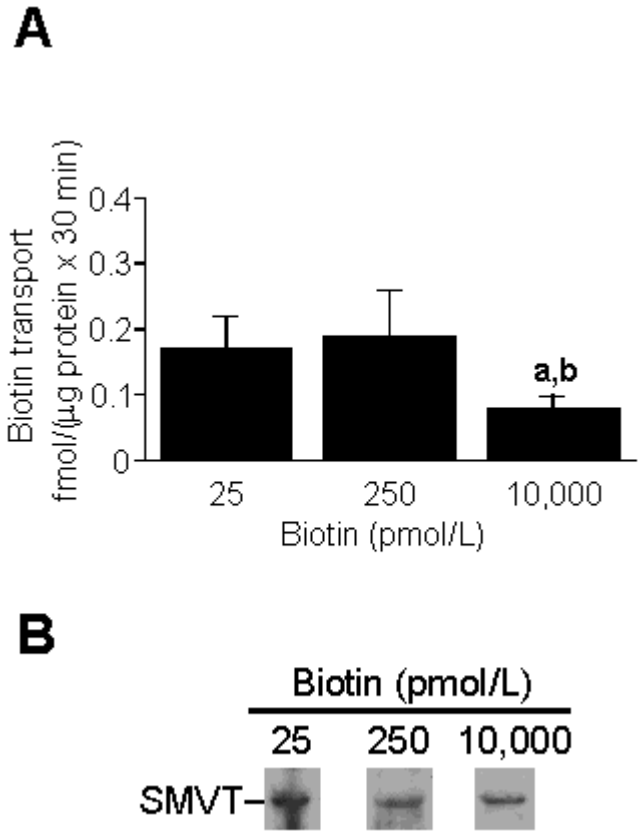

Fig. 1 Expression of biotin transporters correlated negatively with biotin supply in JAr cells. Cells were cultured in media containing either 25 (deficient), 250 (physiological), or 10,000 (pharmacological) pmol/L of biotin for 3 wk. **A** Cells were transferred into medium free of unlabeled biotin, and transport rates were measured using [^{3}H]biotin (475 pmol/L). [a] $P < 0.05$ versus 25 pmol/L biotin; [b] $P < 0.01$ versus 250 pmol/L biotin. Values are means ± SD (n = 5). **B** Cellular proteins were extracted and SMVT protein was quantified by Western blot analysis, using a polyclonal antibody to SMVT

SMVT was quantified using reporter-gene constructs. If cells were cultured in biotin-defined media for 3 wk, transcriptional activity of the reporter-gene constructs pGL3-P2 and pGL3-P1P2 correlated negatively with biotin supply (Table 1). For example, luciferase activity of pGL3-P2 in biotin-deficient cells was 291 ± 13 % of the transcriptional activity observed in cells cultured in physiological medium (100 %); transcriptional activity of pGL3-P2 was 37 ± 2 % if cells were cultured in pharmacological medium. Transcriptional activity of pGL3-P1P2 also correlated negatively with biotin supply (Table 1); however, the difference between cells cultured in physiological and pharmacological media was not statistically significant. No luciferase activity was detectable in cells transfected with pGL3-P1, suggesting that sequences in P2 are essential for expression of SMVT in human placenta. Taken together, analysis of transport rates, SMVT protein abundance, and transcriptional activity of the SMVT gene suggest that JAr cells utilize expression of biotin transporter genes as a homeostatic mechanism to reduce fluctuations in cellular biotin concentrations.

Cellular riboflavin transport

Transport rates of riboflavin were not significantly different among treatment groups [amol of riboflavin/(µg protein × 10 min)]: 4.9 ± 2.2 (25 pmol biotin/L medium) versus 6.8 ± 3.3 (250 pmol biotin/L medium) versus 6.8 ± 2.4 (10,000 pmol biotin/L medium; $P > 0.05$; n = 5). This is consistent with the hypothesis that biotin supply of cells specifically affects the cellular uptake of biotin rather than nutrient uptake in general.

Biotin-dependent carboxylases

Biotin concentrations in culture medium correlated with carboxylase activities in cells, despite homeostatic mechanisms regulating expression of biotin transporters in response to biotin supply. When cells were cultured in biotin-deficient medium for 3 wk, activities of PCC were 1.8 ± 0.2 pmoles bicarbonate/(mg protein × min) compared to 23 ± 1.3 pmoles bicarbonate/(mg protein x min) in cells that were cultured in medium containing physiological concentrations of biotin; activity of PCC was 84 ± 4.6 pmoles bicarbonate/(mg protein × min) if cells were cultured in medium containing phar-

Table 1 Effects of cellular biotin supply on transcriptional activity of the SMVT gene[1]

	Biotin concentration in culture medium (pmol/L)		
Reporter-gene construct	25	250	10,000
	Counts per second/2.3 x 10^6 cells		
pGL3-P1	ND[2]	ND	ND
pGL3-P2	13.9 x 10^6 ± 0.6 x 10^6*	4.8 x 10^6 ± 0.3 x 10^6*	1.7 x 10^6 ± 0.8 x 10^6*
pGL3-P1P2	21.5 x 10^6 ± 0.7 x 10^6*	17.2 x 10^6 ± 1.4 x 10^6	15.8 x 10^6 ± 0.6 x 10^6

[1] Values are means ± SD, n = 3
[2] ND, not detectable
* Significantly different from other treatment groups transfected with the same construct ($P < 0.01$)

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macological concentrations of biotin ($P < 0.01$ among treatment groups; n = 4–5).

PCC activities were paralleled by biotinylation of biotin-dependent carboxylases. After 3 wk of culturing in biotin-defined media, the cellular abundance of holo-PCC, holo-3-methylcrotonyl-CoA carboxylase, holo-pyruvate carboxylase, and holo-acetyl-CoA carboxylase correlated with the concentrations of biotin in culture media and with PCC activities: biotin-deficient medium < physiological medium < pharmacological medium (Fig. 2). Please note that the biotin-containing alpha chains of PCC (mol. wt. = 80 kDa) and 3-methylcrotonyl-CoA carboxylase (mol. wt. = 83 kDa) migrated as one single band in the assay used here. Likewise, the two isoforms of acetyl-CoA carboxylase (α = 267 kDa; β = 283 kDa) migrated as one single band. Taken together, PCC activities and levels of holocarboxylases are consistent with the hypothesis that biotin supply affects activities of biotin-dependent metabolic pathways in cells.

Biotinidase

Biotinidase activities in JAr cells were not significantly different among treatment groups (units/μg of protein): 0.12 ± 0.02 (at 25 pmol/L of biotin) versus 0.22 ± 0.07 (at 250 pmol/L of biotin) versus 0.14 ± 0.04 (at 10,000 pmol/L of biotin; $P > 0.05$). Biotinidase activities were only about five times greater than the detection limit of the assay; also, biotinidase activities were several orders of magnitude smaller than activities measured in human plasma [4, 30]. These findings are consistent with the hypothesis that recycling of biotin by biotinidase does not depend on biotin supply in JAr cells. Consistent with this hypothesis, transfection of cells with BTD-pGL3 did not generate luciferase activities that were greater than in mock-transfected controls (data not shown). This finding suggests that transcriptional activity of the biotinidase gene is low in JAr cells.

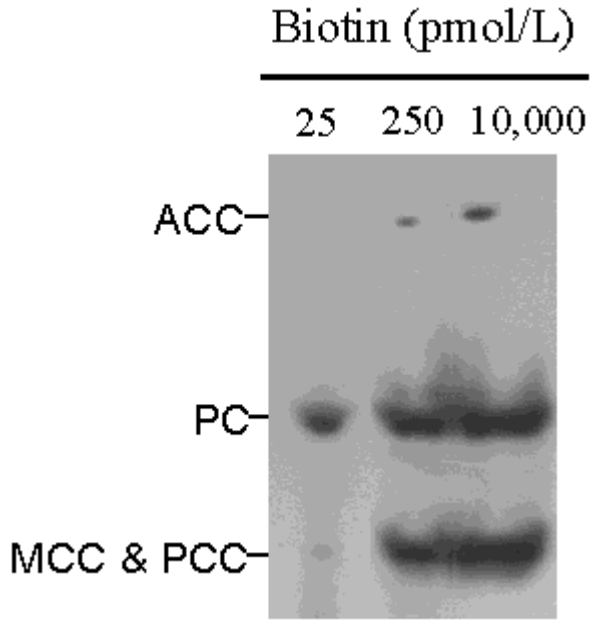

Fig. 2 Biotin supply affects abundance of holocarboxylases in JAr cells. Cells were cultured in media containing either 25 (deficient), 250 (physiological), or 10,000 (pharmacological) pmol/L of biotin for 3 wk. Cells were harvested and the following holocarboxylases were quantified by gel electrophoresis and probing of biotin with streptavidin peroxidase: acetyl-CoA carboxylases (ACC), pyruvate carboxylase (PC), alpha chain of 3-methylcrotonyl-CoA carboxylase (MCC), and alpha chain of propionyl-CoA carboxylase (PCC). Alpha chains of MCC and PCC have similar molecular weights (83 and 80 kDa, respectively) and migrated as one single band

Biotinylation of histones

The abundance of biotinylated histones H1, H2A, H2B, H3, and H4 in cell nuclei correlated with biotin concentrations in media after 3 wk of culturing: biotin-deficient medium < physiological medium < pharmacological medium (Fig. 3). Histones H2A and H2B electrophoresed as one single band. Thus, it remains uncertain whether the probe (streptavidin) bound to histone H2A, H2B, or both.

Proliferation

Thymidine uptake into JAr cells correlated with concentrations of biotin in culture media [pmol/(10^6 cells × 24 h)]: 1.6 ± 0.1 (at 25 pmol/L of biotin) versus 2.3 ± 0.2 (at 250 pmol/L of biotin) versus 3.7 ± 0.4 (at 10,000 pmol/L of biotin; $P < 0.05$ among groups; n = 3). This observation is consistent with the hypothesis that biotin supply affects proliferation rates of JAr cells.

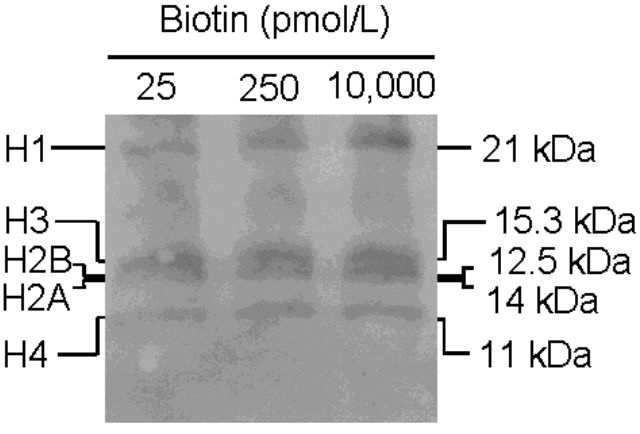

Fig. 3 Biotin supply affects biotinylation of histones in cell nuclei. JAr cells were cultured in media containing either 25 (deficient), 250 (physiological), or 10,000 (pharmacological) pmol/L of biotin for 3 wk. Cell nuclei were harvested to quantify biotin in histones H1, H2A, H2B, H3, and H4 by gel electrophoresis and probing with streptavidin peroxidase

Synthesis of cellular protein and secretion of progesterone

Synthesis of cellular protein (as marker for cell mass) correlated with concentrations of biotin in culture media; biotin-deficient cells synthesized significantly less protein (0.58 ± 0.01 mg/48 h) than cells that were cultured in media containing physiological (0.88 ± 0.01 mg/48 h) and pharmacological concentrations (0.90 ± 0.04 mg/48 h) of biotin ($P < 0.01$ versus biotin-deficient cells; n = 3); protein synthesis was not significantly different in cells cultured in media containing physiological and pharmacological concentrations of biotin ($P > 0.05$).

Secretion of progesterone was reduced in biotin-deficient cells compared to other treatment groups if secretion was normalized by the number of cells per culture flask at t = 0 h. For example, secretion of progesterone by biotin-deficient cells was 72 ± 4 % of secretion by physiological controls; secretion of progesterone was not significantly different in cells that were cultured in media containing either physiological or pharmacological concentrations of biotin. Likely, reduced secretion of progesterone by biotin-deficient cells was caused by a decreased number of cells during the collection period (t = 24 to 48 h) rather than by reduced secretion of progesterone at the single-cell-level: if secretion of progesterone was normalized by the mass of cellular protein at t = 48 h, secretion of progesterone was not significantly different among groups (units = pmol of progesterone/μg of protein): 0.15 ± 0.006 (at 25 pmol/L of biotin) versus 0.14 ± 0.005 (at 250 pmol/L of biotin) versus 0.13 ± 0.003 (at 10,000 pmol/L of biotin; $P > 0.05$ among groups; n = 5). These findings are consistent with the hypothesis that secretion of progesterone per cell was not affected by biotin supply but that total secretion of progesterone may decrease in biotin-deficient cells, caused by a decreased rate of synthesis of new cells.

Discussion

In this study JAr cells were cultured in media containing concentrations of biotin that are representative of plasma concentrations in biotin-deficient individuals, normal individuals, and biotin-supplemented individuals. Expression of the biotinidase gene was not affected by biotin supply, suggesting that cells do not use recycling of biotin as a mechanism to reduce fluctuations in intracellular biotin levels. In contrast, expression of biotin transporters was affected by biotin supply. In the present study three variables were measured in order to quantify expression of biotin transporters: rates of cellular biotin transport, abundance of biotin transporter (SMVT) protein, and transcriptional activity of regulatory regions of the SMVT gene. All of these variables suggested that biotin concentrations in culture media correlate negatively with expression of biotin transporters. However, we noted variations among these variables with regard to biotin dependency. For example, rates of biotin transport were similar in cells cultured in deficient and physiological media (and decreased in pharmacological medium), whereas abundance of SMVT protein was greater in cells cultured in deficient medium compared to physiological medium. We offer the following explanations for the slight discrepancy among these markers for transporter gene expression. (i) SMVT may not be the sole transporter for biotin in human cells. Evidence has been provided that transporters other than SMVT may also mediate biotin uptake [21, 31]; these putative transporters have not yet been identified. (ii) In the present study whole cell extracts were used to quantify SMVT protein. This procedure does not distinguish among SMVT protein located in various cellular compartments. Theoretically, biotin supply of cells might affect the localization of SMVT in intracellular compartments. For example, trafficking of SMVT from intracellular compartments to the plasma membrane might be decreased in biotin-supplemented cells, mediating reduced rates of biotin uptake. These limitations notwithstanding, our findings are consistent with the hypotheses that expression of the SMVT gene correlates negatively with biotin supply in JAr cells, and that changes in SMVT expression in placenta might help to reduce fluctuations in fetal biotin supply in vivo.

The findings presented here suggest that increased expression of biotin transporters is not sufficient to maintain normal biotinylation of carboxylases if cells are incubated in biotin-deficient medium. Both abundances of holo-carboxylases and activities of PCC decreased substantially when cells were cultured in biotin-deficient medium compared to physiological controls. Previous studies in human lymphoid cells (Jurkat cells) and rat tissues produced similar results: activities of PCC and abundances of holocarboxylases correlated with biotin supply [17, 32, 33]. Decreased activities of biotin-dependent carboxylases causes accumulation of odd-chain fatty acids and 3-hydroxyisovaleric acid in cells and extracellular fluids [10]. It remains to be determined whether placental cells secrete these metabolites into fetal circulation, and whether these metabolites impair fetal development.

In the present study, biotin concentrations in culture media correlated with biotinylation of histones in JAr cells. Although the magnitude of the effect was quantitatively moderate, it may be physiologically significant. Previous studies have provided evidence that quantitatively small changes in covalent modifications of histones may affect transcriptional activity of genes. For example, methylation of arginine 3 in histone H4 facilitates subsequent acetylation of histone H4 tails, leading to transcriptional activation [34]; numerous other ex-

amples for synergistic and antagonistic modifications of histones have been identified [35]. By analogy, a moderate increase in biotinylation of histones might affect other modifications of histones.

In the present study, biotinylation of histones was quantified by using acid extracts of total chromatin. Using this approach it is unlikely to detect small (yet meaningful) changes in the biotinylation pattern of histones as they may occur in a confined region of chromatin. Changes in the biotinylation of histones might affect processes such as gene expression within confined regions of chromatin without having a substantial effect on the overall biotinylation of histones in total chromatin. Previous studies suggested that biotinylation of histones might play a role in cell proliferation, silencing of genes, and repair of damaged DNA or apoptosis [4, 5].

The present study provided evidence that biotin supply affected functional variables such as proliferation and, perhaps, hormone secretion in choriocarcinoma cells. Rates of cell proliferation correlated with biotin status, suggesting that placental growth might be impaired in biotin-deficient women. There is precedence for effects of biotin on cell proliferation: biotin-depleted HeLa cells arrest in G1 phase of the cell cycle [36] and Jurkat cells show a transient decrease in proliferation rates if cultured in biotin-deficient medium [17]. Note that in the present study the biotin-deficient medium contained 25 pmol of biotin per liter; effects of biotin-free medium were not investigated because biotin-free nutrition over an extended period of time is very unusual in the general population.

We conclude that expression of biotin transporters is the primary mechanism by which placental cells seek to maintain biotin homeostasis. Notwithstanding homeostatic mechanisms, biotin-deficient culture conditions cause decreased activities of biotin-dependent pathways, decreased cell proliferation and, perhaps, decreased secretion of progesterone. It remains to be determined whether biotin deficiency has similar effects in vivo, leading to impaired fetal development.

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