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Biotin Supplementation Decreases the Expression of the *SERCA3* Gene (*ATP2A3*) in Jurkat Cells, thus Triggering Unfolded Protein Response*

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Abstract

Protein folding in the endoplasmic reticulum (ER) depends on Ca^{2+} ; uptake of Ca^{2+} into the ER is mediated by *SERCA3*. The 5'-flanking region of the *SERCA3* gene (*ATP2A3*) contains numerous binding sites for the transcription factors Sp1 and Sp3. Biotin affects the nuclear abundance of Sp1 and Sp3, which may act as transcriptional activators or repressors. Here we determined whether biotin affects the expression of the *SERCA3* gene and, thus, protein folding in human lymphoid cells. Jurkat cells were cultured in media containing 0.025 nmol/L biotin (denoted "deficient") or 10 nmol/L biotin ("supplemented"). The transcriptional activity of the full-length human *SERCA3* promoter was 50% lower in biotin-supplemented cells compared to biotin-deficient cells. Biotin-dependent repressors bind to elements located 731 to 1312 basepairs upstream from the transcription start site in the *SERCA3* gene. The following lines of evidence suggest that low expression of *SERCA3* in biotin-supplemented cells impaired folding of secretory proteins in the ER, triggering Unfolded Protein Response: (i) sequestration of Ca^{2+} in the ER decreased by 14–24% in response to biotin supplementation; (ii) secretion of interleukin-2 into the extracellular space decreased by 75% in response to biotin supplementation; (iii) the nuclear abundance of stress-induced transcription factors increased in response to biotin supplementation; and (iv) the abundance of stress-related proteins such UBE1, GADD153, XBP1, and phosphorylated eIF2 α increased in response to biotin supplementation. Collectively, this study suggests that supplements containing pharmacological doses of biotin may cause cell stress by impairing protein folding in the ER.

Keywords

biotin; calcium; human; Jurkat cells; protein folding

1. Introduction

Biotin has the following biological functions in mammals. First, biotin serves as a coenzyme for acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase (PCC)¹, and 3-methylcrotonyl-CoA carboxylase [1]. These carboxylases catalyze steps in the metabolism of glucose, amino acids, and fatty acids [1]. Second, histones (DNA-binding proteins) contain

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covalently bound biotin [2]; biotinylation of histones might play a role in gene silencing [3], cell proliferation [2,4], and DNA repair or apoptosis [3]. Third, biotin affects gene expression at both the transcriptional [5–7] and the posttranscriptional level [6,8,9]. Numerous cell signals have been identified that mediate effects of biotin on gene expression: biotinyl-AMP [10], transcription factors such as NF- κ B [11], Sp1 and Sp3 [12], and biotinylation of histones [13].

Approximately 270 biotin-dependent genes have been identified in human lymphocytes by using DNA microarrays [14]. In these previous studies, gene expression profiles were obtained before and after supplementation of healthy adults with 8.8 μ mol of biotin per day for 21 d. Sarco/endoplasmic reticulum Ca^{2+} -ATPase 3 (SERCA3) was among the genes that were down-regulated substantially in response to biotin supplementation: the abundance of mRNA encoding SERCA3 (isoform a) decreased by >80% in post-supplementation compared to pre-supplementation lymphocytes; expression of isoforms b, c, d, and e was quantitatively negligible in lymphocytes. The 5'-flanking region of the *SERCA3* gene contains 25 consensus binding sites for the biotin-dependent transcription factors Sp1 and Sp3 [12,15]. Sp1 and Sp3 may act as transcriptional activators or repressors, depending on the context [16,17]. Based on these lines of evidence it seems likely that the decreased expression of SERCA3 in biotin-supplemented cells is mediated by increased abundance of Sp1 and Sp3.

Three homologous genes encode for the calcium transporters SERCA1, SERCA2, and SERCA3 [18,19]. These proteins transport calcium from the cytoplasm into the endoplasmic reticulum (ER), maintaining a resting concentration of free Ca^{2+} in the ER that is 3–4 orders of magnitude greater than in the cytoplasm [19]. SERCA3 is abundantly expressed in lymphoid cells [20], suggesting a pivotal role for this transporter in the calcium homeostasis in immune cells.

Decreased expression of SERCA3 in response to biotin supplementation is likely to affect the oxidative folding (formation of disulfide bonds) of secretory proteins in the ER, based on the following lines of reasoning. Secretory proteins enter the ER co-translationally for glycosylation and oxidative folding by calcium-dependent proteins such as calnexin [21,22], BiP [23], and protein disulfide isomerase [23]. Glycosylation and folding in the ER are essential for subsequent protein export [24,25]. Depletion of ER-resident Ca^{2+} by ionophores, chelators, or inhibitors of SERCA results in accumulation of immature proteins in the ER [25–29]. Accumulation of immature proteins triggers stress-response systems collectively referred to as Unfolded Protein Response (UPR) [30]. UPR is characterized by high proteolytic activity [31], increased expression of chaperones that mediate protein folding [32,33], low overall translational activity [30], and transcriptional activation of *SERCA* genes [34]. Continued accumulation of unfolded proteins causes growth arrest and apoptosis [35].

In the present study we tested the hypothesis that supplementation of human lymphoma (Jurkat) cells with pharmacological doses of biotin impairs the posttranslational processing of secretory proteins, causing cell stress. Specifically we determined (i) whether biotin supplementation decreases the expression of SERCA3, impairing the sequestration of Ca^{2+} in the ER; (ii) whether the biotin-dependent disturbance of calcium homeostasis is associated with decreased secretion of proteins into the extracellular space; and (iii) whether biotin supplementation activates the UPR.

¹Abbreviations: ATF6, activating transcription factor 6; eIF-2 α , eukaryotic translation initiation factor 2 α ; EMSA, electrophoretic mobility shift assay; GADD153, growth arrest and DNA damage gene 153; Grp78, glucose regulated protein 78; IL-2, interleukin-2; PCC, propionyl-CoA carboxylase; PHA, phytohemagglutinin; PMA, phorbol-12-myristate-13-acetate; RT-PCR, reverse transcriptase polymerase chain reaction; SERCA3, sarco/endoplasmic reticulum Ca^{2+} -ATPase 3; UBE1, ubiquitin activating enzyme 1; UPR, unfolded protein response; XBP1, X-box binding protein 1.

Jurkat cells were selected as a cell model for the following reasons. First, biotin-dependent signaling cascades have been characterized in Jurkat cells [11,12]. Second, biotin-dependent repression of the *SERCA3* gene [14] is likely to affect Ca^{2+} homeostasis in lymphoma cells [20]. Third, lymphoid cells such as Jurkat cells secrete proteins that play essential roles in immune function [36]. Fourth, Jurkat cells have been used successfully in previous studies to investigate effects of nutrients on UPR [37].

2. Materials and Methods

2.1. Cell culture

Jurkat cells (clone E6-1) were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured in the following biotin-defined media for at least 5 wk prior to sample collection [38]: (i) 0.025 nmol/L of biotin (denoted “deficient”), representing the plasma level in biotin-deficient individuals; or (ii) 10 nmol/L of biotin (“supplemented”), representing the plasma level in individuals who take typical over-the-counter supplements providing 20 times the Adequate Intake of biotin [39–41]. Culturing Jurkat cells in biotin-defined media for 5 wk provides for sufficient time to achieve new steady-state concentrations of biotin, as judged by activities of PCC and biotinylation of carboxylases [38]. Culture media were replaced with fresh media every 48 h. Media were prepared by using customized RPMI-1640 and biotin-depleted fetal bovine serum as described [38]; biotin concentrations in media were confirmed by avidin-binding assay [42] with modifications [38]. For some control experiments, we cultured human HepG2 hepatocarcinoma cells in biotin-defined media as described [43].

2.2. Biotin-dependent carboxylases

Biotin-dependent carboxylases are good markers for cellular biotin [1]. Biotinylated carboxylases in cell extracts were resolved by polyacrylamide gel electrophoresis and were probed using streptavidin peroxidase [38]. Densitometric analysis of gels was conducted as described [12]. Activities of PCC in cell lysates were quantified as described [38].

2.3. Reporter-gene constructs

The following reporter gene constructs were used to quantify effects of biotin on the transcriptional activity of the *SERCA3* gene [15]: (i) basepairs –1312 to +55 (+1 = transcription start site) from the 5'-flanking region of the human *SERCA3* gene (*ATP2A3*) driving luciferase expression (denoted “EcoRV-del.”); (ii) basepairs –1043 to +55 of *ATP2A3* driving luciferase expression (“HindIII-del.”); (iii) basepairs –731 to +55 of *ATP2A3* driving luciferase expression (“SacI-del.”); (iv) basepairs –351 to +55 of *ATP2A3* driving luciferase expression (“Eco-RI-del.”); (v) basepairs –135 to +55 of *ATP2A3* driving luciferase expression (“PstI-del.”); and (vi) basepairs –31 to +55 of *ATP2A3* driving luciferase expression (“SmaI-del.”). A promoter-free plasmid containing the luciferase gene (denoted “pGL3-Basic”) was purchased from Promega, Inc. (Madison, WI); a construct of the β -galactosidase reporter gene driven by the RSV promoter (denoted “RSV β gal”) was obtained from B. R. White (University of Nebraska-Lincoln). Transfections and reporter-gene analyses were conducted as described, including stimulation with phorbol-12-myristate-13-acetate (PMA) and phytohemagglutinin (PHA) [44]. Reporter-gene activities were quantified 48 h after transfection. Luciferase activities were normalized for transfection efficiency by using β -galactosidase activities. Data are expressed as ratios of luciferase activities in cells transfected with promoter-driven constructs to activities in cells transfected with pGL3-Basic.

Accumulation of unfolded proteins in the ER causes nuclear translocation of activating transcription factor 6 (ATF6) [45] and X-box binding protein 1 (XBP1) [32]; these transcription factors bind to ER stress elements (ERSE) in 5'-flanking regions of stress-response genes [32,45,46]. The following reporter gene constructs were used to determine whether

transcriptional activities in biotin-supplemented cells are consistent with UPR mediated by ERSE. (i) A reporter construct in which expression of luciferase is driven by five repeats of a consensus binding site for ATF6 [47]: “p5xATF6-GL3”. This plasmid was provided R. Prywes (Columbia University, NY) [47]. (ii) Reporter constructs in which expression of luciferase is driven by the 169 and 457 basepairs, respectively, upstream from the transcription start site in the *glucose regulated protein 78 (Grp78)* gene: “-169GRP78-luc” and “-457GRP78-luc” [48]. These plasmids were provided by A. S. Lee (University of Southern California Los Angeles, CA). The GRP78/BiP protein binds misfolded proteins in the ER, mediating their re-folding or degradation [33]. (iii) A reporter construct in which expression of luciferase is driven by the ERSE from the human *growth arrest and DNA damage 153 (GADD153)* gene: “CHOP-ERSE1”; baseline luciferase activity was quantified by using plasmid “CHOP-ERSE1(mut8)” which is not inducible by ER stress [49]. Both CHOP-ERSE1 and CHOP-ERSE1(mut8) were provided by C. C. Glembotski (San Diego University, CA) [49]. GADD153 is a pro-apoptotic transcription factor (synonymous to CHOP); abundance of GADD153 increases in response to accumulation of unfolded proteins [30]. Transfections and reporter gene analyses were conducted as described above.

2.4. Northern blots

Expression of SERCA3 and histone H4 (control) was quantified by Northern blot analysis as described [50]. Gene-specific probes for Northern blots were generated by polymerase chain reaction as described [50], using the following primers: (i) 5'-GAG TCA CGC TTC CCC ACC ACC-3' and 5'-GGC TCA TTT CTT CGT GCA TGT GGT TC-3' for human SERCA3 (GenBank accession number NM_005173); and (ii) 5'-ATG TCT GGT AGA GGC AAA GGT GGT AAA-3' and 5'-TCA GCC ACC AAA GCC GTA CAG AGT GCG-3' for histone H4 (GenBank accession number M60749). Identities of probes were confirmed by sequencing in the Genomic Research Core Facility at the University of Nebraska-Lincoln (data not shown). Abundance of mRNA was quantified by gel densitometry [50]; the abundance of SERCA3 mRNA was normalized by the abundance of histone H4 mRNA.

2.5. Cytoplasmic Ca²⁺

Incubation of lymphoid cells with PMA and PHA causes release of Ca²⁺ from intracellular stores into the cytoplasm [36]. Here, cytoplasmic free Ca²⁺ was quantified at timed intervals before and after treatment of cells with 50 µg/L of PMA and 2 mg/L of PHA; Ca²⁺ was quantified using the Fluo-3 AM kit (Molecular Probes, Inc.; Eugene, OR) in a 96-well plate format according to the manufacturer's instructions. In addition, cytoplasmic Ca²⁺ was visualized by using confocal microscopy and the Fluo-3 AM kit (Microscopy Core Facility at the University of Nebraska-Lincoln).

2.6. Secretion of interleukin-2 (IL-2)

Jurkat cells secrete IL-2 in response to appropriate stimulation; IL-2 contains one disulfide bond and, thus, requires oxidative folding prior to secretion [37]. Here cells were stimulated with PMA and PHA to induce secretion of IL-2 [37]. IL-2 in culture media was quantified using ELISA (“hIL-2 EASIA”, Biosource; Camarillo, CA) as described previously [38].

2.7. Electrophoretic mobility shift assays (EMSA)

EMSA were used to determine whether biotin supplementation affects the nuclear abundance of transcription factors with affinity for ERSE. Nuclear extracts from Jurkat cells were prepared as described previously [12]. ERSE-binding factors in nuclear extracts were probed by EMSA [11], using a ³²P-labeled double-stranded oligonucleotide probe with an ERSE consensus site for ATF6 and XBP1: 5'-GAG GGC CTT CAC CAA TCG GCG GCC TCC ACG ACG GGG CTG G-3' and 5'-CCA GCC CCG TCG TGG AGG CCG CCG ATT GGT GAA GGC CCT

C-3' [32]. The nuclear abundance of the transcription factor Oct-1 does not depend on biotin and was quantified as a control [12]. Additional controls were prepared by omitting nuclear extracts from incubation mixtures, by incubating nuclear extracts with radiolabeled probe in the presence of a molar excess of unlabeled probe, and by using nuclear extracts from HepG2 cells. Binding of transcription factors to ERSE was quantified by using gel densitometry [12].

In some cases, transcription factor-oligonucleotide-complexes were supershifted by using polyclonal rabbit IgG anti-human antibodies to XBP1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). These samples were prepared by incubating 5 µg of nuclear protein with 2 µg of antibody to XBP1 for 60 min at 4°C prior to adding oligonucleotide probes. Note that incubation of nuclear proteins with antibodies (*i*) may prevent formation of transcription factor-oligonucleotide complexes (if antibodies block oligonucleotide-binding sites of transcription factors); or (*ii*) may decrease the electrophoretic mobility of the transcription factor-oligonucleotide-complex (if antibodies bind to transcription factors without blocking their oligonucleotide-binding sites).

2.8. Reverse transcriptase polymerase chain reaction (RT-PCR)

Expression of genes encoding XBP1 and β -actin (control) was quantified by RT-PCR as described [51]. The following customized primers were used for PCR (Integrated DNA Technologies, Coralville, IA): (*i*) 5'-TGG TAG ATT TAG AAG AAG AGA ACC AAA-3' and 5'-AAT CAG CTG GGG AAA GAG TTC ATT GGC-3' for XBP1 (GenBank accession number AB_76384); and (*ii*) 5'-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3' and 5'-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC-3' for β -actin (GenBank accession number NM_001101). cDNA was quantified by gel densitometry using the Kodak EDAS 290 Documentation and Analysis System (Rochester, NY); only values from within the exponential phase of PCR amplification were considered for data analysis. Abundance of cDNA encoding XBP1 was normalized by the abundance of cDNA encoding β -actin.

2.9. Western blot analyses

The following proteins play a role in the cellular response to ER stress. (*i*) The transcription factor XBP1 (see above). (*ii*) The pro-apoptotic transcription factor GADD153 (see above). (*iii*) Ubiquitin-activating enzyme 1 (UBE1): UBE1 catalyzes the first step in the proteasome-dependent degradation of unfolded proteins [52,53]. Accumulation of unfolded proteins is associated with increased expression of UBE1 [37]. (*iv*) Eukaryotic translation initiation factor 2 α (eIF2 α): eIF2 α is part of the active 40S translation preinitiation complex [54]. Cells respond to ER stress with phosphorylation of a serine residue in eIF2 α ; phosphorylated eIF2 α cannot participate in the formation of the preinitiation complex [54], decreasing both translational activity and the abundance of unfolded proteins in the ER [26,28].

For analysis of GADD153 and UBE1, protein extracts were prepared from whole cells by using detergents and protease inhibitors [55]. Nuclear extracts for analysis of XBP1 were prepared by using the Nuclear Extract Kit (Active Motif, Carlsbad, CA) according to the manufacturer's instructions. For analysis of phosphorylated eIF2 α , cytoplasmic protein extracts were prepared in the presence of phosphatase inhibitors [56]. Nuclear histone H3 (control) was prepared by acid extraction as described [2]. The following gels were used to resolve proteins (Invitrogen, Carlsbad, CA) as described previously [2,12,55]: 18% Tris glycine gels (GADD153, UBE1, and histone H3) and 4–12% Bis Tris gels (XBP1 and eIF2 α). Proteins were probed with the following antibodies using standard Western blot procedures [55]: rabbit polyclonal anti-human XBP1 (Santa Cruz, Inc., Santa Cruz, CA), mouse monoclonal anti-human GADD153 (Santa Cruz, Inc.), mouse monoclonal anti-human UBE1 (Upstate Biotechnologies, Inc.; Lake

Placid, NY), rabbit polyclonal anti-human phospho-eIF2 α (Upstate Biotechnologies, Inc.), and goat polyclonal anti-human histone H3 (Santa Cruz, Inc.).

2.10. Statistical Analysis

The paired t-test was used to test for significance of differences [57]. StatView 5.0.1 (SAS Institute; Cary, NC) was used to perform all calculations. Differences were considered significant if $P < 0.05$. Data are expressed as mean $\pm$ SD.

3. Results

3.1. Biotin-dependent carboxylases

Biotinylation of carboxylases in Jurkat cells paralleled biotin concentrations in culture media. If cells were cultured in biotin-deficient medium, biotinylated pyruvate carboxylase, PCC, and 3-methylcrotonyl-CoA carboxylase were barely detectable by Western blot analysis of cell extracts using streptavidin as a probe (Fig. 1A). In contrast, holocarboxylases were abundant in extracts from cells cultured in biotin-supplemented medium. Note that the biotin-containing α -chains of PCC (molecular mass = 80 kDa) and 3-methylcrotonyl-CoA carboxylase (molecular mass = 83 kDa) migrate as one single band on the gels used here. Holocarboxylases were 49 to 78 times more abundant in biotin-supplemented cells than in biotin-deficient cells, as judged by gel densitometric analysis of Western blots (Fig. 1B). Acetyl-CoA carboxylase was not detectable in cell extracts, consistent with previous studies in Jurkat cells [38].

PCC activities paralleled the abundance of holocarboxylases in Jurkat cells. PCC activity was 60 ± 5.1 pmol bicarbonate fixed/(min $\times 10^6$ cells) and 24 ± 2.8 pmol bicarbonate fixed/(min $\times 10^6$ cells) in biotin-supplemented and biotin-deficient cells, respectively ($P < 0.01$; $n = 5$). Collectively, these findings suggest that biotin concentrations in culture media affected intracellular biotin concentrations.

3.2. SERCA3 expression

Biotin concentrations in culture media affected the transcriptional activity of the *SERCA3* promoter. The transcriptional activity of the full-length construct (basepairs -1312 to +55 bp) was 50% smaller in biotin-supplemented cells compared to biotin-deficient cells (Fig. 2). Effects of biotin on transcriptional activity decreased if basepairs -1312 to -731 were deleted, consistent with the binding of biotin-dependent repressors to this region. This region contains three Sp1 sites, one Ets-1 site, one GATA box, and one Oct-1 binding site [15]. Further deletion of basepairs -731 to -135 reversed effects of biotin on transcriptional activity: biotin supplementation was associated with a moderate transcriptional activation of *SERCA3*. Likely, the region spanning basepairs -731 to -135 contains binding sites for biotin-dependent activators of transcription; this region contains nine Sp1 sites [15]. Note that plasmid EcoRV-del (-1312/+55) is the full-length construct and, thus, is most likely to represent effects of biotin on transcriptional activity of the *SERCA3* gene *in vivo*.

Next the abundance of *SERCA3* mRNA in Jurkat cells was quantified by Northern blot analysis to confirm biotin-dependent transcription of the *SERCA3* gene in human lymphoid cells. The abundance of *SERCA3* mRNA was approximately 50% smaller in biotin-supplemented cells compared to biotin-deficient cells (Fig. 3A & B). Collectively, previous studies in human lymphocytes [14] and the present studies in Jurkat cells are consistent with decreased expression of the *SERCA3* gene in biotin-supplemented compared to biotin-deficient lymphoid cells.

3.3. Cytoplasmic Ca^{2+}

The concentration of free Ca^{2+} in the cytoplasm depended on biotin. The cytoplasmic Ca^{2+} concentration was approximately 14% greater in resting biotin-supplemented cells compared to resting biotin-deficient cells (Fig. 4). Cytoplasmic concentrations of Ca^{2+} increased if cells were stimulated with PMA and PHA, consistent with release of Ca^{2+} from intracellular stores. The cytoplasmic concentration of Ca^{2+} was approximately 24% greater in biotin-supplemented compared to biotin-deficient cells after stimulation with PMA and PHA. These observations are consistent with decreased expression of the SERCA3 pump in biotin-supplemented cells, reducing the transport of Ca^{2+} from the cytoplasm into the ER.

3.4. Secretion of IL-2

Secretion of IL-2 requires oxidative folding in the ER, leading to the formation of a disulfide bond between cysteine-58 and cysteine-105 [58]. In the present study, secretion of IL-2 was smaller in biotin-supplemented compared to biotin-deficient cells [kU of IL-2/($\text{L} \times 10^6$ cells $\times$ 6 h)]: 3.4 ± 0.3 versus 13.6 ± 0.3 ($n = 7$; $P < 0.01$). This finding suggests that the oxidative folding of secretory proteins is impaired in biotin-supplemented cells.

3.5. EMSA

ERSE-binding transcription factors were detected in nuclear extracts from both biotin-deficient and biotin-supplemented Jurkat cells, but the DNA-binding activity of these transcription factors was not affected by biotin (Fig. 5, lanes 1 and 2). The following controls suggested that the observed effects were specific. First, binding of transcription factors to ERSE was eliminated by pre-treating nuclear extracts with an antibody to XBP1 (lanes 3 and 4). Second, no bands were detectable in the absence of nuclear extract (lane 5) or in the presence of a 50-fold molar excess of unlabeled probe (lane 6). Finally, biotin concentrations in culture media did not affect the nuclear abundance of Oct-1 (Fig. 5, lanes 7 and 8).

Previous studies suggested that Jurkat cells are less sensitive than HepG2 cells to diet-induced ER stress [37,59]. Hence, we tested HepG2 cells to determine whether biotin supplementation is associated with increased nuclear ERSE-binding activity in biotin-supplemented cells. Indeed, ERSE-binding activity was greater in biotin-supplemented HepG2 cells compared with biotin-deficient cells (Fig. 5, lanes 9 and 10). This observation was corroborated by using gel densitometric analysis of EMSA. ERSE-binding activity equaled 238 ± 19 arbitrary units in biotin-supplemented HepG2 cells compared with 155 ± 32 arbitrary units in biotin-deficient cells ($P < 0.05$; $n = 3$).

3.6. Transcriptional activities of ERSE-dependent reporter genes

Jurkat cells responded to supplementation with pharmacological doses of biotin by increased transcriptional activities of the following ERSE-dependent reporter genes. (i) The transcriptional activity (luciferase activity) of a reporter gene (p5xATF6-GL3) driven by five ERSE was 22% greater in biotin-supplemented compared to biotin-deficient cells (Table 1). (ii) The transcriptional activities (luciferase activity) of reporter genes driven by the 5'-flanking region of the *Grp78* gene were 271% (plasmid -169GRP78-luc) and 196% (plasmid -457GRP78-luc) greater in biotin-supplemented compared to biotin-deficient cells (Table 1). Collectively, these findings are consistent with ER stress caused by biotin supplementation. (iii) The transcriptional activity (luciferase activity) of a reporter gene (CHOP-ERSE1) driven by the ERSE in the *GADD153* gene was 53% greater in biotin-supplemented compared to biotin-deficient cells (Table 1).

3.7. Abundance of stress response proteins

The cellular abundance of the following stress-related proteins was greater in biotin-supplemented compared to biotin-deficient cells (Figure 6): UBE1 in whole cell extracts; GADD153 in whole cell extracts; XBP1 in nuclear extracts; and phosphorylated eIF2 α in cytoplasmic extracts. The abundance of a control protein (nuclear histone H3) was not affected by biotin. These findings are consistent with ER stress caused by biotin supplementation.

3.8. XBP1 expression

The abundance of mRNA encoding XBP1 was moderately greater in biotin-supplemented compared to biotin-deficient cells, as judged by RT-PCR and gel densitometric analysis (arbitrary units): 478 ± 118 versus 438 ± 152 ($n = 3$). However, the difference was not statistically significant ($P = 0.20$). The abundance of β -actin mRNA (control) was not affected by biotin. The increased nuclear abundance of XBP1 protein observed in biotin-supplemented cells might be caused by mechanisms other than increased transcription of the *XBP1* gene.

4. Discussion

The present study provides evidence that biotin supplementation is associated (i) with decreased expression of the *SERCA3* gene in human lymphoid cells; (ii) with decreased sequestration of calcium in the ER; (iii) with decreased secretion of IL-2 into the extracellular space; (iv) with increased nuclear abundance of transcription factors binding to ERSE; (v) with increased transcriptional activity of ERSE-dependent genes; and (vi) with increased cellular abundance of proteins that play roles in UPR. Collectively, these findings are consistent with the hypothesis that supplementation with pharmacological doses of biotin may cause cell stress by impairing protein folding in the ER.

Effects of biotin on calcium flux and protein folding are physiologically important, based on the following lines of reasoning. First, some cell types depend on rapid proliferation in order to maintain normal physiological responses, e.g., antibody-secreting cells [36]. Cell stress triggering UPR is associated with decreased cell proliferation and increased cell death [30]. Thus, biotin-induced UPR might decrease the number of secretory cells, affecting processes such as the immune response. Second, UPR is associated with decreased secretion of disulfide-containing proteins into the extracellular space [29]. Secretory proteins such as IL-2 play essential roles in various physiological processes. For example, extracellular IL-2 binds to receptors located on the surface of target cells such as T and B cells and natural killer cells, triggering proliferation and differentiation [36]. Decreased abundance of IL-2 is likely to impair immune function. Third, cytoplasmic free Ca^{2+} enhances the generation of the second messenger nitric oxide in Jurkat cells [60]. Ongoing studies in our laboratory suggested that biotin supplementation is associated with increased synthesis of nitric oxide (R. Rodriguez-Melendez & J. Zemleni, unpublished observation); biological effects of biotin-induced generation of nitric oxide are currently being investigated.

Classically, the use of dietary supplements containing pharmacological doses of biotin has been considered safe [1]. The findings presented here suggest that a careful re-evaluation of the safety of biotin supplementation might be in order. Biotin supplementation may be associated with an undesired pattern of gene expression such as decreased expression of *SERCA3* (present study) and increased expression of cytochrome P450 1B1 [61]. Cytochrome P450 1B1 catalyzes hydroxylation of pro-carcinogens such as polycyclic aromatic hydrocarbons, aryl amines [62], and 17 β -estradiol [63]. This metabolic activation of pro-carcinogens is a key factor in carcinogenesis of the human prostate [64,65], breast [66], and uterus [67]. Collectively, effects of biotin on gene expression [5,14] and cell signaling [11, 12] need to be considered when establishing Tolerable Upper Intake Levels for biotin [40].

The present study suggests that biotin-dependent repressors bind to elements located 731 to 1312 basepairs upstream from the transcription start site in the *SERCA3* gene. The identity of these repressors is unknown. The following response elements have been identified in the region spanned by basepairs -731 to -1312: three Sp1 sites, one Ets-1 site, one GATA box, and one Oct-1 binding site [15]. The present study and previous studies [11,12] provided evidence that biotin does not affect the nuclear abundance of Oct-1, suggesting that effects of biotin on *SERCA3* expression are not mediated by Oct-1. In contrast, the nuclear abundance of Sp1 and Sp3 correlates positively with biotin supply [12]. Given that Sp3 may act as a transcriptional repressor [16,17,68], Sp3 might mediate the decreased expression of *SERCA3* in biotin-supplemented cells. However, this is an untested speculation, and unidentified proteins and signaling pathways might also play a role in biotin-dependent repression of *SERCA3*.

The present study provides evidence that biotin supplementation might impair protein folding, decreasing the secretion of proteins such as IL-2 into the extracellular space. Note that factors other than protein folding may also affect the extracellular concentration of IL-2. Previously we have demonstrated that expression of IL-2 receptor γ correlates positively with biotin supply in Jurkat cells [44]. Increased expression of IL-2 receptor γ is associated with increased endocytosis of IL-2, decreasing the concentration of IL-2 in the culture medium.

Notwithstanding the above-mentioned uncertainties, the present study is consistent with the hypothesis that biotin overdose may interfere with Ca^{2+} homeostasis in Jurkat cells. Jurkat cells fold and secrete relatively moderate amounts of proteins, i.e., <100 pg of IL-2/(10^6 cells $\times$ h) [44]. It will be of interest to determine whether cells that secrete larger amounts of proteins (e.g., antibody-producing cells) are more susceptible to triggering UPR in response to biotin supplementation.

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References

1. Zemleni, J. (2001) Biotin. In: Present Knowledge in Nutrition (Bowman, B.A. and Russell, R.M., eds.), ILSI Press, Washington, D.C.
2. Stanley JS, Griffin JB, Zemleni J. Biotinylation of histones in human cells: effects of cell proliferation. *Eur J Biochem* 2001;268:5424–5429. [PubMed: 11606205]
3. Peters DM, Griffin JB, Stanley JS, Beck MM, Zemleni J. Exposure to UV light causes increased biotinylation of histones in Jurkat cells. *Am J Physiol Cell Physiol* 2002;283:C878–C884. [PubMed: 12176744]
4. Narang MA, Dumas R, Ayer LM, Gravel RA. Reduced histone biotinylation in multiple carboxylase deficiency patients: a nuclear role for holocarboxylase synthetase. *Hum Mol Genet* 2004;13:15–3. [PubMed: 14613969]
5. Wiedmann S, Eudy JD, Zemleni J. Biotin supplementation causes increased expression of genes encoding interferon- γ , interleukin-1 β , and 3-methylcrotonyl-CoA carboxylase, and causes decreased expression of the gene encoding interleukin-4 in human peripheral blood mononuclear cells. *J Nutr* 2003;133:716–719. [PubMed: 12612142]
6. Dakshinamurti, K. (2003) Regulation of gene expression by biotin, vitamin B6 and vitamin C. In: Molecular Nutrition (Daniel, H. and Zemleni, J., eds.), CABI Publishing, Oxfordshire, UK.
7. Rodriguez-Melendez R, Zemleni J. Regulation of gene expression by biotin. *J Nutr Biochem* 2003;14:680–690. [PubMed: 14690760]
8. Collins JC, Paietta E, Green R, Morell AG, Stockert RJ. Biotin-dependent expression of the asialoglycoprotein receptor in HepG2. *J Biol Chem* 1988;263:11280–11283. [PubMed: 2457019]

9. Rodriguez-Melendez R, Perez-Andrade ME, Diaz A, Deolarte A, Camacho-Arroyo I, Ciceron I, Ibarra I, Velazquez A. Differential effects of biotin deficiency and replenishment on rat liver pyruvate and propionyl-CoA carboxylases and on their mRNAs. *Mol Genet Metabol* 1999;66:16–23.
10. Solorzano-Vargas RS, Pacheco-Alvarez D, Leon-Del-Rio A. Holocarboxylase synthetase is an obligate participant in biotin-mediated regulation of its own expression and of biotin-dependent carboxylases mRNA levels in human cells. *Proc Natl Acad Sci USA* 2002;99:5325–5330. [PubMed: 11959985]
11. Rodriguez-Melendez R, Schwab LD, Zemleni J. Jurkat cells respond to biotin deficiency with increased nuclear translocation of NF- κ B, mediating cell survival. *Int J Vitam Nutr Res* 2004;74:209–216. [PubMed: 15296080]
12. Griffin JB, Rodriguez-Melendez R, Zemleni J. The nuclear abundance of transcription factors Sp1 and Sp3 depends on biotin in Jurkat cells. *J Nutr* 2003;133:3409–3415. [PubMed: 14608051]
13. Camporeale, G., Kothapalli, N., Sarath, G., and Zemleni, J. (2004) Biotinylation of histones in human cells. In: *Nutrients and Cell Signaling* (Zemleni, J. and Dakshinamurti, K., eds.), Marcel Dekker, New York, NY.
14. Wiedmann S, Rodriguez-Melendez R, Ortega-Cuellar D, Zemleni J. Clusters of biotin-responsive genes in human peripheral blood mononuclear cells. *J Nutr Biochem* 2004;15:433–439. [PubMed: 15219929]
15. Dode L, Greef CD, Mountian I, Attard M, Town MM, Casteels R, Wuytack F. Structure of the human sarco-endoplasmic reticulum Ca^{2+} -ATPase 3 gene. *J Biol Chem* 1998;273:13982–13994. [PubMed: 9593748]
16. Birnbaum MJ, Wijnen AJv, Odgren PR, Last TJ, Suske G, Stein GS, Stein JL. Sp1 trans-activation of cell cycle regulated promoters is selectively repressed by Sp3. *Biochemistry* 1995;34:16503–16508. [PubMed: 8845379]
17. Black A, Black JD, Azizkhan-Clifford J. Sp1 and Krüppel-like factor family of transcription factors in cell growth regulation and cancer. *J Cell Physiol* 2001;188:143–160. [PubMed: 11424081]
18. Burk SE, Lytton J, MacLennan DH, Shull GE. cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca^{2+} pump. *J Biol Chem* 1989;264:18561–18568. [PubMed: 2553713]
19. Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* 1992;267:14483–14489. [PubMed: 1385815]
20. Martin V, Bredoux R, Corvazier E, Gorp Rv, Kovacs T, Gelebart P, Enouf J. Three novel sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) 3 isoforms. *J Biol Chem* 2002;277:24442–24452. [PubMed: 11956212]
21. Bergeron JJM, Brenner MB, Thomas DY, Williams DB. Calnexin: a membrane-bound chaperone of the endoplasmic reticulum. *Trends Biochem Sci* 1994;19:124–128. [PubMed: 8203019]
22. Hebert DN, Foellmer B, Helenius A. Glucose trimming and reglycosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. *Cell* 1995;81:425–433. [PubMed: 7736594]
23. Macher DRJ, Koch GLE. Identification of a set of calcium-binding proteins in reticuloplasm, the luminal content of the endoplasmic reticulum. *J Cell Sci* 1988;91:61–70. [PubMed: 3253304]
24. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000;1:11–21. [PubMed: 11413485]
25. Lodish HF, Kong N. Perturbation of cellular calcium blocks exit of secretory proteins from the rough endoplasmic reticulum. *J Biol Chem* 1990;265:10893–10899. [PubMed: 2162823]
26. Prostko CR, Brostrom MA, Malara EM, Brostrom CO. Phosphorylation of eukaryotic initiation factor (eIF) 2 α and inhibition of eIF-2B in GH₃ pituitary cells by perturbants of early protein processing that induce GRP78. *J Biol Chem* 1992;267:16751–16754. [PubMed: 1512215]
27. Lodish HF, Kong N, Wikstrom L. Calcium is required for folding of newly made subunits of the asialoglycoprotein receptor within the endoplasmic reticulum. *J Biol Chem* 1992;267:12753–12760. [PubMed: 1618778]
28. Srivastava SP, Davies MV, Kaufman RJ. Calcium depletion from the endoplasmic reticulum activates the double-stranded RNA-dependent protein kinase (PKR) to inhibit protein synthesis. *J Biol Chem* 1995;270:16619–16624. [PubMed: 7622470]

29. Cooper GR, Brostrom CO, Brostrom MA. Analysis of the endoplasmic reticular Ca^{2+} requirement for $\alpha 1$ -antitrypsin processing and transport competence. *Biochem J* 1997;325:601–608. [PubMed: 9271078]
30. Sidrauski, C., Brickner, J.H., and Walter, P. (2002) The unfolded protein response. In: *Protein Targeting, Transport & Translocation* (Dalbey, R.E. and Heijne, G.v., eds.), Academic Press, San Diego, CA.
31. Pahl HL. Signal transduction from the endoplasmic reticulum to the cell nucleus. *Physiol Rev* 1999;79:683–700. [PubMed: 10390516]
32. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001;107:881–891. [PubMed: 11779464]
33. Rao RV, Peel A, Logviniva A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, Bredesen DE. Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. *FEBS Lett* 2002;514:122–128. [PubMed: 11943137]
34. Caspersen C, Pedersen PS, Treiman M. The sarco/endoplasmic reticulum calcium-ATPase 2b is an endoplasmic reticulum stress-inducible protein. *J Biol Chem* 2000;275:22363–22372. [PubMed: 10748035]
35. Brewer JW, Diehl JA. PERK mediates cell-cycle exit during the mammalian unfolded protein response. *Proc Natl Acad Sci USA* 2000;97:12625–12630. [PubMed: 11035797]
36. Klein, J., and Horejsi, V. (1997) Immunology Blackwell Science Ltd., Oxford, UK.
37. Camporeale G, Zemleni J. Oxidative folding of interleukin-2 is impaired in flavin-deficient Jurkat cells, causing intracellular accumulation of interleukin-2 and increased expression of stress response genes. *J Nutr* 2003;133:668–672. [PubMed: 12612135]
38. Manthey KC, Griffin JB, Zemleni J. Biotin supply affects expression of biotin transporters, biotinylation of carboxylases, and metabolism of interleukin-2 in Jurkat cells. *J Nutr* 2002;132:887–892. [PubMed: 11983808]
39. Mock DM, Lankford GL, Mock NI. Biotin accounts for only half of the total avidin-binding substances in human serum. *J Nutr* 1995;125:941–946. [PubMed: 7722698]
40. National Research Council (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline (Food and Nutrition Board, Institute of Medicine). National Academy Press, Washington, DC.
41. Zemleni J, Helm RM, Mock DM. In vivo biotin supplementation at a pharmacologic dose decreases proliferation rates of human peripheral blood mononuclear cells and cytokine release. *J Nutr* 2001;131:1479–1484. [PubMed: 11340103]
42. Mock, D.M. (1997) Determinations of biotin in biological fluids. In: *Methods in Enzymology* (McCormick, D.B., Suttie, J.W. and Wagner, C., eds.), Academic Press, New York, NY.
43. Rodriguez-Melendez, R., Griffin, J.B., and Zemleni, J., The expression of genes encoding ribosomal subunits and eukaryotic translation initiation factor 5A depends on biotin and bisnorbiotin in HepG2 cells. *J. Nutr. Biochem.* (in press) (2005)
44. Rodriguez-Melendez R, Camporeale G, Griffin JB, Zemleni J. Interleukin-2 receptor γ -dependent endocytosis depends on biotin in Jurkat cells. *Am J Physiol Cell Physiol* 2003;284:C415–C421. [PubMed: 12388078]
45. Yoshida H, Haze K, Yanagi H, Yura T, Mori K. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. *J Biol Chem* 1998;273:33741–33749. [PubMed: 9837962]
46. Roy B, Lee AS. The mammalian endoplasmic reticulum stress response element consists of an evolutionary conserved tripartite structure and interacts with a novel stress-inducible complex. *Nucl Acids Res* 1999;27:1437–1443. [PubMed: 10037803]
47. Wang Y, Shen J, Arenzana N, Tirasophon W, Kaufman RJ, Prywes R. Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. *J Biol Chem* 2000;275:27013–27020. [PubMed: 10856300]
48. Luo S, Baumeister P, Yang S, Abcouwer SF, Lee AS. Induction of Grp78/BiP by translational block: activation of the Grp78 promoter by ATF4 through and upstream ATF/CRE site independent of the endoplasmic reticulum stress elements. *J Biol Chem* 2003;278:37375–37385. [PubMed: 12871976]

49. Thuerauf DJ, Hoover H, Meller J, Hernandez J, Su L, Andrews C, Dillmann WH, McDonough PM, Glembotski CC. Sarco/endoplasmic reticulum calcium ATPase-2 expression is regulated by ATF6 during the endoplasmic reticulum stress response: intracellular signaling of calcium stress in a cardiac myocyte model system. *J Biol Chem* 2001;276:48309–48317. [PubMed: 11595740]
50. Scheerger SB, Zemleni J. Expression of oncogenes depends on biotin in human small cell lung cancer cells NCI-H69. *Int J Vitam Nutr Res* 2003;73:461–467. [PubMed: 14743551]
51. Zemleni J, Stanley JS, Mock DM. Proliferation of peripheral blood mononuclear cells causes increased expression of the sodium-dependent multivitamin transporter gene and increased uptake of pantothenic acid. *J Nutr Biochem* 2001;12:465–473. [PubMed: 11834205]
52. Attaix, D., Combaret, L., Kee, A.J., and Taillandier, D. (2003) Mechanisms of ubiquitination and proteasome-dependent proteolysis in skeletal muscle. In: *Molecular Nutrition* (Daniel, H. and Zemleni, J., eds.), CAB International, Wallingford, UK.
53. Bonifacino JS, Weissman AM. Ubiquitin and the control of protein fate in the secretory and endocytic pathways. *Annu Rev Cell Dev Biol* 1998;14:19–57. [PubMed: 9891777]
54. Garrett, R.H., and Grisham, C.M. (1995) *Biochemistry* Saunders College Publishing, Fort Worth, TX.
55. Griffin JB, Stanley JS, Zemleni J. Synthesis of a rabbit polyclonal antibody to the human sodium-dependent multivitamin transporter. *Int J Vitam Nutr Res* 2002;72:195–198. [PubMed: 12214555]
56. Lynch CJ, Fox HL, Vary TC, Jefferson LS, Kimball SR. Regulation of amino acid-sensitive TOR signaling by leucine analogues in adipocytes. *J Cell Biochem* 2000;77:234–251. [PubMed: 10723090]
57. SAS Institute (1999) *StatView Reference* SAS Publishing, Cary, NC.
58. Goldsby, R.A., Kindt, T.J., and Osborne, B.A. (2000) *Immunology* W. H. Freeman and Company, New York, NY.
59. Manthey KC, Chew YC, Zemleni J. Riboflavin deficiency impairs oxidative folding and secretion of apolipoprotein B-100 in HepG2 cells, triggering stress-response systems. *J Nutr* 2005;135:978–982. [PubMed: 15867268]
60. Srivastava RK, Sollott SJ, Khan L, Hansford R, Lakatta EG, Longo DL. Bcl-2 and Bcl-X(L) block thapsigargin-induced nitric oxide generation, c-Jun NH(2)-terminal kinase activity, and apoptosis. *Mol Cell Biol* 1999;19:5659–5674. [PubMed: 10409755]
61. Rodriguez-Melendez R, Griffin JB, Zemleni J. Biotin supplementation increases expression of the cytochrome P450 1B1 gene in Jurkat cells, increasing the occurrence of single-stranded DNA breaks. *J Nutr* 2004;134:2222–2228. [PubMed: 15333708]
62. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. *Cancer Res* 1996;56:2979–2984. [PubMed: 8674051]
63. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Stutter TR. 17 β -estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc Natl Acad Sci USA* 1996;93:9776–9781. [PubMed: 8790407]
64. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. *Carcinogenesis* 2000;21:1683–1689. [PubMed: 10964100]
65. Cavalieri EL, Devanesan P, Bosland MC, Badawi AF, Rogan EG. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. *Carcinogenesis* 2002;23:329–333. [PubMed: 11872641]
66. Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proc Natl Acad Sci USA* 1996;93:3294–3296. [PubMed: 8622931]
67. Liehr JG, Ricci MJ, Jefcoate CR, Hannigan EV, Hokanson JA, Zhu BT. 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: implications for the mechanism of uterine tumorigenesis. *Proc Natl Acad Sci USA* 1995;92:9220–9224. [PubMed: 7568105]
68. Ghayor G, Chadjichristos C, Herrouin JF, Ala-Kokko L, Suske G, Pujol JP, Galera P. Sp3 represses the Sp1-mediated transactivation of the human COL2A1 gene in primary and de-differentiated chondrocytes. *J Biol Chem* 2001;276:36881–36895. [PubMed: 11447232]

Fig. 1. Biotin supply affects the abundance of holocarboxylases in Jurkat cells. Cells were cultured in biotin-deficient (“D”; 0.025 nmol/L) and biotin-supplemented (“S”; 10 nmol/L) media for 5 wk. Panel A: Representative Western blot depicting pyruvate carboxylase (PC), α -chain of propionyl-CoA carboxylase (PCC), and α -chain of 3-methylcrotonyl-CoA carboxylase (MCC). Panel B: Gel densitometric analysis of Western blots. Values are means $\pm$ SD ($n = 6$). ^aSignificantly different from the abundance of the same carboxylase in cells cultured in biotin-supplemented medium ($P < 0.05$).

Fig. 2.

The transcriptional activity of the human SERCA3 promoter depends on biotin. Biotin-supplemented (hatched bars) and biotin-deficient (solid bars) Jurkat cells were transfected with reporter (luciferase) plasmids driven by the full-length or truncated forms of the 5'-flanking region of SERCA3; 48 h after transfection cells were stimulated with PMA and PHA for 6 h. Data are expressed as ratios of luciferase activities in cells transfected with SERCA3 constructs to activities in cells transfected with promoter-free vector (n = 5 – 6).

Fig. 3.

Biotin affects the abundance of SERCA3 mRNA in Jurkat cells. Cells were cultured in biotin-deficient ("D" = 0.025 nmol/L) and biotin-supplemented ("S" = 10 nmol/L) media for 5 wk. Panel A: Representative Northern blots of SERCA3 and histone H4 (control). Panel B: Gel densitometric quantitation of SERCA3 mRNA, normalized by the abundance of histone H4 mRNA. Values are means $\pm$ SD ($n = 3$). ^aSignificantly different from cells cultured in biotin-supplemented medium ($P < 0.05$).

Fig. 4. Biotin affects the concentration of free calcium in Jurkat cell cytoplasm. Cells were cultured in biotin-deficient (0.025 nmol/L biotin) and biotin-supplemented (10 nmol/L biotin) media for 5 wk. Panel A: Cells were stimulated with PMA and PHA, and cytoplasmic free calcium was probed at timed intervals using a fluorophore. Values are means $\pm$ SD ($n = 5$). ^aSignificantly different from cells cultured in biotin-supplemented medium ($P < 0.01$). Panel B: Images were obtained by confocal microscopy one minute after stimulation of cells with PMA and PHA. Arrows depict cells with low cytoplasmic calcium.

Fig. 5.

Nuclear translocation of ERSE-binding factors increases in response to biotin supplementation in HepG2 cells but not in Jurkat cells. Cells were cultured in biotin-deficient ("D" = 0.025 nmol/L) and biotin-supplemented ("S" = 10 nmol/L) media for 10 d (HepG2 cells) and 5 wk (Jurkat cells), respectively. Nuclear extracts were analyzed by EMSA for DNA-binding activities of transcription factors: lanes 1 and 2 = ERSE-binding activity in Jurkat nuclear extracts; lanes 3 and 4 = Jurkat nuclear were pre-treated with anti-XBP1 antibody; lanes 5 = absence of nuclear extract; lane 6 = ERSE-binding activity in Jurkat nuclear extracts in the presence of a 50-fold molar excess of unlabeled ERSE; lanes 7 and 8 = DNA-binding activity of Oct-1 in Jurkat nuclear extracts; lanes 9 and 10 = ERSE-binding activity in HepG2 nuclear extracts. NE = no nuclear extract; Ab = antibody treated (supershift experiment).

Fig. 6.

Abundance of ER stress-related proteins depends on biotin in Jurkat cells. Cells were cultured in biotin-deficient (“D” = 0.025 nmol/L) and biotin-supplemented (“S” = 10 nmol/L) media for 5 wk. The following proteins were probed by Western blot analysis: UBE1 in whole cell extracts; GADD153 in whole cell extracts; XBP1 in nuclear extracts; and phosphorylated eIF2 α (P-eIF2 α) in cytoplasmic extracts. Histone H3 in nuclear extracts was used as a control.

TABLE 1

Effects of biotin concentrations in culture media on the transcriptional activities of ERSE-dependent luciferase constructs in Jurkat cells

Plasmid	Biotin in culture medium (nmol/L)	
	0.025	10
	<i>Stimulation of luciferase activity^I</i>	
p5xATF6-GL3	2.6 ± 0.22 ^a	3.2 ± 0.04
-169GRP78-luc	49 ± 3.5 ^b	182 ± 15.4
-457GRP78-luc	114 ± 21 ^b	338 ± 68
CHOP-ERSE1	1.5 ± 0.07 ^b	2.3 ± 0.02

^I Values are ratios of luciferase activities in cells transfected with ERSE-dependent luciferase constructs to activities in cells transfected with promoter-free luciferase constructs.

^{a,b} Significantly different compared to cells cultured in medium containing 10 nmol/L biotin (^a*P* = 0.05; ^b*P* < 0.01; n = 3 independent experiments).