In Vivo Biotin Supplementation at a Pharmacologic Dose Decreases Proliferation Rates of Human Peripheral Blood Mononuclear Cells and Cytokine Release[1]

Janos Zempleni, Ricki M. Helm* and Donald M. Mock†[2]

*Department of Nutritional Science and Dietetics, University of Nebraska at Lincoln, Lincoln, NE 68583; *Department of Pediatrics, Division of Allergy and Immunology, Arkansas Children's Hospital Research Institute, Little Rock, AR 72202; and †Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205*

ABSTRACT Theoretically, vitamin supplements may either enhance or reduce protein synthesis and proliferation in peripheral blood mononuclear cells (PBMC). In the present study, we determined whether administration of a pharmacologic dose of biotin affects proliferation rates of PBMC and cytokine release. Healthy adults ($n = 5$) ingested 3.1 μmol biotin/d for 14 d; blood and urine were collected pre- and postsupplementation. PBMC were isolated by density gradient and incubated with the mitogen concanavalin A for up to 3 d. At timed intervals during mitogen stimulation, we measured the following: *1*) cellular uptake of [^{3}H]thymidine to determine proliferation rates; *2*) concentrations of various cytokines released into the medium; and *3*) the percentages of PBMC subsets as judged by CD surface markers. Biotin supplementation caused a significant decrease of PBMC proliferation. At 2 d after mitogen stimulation, [^{3}H]thymidine uptake by postsupplementation PBMC was 66 $\pm$ 21% of the uptake by presupplementation PBMC ($P < 0.05$). Similarly, concentrations of interleukin-1β (2 d after mitogen) and interleukin-2 (1 d after mitogen) in media from postsupplementation PBMC were 65 $\pm$ 28% and 44 $\pm$ 23%, respectively, of those for presupplementation PBMC ($P < 0.01$). Percentages of PBMC subsets were not affected by 14 d of biotin supplementation. Overall, this study provides evidence that administration of pharmacologic doses of biotin for 14 d decreases PBMC proliferation and synthesis of interleukin-1β and interleukin-2. J. Nutr. 131: 1479–1484, 2001.

KEY WORDS: • *biotin* • *cytokines* • *humans* • *lymphocytes* • *proliferation*

Peripheral blood mononuclear cells (PBMC)[3] represent a heterogeneous population of immune cells (T cells, B cells and various granulocytes) that arise from pluripotent hematopoietic stem cells in the bone marrow (Janeway et al. 1999). PBMC account for cellular and humoral immune responses; some PBMC (T and B cells) proliferate rapidly after either antigenic or mitogenic stimulation. In addition, stimulated PBMC synthesize and release various cytokines. Cytokines serve as messengers between immune cells and have various functions in the immune response such as induction of proliferation and differentiation of T and B cells, and inhibition of viral replication (Klein and Horejsi 1997). Thus, stimulation of immune cells triggers a substantial increase of metabolic activity, leading to cell proliferation and synthesis of cytokines.

Proliferation and cytokine synthesis by PBMC may cause a substantial increase in nutrient demand by any of the following mechanisms: *1*) increased nutrients are required to provide metabolic energy (e.g., glucose); *2*) increased nutrients are required in biosynthetic pathways (e.g., amino acids for protein synthesis); *3*) increased nutrients are required as coenzymes (e.g., water-soluble vitamins); and *4*) increased nutrients are required for DNA synthesis (e.g., folic acid). For example, the cellular uptake of alanine, proline and leucine (Segel and Lichtman 1981) and the rate of catabolism of glucose (Loos and Roos 1973, Roos et al. 1972, Roos and Loos 1973) increase in response to mitogenic stimulation of PBMC. In mitogen-stimulated PBMC, 14 amino acids are essential to maintain protein synthesis and a normal rate of proliferation (Waithe et al. 1975). Similarly, mitogen stimulation increases the uptake of biotin (Zempleni and Mock 1999c) and other water-soluble vitamins (Hall 1984, Williams et al. 1985, Zempleni and Mock 2000b) by PBMC, perhaps to provide coenzymes for metabolic pathways, e.g., biotin-dependent carboxylases (Zempleni and Mock 2000c). A deficiency of nutrients can cause arrest of cells in the G_0 phase; cells do not enter the cell cycle and do not divide. For example, HeLa cells arrest in G_0 phase if incubated in biotin-free medium (Dakshinamurti et al. 1985). These findings are consistent with the hypothesis that proliferating cells have an increased demand for nutrients.

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[2] To whom correspondence should be addressed.
E-mail: mockdonaldm@exchange.uams.edu.
[3] Abbreviations: con A, concanavalin A; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; IL, interleukin; INF, interferon; PBMC, peripheral blood mononuclear cells.

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Various studies have demonstrated adverse effects of biotin deficiency on immune function. For example, biotin deficiency caused decreased antibody synthesis (Kumar and Axelrod 1978), reduced thymus size and cellularity (Rabin 1983) and decreased proliferation of spleen cells (Báez-Saldaña et al. 1998) in rats and mice. Administration of biotin supplements at pharmacologic doses to biotin-deficient animals caused immediate partial restoration of immune function (Kumar and Axelrod 1978, Petrelli et al. 1981). In contrast, the effects of biotin supplements given to normal animals may vary with species or immune function or both. One report demonstrated that biotin supplementation resulted in increased phagocytic activity of the reticuloendothelial system in rats (Petrelli and Marsili 1971). In another report, biotin supplementation of pigs depressed the humoral immune response (Kornegay et al. 1989). Thus, we considered that in vivo supplementation of pharmacologic doses of biotin for 14 d might enhance or repress proliferation and function of PBMC in healthy adults. To test these alternate possibilities, we examined the effect of biotin supplementation on uptake of thymidine (as a proliferation marker) into PBMC and on release of cytokines by PBMC (as a marker of function).

SUBJECTS AND METHODS

Subjects. Healthy adults (n = 5; 2 men, 3 women) aged 35 to 47 y participated in this study. All subjects were nonsmokers; none had knowingly consumed any vitamin supplements for at least 2 wk before initiation of the study. Anticonvulsants alter biotin status by impairing biotin uptake from the intestine or accelerating biotin catabolism or both (Krause et al. 1982, Mock and Dyken 1997, Said et al. 1989). Thus, individuals receiving anticonvulsant treatment were excluded from study participation. This study was approved by the Human Research Advisory Committee at the University of Arkansas for Medical Sciences. Informed written consent was obtained from each subject.

Study design. A 24-h urine sample and a heparinized blood sample (~150 mL) were collected from each subject before biotin supplementation (denoted "presupplementation"). A second set of samples (denoted "postsupplementation") was collected ~24 h after subjects had completed a 14-d supplementation with biotin; one capsule of "Biotin Caps" (Twinlab, Ronkonkoma, NY) was taken per day. The manufacturer states that one capsule does not contain < 600 μg (2.46 μmol) of biotin. The biotin content of the supplement was determined by avidin-binding assay (Mock 1997); biotin content was 3.1 ± 0.4 μmol/capsule. The normal dietary intake of biotin is < 0.4 μmol/d (Food and Nutrition Board/Institute of Medicine Standing Committee 1998).

A supplementation period of 14 d was chosen based on the following line of reasoning: in a previous study, we administered 4.9 μmol of biotin orally to normal adults for 14 d and measured the time course of the concentrations of biotin and metabolites in serum (Mock and Mock 1997). Concentrations of biotin and metabolites appeared to reach a steady state by d 3 on the basis of the observation that fasting serum concentrations were essentially unchanged from d 3 to 14 of supplementation. This suggests that 14 d of supplementation are sufficient to achieve equilibration of biotin throughout the volume of distribution.

PBMC culturing. PBMC were isolated aseptically from blood using gradient centrifugation as previously described (Zempleni and Mock 1999c); plasma was saved for determination of biotin concentration and for use as a culture supplement as described below. PBMC (4×10^9 cells/L) were suspended in custom-manufactured RPMI-1640 (Atlanta Biologicals, Norcross, GA) that was compounded from pure ingredients without biotin; the culture medium was supplemented with 10% autologous plasma (by volume), 1×10^5 IU/L penicillin, and 100 mg/L streptomycin (final concentrations). Antibiotics and culture medium from the same stock solution and powder, respectively, were used in all experiments.

For in vitro culture studies, we attempted to simulate the likely plasma levels of biotin before and during supplementation. The biotin concentration in the culture medium was adjusted to 0.25 nmol/L for presupplementation PBMC and to 10 nmol/L for postsupplementation samples on the basis of the following pilot data. Plasma concentration of biotin from unsupplemented subjects was ~0.25 nmol/L, whereas the biotin concentration in plasma from a supplemented subject (3.1 μmol/d for 14 d) was ~10 nmol/L ~1 h after the last supplement was taken. After adjustment of biotin concentration in the medium, an aliquot of the suspension was collected (denoted "d 0"), and concanavalin A (con A; cat.# C 2010; Sigma Chemical, St. Louis, MO) was added to the remainder at a final concentration of 20 mg/L to stimulate proliferation and cytokine synthesis; con A from the same stock solution was used in all experiments. At timed intervals (1, 2 and 3 d after addition of con A), aliquots of PBMC suspended in medium were collected and assayed as described below.

Biotin analysis. In urine, biotin was measured by avidin-binding assay (Mock 1997, Zempleni and Mock 2000a). This assay does not distinguish between biotin and biotin metabolites but rather quantitates the total of biotin and biotin metabolites. Hence, this is a semiquantitative estimate of biotin status. In contrast, biotin and biotin metabolites in plasma were separated by HPLC before biotin-containing fractions were assayed by avidin-binding assay (Mock 1997, Zempleni and Mock 2000a); this allows accurate quantitation of biotin.

PBMC subsets. The percentage of individual PBMC subsets was determined by flow cytometry in freshly isolated and in cultured cells using monoclonal antibodies against cell surface markers. The following monoclonal antibodies (PharMingen, San Diego, CA) were used as previously described (Helm et al. 1996): fluorescein isothiocyanate (FITC) mouse anti-human CD3 (cat.# 30104X); FITC mouse anti-human CD4 (cat.# 30154X); FITC mouse anti-human CD8 (cat.# 30324X); FITC mouse anti-human CD19 (cat.# 30654X); R-phycoerythrin mouse anti-human CD56 (cat.# 31665X). These CD antibodies bind to the following PBMC subsets: CD3 positive = T cells; CD4 positive = T-cell subset (T-helper cells) and monocytes (weak); CD8 positive = T-cell subset (T-suppressor cells); CD19 positive = B cells; CD56 positive = natural killer cells (Klein and Horejsi 1997). As controls for nonspecific PBMC binding, FITC mouse immunoglobulin (Ig)G1 (cat.# 03214C) and R-phycoerythrin mouse IgG1 (cat.# 03215A) were used.

Briefly, 1 volume of mouse IgG1 antibodies (controls) was mixed with 10 volumes of PBS (4°C); the anti-human antibodies were used without dilution. PBMC (~100,000, typically in < 25 μL of suspension) were mixed with 10 μL of a given antibody; PBS was added to produce a volume of 100 μL. After vortexing, samples were incubated in the dark for 15 min. PBS (4 mL, 4°C) was added and the PBMC were sedimented at 500 × g for 5 min. The supernatant was discarded. After the addition of 300 μL of formaldehyde 1% (by volume) in PBS, the resuspended PBMC were analyzed by flow cytometry as described previously (Helm et al. 1996).

Cytokines. Timed aliquots from the PBMC suspension were centrifuged at 2260 × g for 90 s; the cell-free supernatant was harvested and used to quantitate cytokines. The following commercially available kits for human cytokines were used according to the manufacturer's instructions (Biosource, Camarillo, CA): IL-1 Beta Easia kit (cat.# KAC1212) for interleukin-1β; IL-2 Easia kit (cat.# KAC1242) for interleukin-2; IL-3 Easia kit (cat.# KAC1272) for interleukin-3; IL-4 Easia kit (cat.# KAC1282) for interleukin-4; IL-6 Easia kit (cat.# KAC1262) for interleukin-6; IFN-gamma Easia kit (cat.# KAC1232) for interferon-γ. Each sample was assayed in duplicate. All samples from a given subject were assayed within the same batch.

The present study was designed to investigate the effects of biotin supplementation on various cells of the immune system. Therefore, we measured cytokines that are produced by several different immune cells. For example, IL-1β is produced by macrophages and epithelial cells; IL-2 is produced by T_H1 lymphocytes; IL-4 is produced by T_H2 lymphocytes; IL-6 is produced by monocytes, macrophages and T_H2 lymphocytes; and INF-γ is produced by T_H1 lymphocytes and natural killer cells (Klein and Horejsi 1997).

Proliferation rate. Uptake of [^{3}H]thymidine (specific radioactivity 1.3 TBq/mmol; ICN; Irvine, CA) into PBMC was measured as

previously described using 96-well plates (Zempleni and Mock 1999c). Briefly, 37 kBq of [^{3}H]thymidine was added per well and incubation was continued for 6 h at 37°C. Then, cells were harvested onto filter papers and radioactivity was determined by scintillation counting.

Pantothenic acid and CoA. Biotin and pantothenic acid share a common transporter for cellular uptake (Wang et al. 1999). At supraphysiologic concentrations (e.g., 10 μmol/L), biotin might displace pantothenic acid from the transporter, leading to decreased cellular uptake of pantothenic acid. Thus, we assessed in vitro whether a biotin concentration equivalent to that in the plasma of biotin-supplemented individuals causes a decreased uptake of physiologic concentrations of [^{3}H]pantothenic acid. Uptake was measured in analogy to the method previously described for biotin (Zempleni and Mock 1998) as modified for pantothenic acid (unpublished data). Also, we measured in vivo intracellular concentrations of endogenous CoA (the coenzyme form of pantothenic acid) in PBMC before and after biotin supplementation. CoA analyses were kindly conducted by Diana M. Downs (University of Wisconsin at Madison) as previously described (Allred and Guy 1969).

Statistics. Because data sets exhibited heterogenous variances (as judged by Bartlett's test), the Wilcoxon signed rank test was used to analyze differences between groups (pre- vs. postsupplementation) (Abacus Concepts 1989 and 1996). For [^{3}H]thymidine uptake and cytokine concentrations, only the peak values were tested for significance of difference. Differences were considered significant if $P < 0.05$. All statistical analyses were performed using Statview 4.5 (SAS Institute, Cary, NC). Data are presented as means ± 1 SD.

RESULTS

Biotin in plasma and urine. Biotin and metabolites were measured in plasma and urine samples from before and after the biotin supplementation period to determine whether subjects complied with the supplementation protocol and to confirm that biotin given at pharmacologic doses was bioavailable. The urinary excretion of total avidin-binding substances increased from 107 ± 26 nmol/24 h (presupplementation) to 1854 ± 288 nmol/24 h (postsupplementation; $P < 0.05$). The plasma concentration of biotin was ~7.4 times greater in postsupplementation plasma compared with presupplementation plasma (2848 ± 846 vs. 340 ± 110 pmol/L; **Fig. 1**). The plasma concentration of the quantitatively most important biotin metabolite, bisnorbiotin, was 15.5 times greater in biotin-supplemented subjects (3786 ± 2896 vs. 230 ± 192 pmol/L). The plasma concentration of biotin-*d,l*-sulfoxides was approximately doubled postsupplementation compared with presupplementation (252 ± 141 vs. 128 ± 113 pmol/L), but the increase was not significant ($P = 0.22$). These data provide evidence that subjects complied with the study protocol (i.e., ingested the biotin supplements). Moreover, on the basis of the relationship between biotin intake and plasma concentrations and urinary excretion, the subjects appeared to have absorbed a substantial fraction of the biotin supplements.

Proliferation rates of PBMC. Cellular uptake of [^{3}H]thymidine is a well-established marker of PBMC proliferation (Stites 1987). In PBMC harvested before and after biotin supplementation, [^{3}H]thymidine uptake was measured before addition of mitogen (con A) to the culture medium (d 0) and 1, 2 and 3 d after addition of mitogen. The mitogenic response was blunted by biotin. On d 2 after mitogen addition, [^{3}H]thymidine uptake in postsupplementation PBMC was 66 ± 21% of that exhibited by presupplementation PBMC (**Fig. 2**).

Cytokine synthesis. Cytokine concentrations in medium supernatant were determined using the sampling schedule described above for thymidine uptake. IL-3 was detectable in only 5 of 20 samples; those data were excluded from further analysis. For five cytokines (IL-1β, IL-2, IL-4, IL-6 and INF-γ), synthesis tended to be lower in PBMC from postsupplementation subjects compared with presupplementation subjects (**Table 1**). Peak cytokine concentrations in postsupplementation samples were 44–88% of presupplementation values. However, the decrease was significant only for IL-1β and IL-2 (for others, $P = 0.22$–0.35). The time courses of the concentrations of these two cytokines are depicted in **Figure 3**. On d 2 after mitogen addition to the medium, the concentration of IL-1β in postsupplementation samples was 65 ± 28% of presupplementation values; on d 1 after mitogen addition, the concentration of IL-2 in postsupplementation samples was 44 ± 23% of presupplementation values. Taken together, these findings provide evidence that biotin supplementation actually impaired the ability of PBMC to produce IL-1β and IL-2 by PBMC in response to mitogen stimulation.

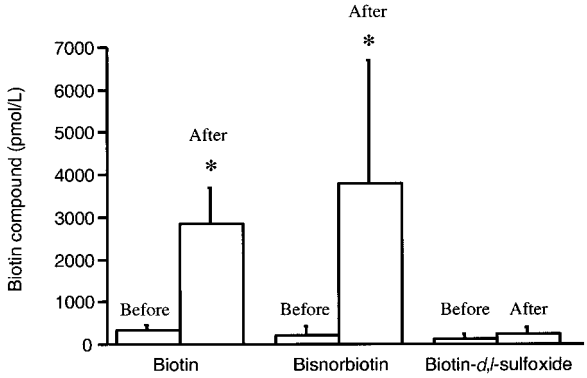

FIGURE 1 Plasma concentrations of biotin, bisnorbiotin and biotin-*d,l*-sulfoxides in healthy adults before and after supplementation with biotin (3.1 μmol/d for 14 d). Data are expressed as means ± SD, $n = 5$. *$P < 0.01$ (pre- vs. postsupplementation; Wilcoxon test).

PBMC subsets. PBMC are a heterogeneous population of immune cells; PBMC subsets can be quantitated on the basis of cell surface antigens (Klein and Horejsi 1997). We measured cell surface markers of freshly isolated (**Table 2**) and cultured (data not shown) PBMC to detect major PBMC subsets (see Subjects and Methods). Biotin supplementation did not significantly affect percentages of PBMC subsets.

Pantothenic acid uptake and intracellular CoA concentrations. Biotin at pharmacologic concentrations might interfere with cellular uptake of pantothenic acid by reversible competition for binding sites (Wang et al. 1999). This might result in decreased intracellular concentrations of pantothenic acid and CoA and perhaps impaired PBMC proliferation as observed here. Theoretically, the biotin concentration in plasma has to exceed the Michaelis-Menten constant of the transporter for pantothenic acid to substantially decrease cellular transport of pantothenic acid. In this study, plasma concentrations of biotin (<10 nmol/L) were consistently below the Michaelis-Menten constant (2 μmol/L) of the transporter for biotin (Prasad et al. 1997), making it rather unlikely that biotin decreased cellular uptake of pantothenic acid. Notwithstanding these theoretical considerations, we measured the uptake of [^{3}H]pantothenic acid at physiologic concentrations (100 nmol/L) in the presence of either a physiologic (0.25 nmol/L) or a pharmacologic concentration of biotin (10 nmol/L). Cellular uptake of [^{3}H]pantothenic acid was 34.8 ± 3.9 fmol/(10^6 cells × 15 min) at the physiologic biotin concentration, and 36.8 ± 3.5 fmol/(10^6 cells × 15 min) at the pharmacologic biotin concentration ($P > 0.05$). Endogenous concentrations of CoA were also determined before supplementation. Unfortunately, the content was below the detection limit of current assays. These data provide evidence that

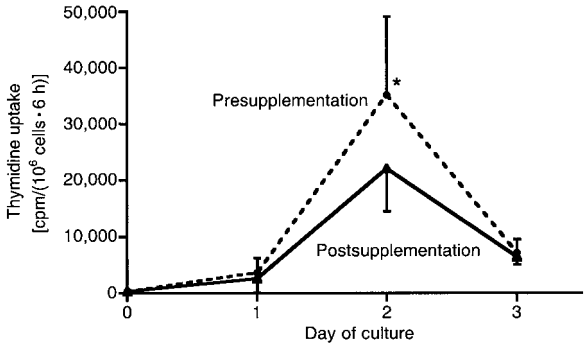

FIGURE 2 Uptake of [^{3}H]thymidine into cultured peripheral blood mononuclear cells (PBMC) isolated from healthy adults before and after supplementation with biotin (3.1 μmol/d for 14 d). PBMC were cultured in concanavalin A–containing medium (20 mg/L) for up to 3 d (d 0 = before addition of concanavalin A). At timed intervals, aliquots were collected to measure [^{3}H]thymidine uptake. Data are expressed as means ± SD, n = 5. *P < 0.05 (pre- vs. postsupplementation; Wilcoxon test).

administration of pharmacologic doses of biotin for 14 d does not impair proliferation of PBMC by decreasing cellular pantothenic acid homeostasis.

DISCUSSION

This study investigated the effects of pharmacologic doses of biotin on proliferation and functional capabilities of immune cells in healthy adults. We chose biotin for the following four reasons: *1*) biotin is accumulated in PBMC by an active transport mechanism (Zempleni and Mock 1998), potentially leading to large intracellular concentrations. *2*) Biotin plays a role in gene expression (Borboni et al. 1996, Chauhan and Dakshinamurti 1991, Dakshinamurti and Cheah-Tan 1968, Maeda et al. 1996) and DNA packaging (Hymes et al. 1995a and 1995b), suggesting a potential role in cell proliferation. *3*) Biotin therapy at pharmacologic doses is justified in some biotin-related inborn errors (Wolf and Heard 1991, Wolf 1995); in addition, treatment with pharmacologic doses of biotin coupled to a radionuclide is an emerging practice in radioimmunotherapy (Breitz et al. 1999, Paganelli et al. 1998). Even though no toxicity for biotin has been reported (Mock 1996), the bioavailability of biotin is ~100% even at pharmacologic doses (Zempleni and Mock 1999a). Hence, high intracellular levels of biotin could likely result from biotin supplementation. *4*) Biotin, pantothenic acid and lipoic acid appear to share a common transporter for cellular uptake; all three substrates bind with similar affinity (Prasad et al. 1997, Wang et al. 1999). Hence, if pharmacologic biotin concentrations lead to saturation of transporter-binding sites, cellular uptake of pantothenic acid might be affected. The importance

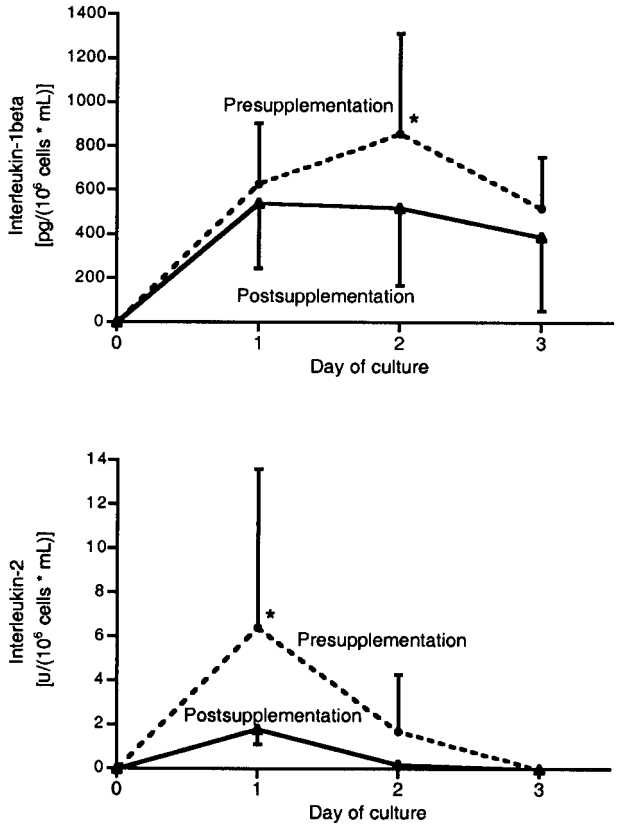

FIGURE 3 Concentrations of interleukin-1β (*upper panel*) and interleukin-2 (*lower panel*) in supernatants from peripheral blood mononuclear cells (PBMC) isolated from healthy adults before and after supplementation with biotin (3.1 μmol/d for 14 d). PBMC were cultured in concanavalin A–containing medium (20 mg/L) for up to 3 d (d 0 = before addition of concanavalin A). At timed intervals, aliquots were collected to measure cytokines in culture supernatants. Data are expressed as means ± SD, n = 5. *P < 0.05 (pre- vs. postsupplementation; Wilcoxon test).

TABLE 1

Peak cytokine concentration in culture supernatants of peripheral blood mononuclear cells (PBMC) from healthy adults before and after biotin supplementation (3.1 μmol/d for 14 d)[1,2]

Cytokine[3]	Unit	Presupplementation	Postsupplementation
IL-1β	ng/(L × 10^6 cells)	859 ± 456	521 ± 352*
IL-2	kU/(L × 10^6 cells)	6.4 ± 7.2	1.8 ± 0.7*
IL-4	ng/(L × 10^6 cells)	7.2 ± 11.0	2.6 ± 2.1
IL-6	ng/(L × 10^6 cells)	305 ± 116	206 ± 105
IFN-γ	kIU/(L × 10^6 cells)	108 ± 122	47 ± 71

[1] Data are expressed as means ± SD, n = 5. *P < 0.05 (pre- vs. post-supplementation; Wilcoxon test).
[2] Peak concentrations were achieved on d 1 of culture for IL-2 and IL-4, and on d 2 of culture for IL-1β, IL-6 and IFN-γ.
[3] Abbreviations used: IL, interleukin; IFN, interferon.

TABLE 2

Cluster of differentiation (CD) subsets in freshly isolated peripheral blood mononuclear cells (PBMC) from healthy adults before and after biotin supplementation (3.1 μmol/d for 14 d)[1,2]

Surface marker	Presupplementation	Postsupplementation
	% of total	
CD3	69.5 ± 7.6	71.4 ± 7.9
CD4	50.5 ± 9.7	51.1 ± 12.1
CD8	17.5 ± 5.9	18.9 ± 6.0
CD19	5.2 ± 3.5	3.2 ± 2.8
CD56	14.8 ± 6.9	13.0 ± 5.8

[1] Data are expressed as means ± SD, n = 5. P > 0.05 for pre- vs. post-supplementation (for all subsets by Wilcoxon test).

of CoA, the coenzyme form of pantothenic acid, for cell proliferation is well documented (McKiernan and Bavister 2000, Plesofsky-Vig 1996, Weimann and Herrmann 1999, Youssef et al. 1996). Given the cases in which large doses of biotin are used legitimately and the potential role of biotin in cell proliferation, investigating the effects of biotin supplements on PBMC proliferation and cytokine synthesis is justified.

The observations of this study provide evidence that administration of pharmacologic doses of biotin for 14 d causes a decrease of thymidine uptake (i.e., proliferation) and a decrease of synthesis of IL-1β and IL-2 by mitogen-stimulated PBMC. The percentages of PBMC cell surface markers were not affected by biotin supplementation.

Why are decreased synthesis rates for IL-1β and IL-2 of potential importance? IL-1β is produced by monocytes; it activates neutrophils and cytotoxic natural killer cells (Klein and Horejsi 1997). The concentration of IL-2 (produced by T_H1 lymphocytes) can determine whether a T cell will proliferate and become an armed effector cell (Klein and Horejsi 1997).

The response of the subjects to biotin was remarkably uniform. Proliferation decreased in five of five subjects and cytokine synthesis decreased in four or five of five subjects for all cytokines tested, providing strong evidence for a biotin effect. Nevertheless, an effect of time on immune function cannot be excluded entirely; this would have required a randomized, placebo-controlled trial.

One mechanistic hypothesis to explain effects of biotin supplementation on PBMC function is that biotin interferes with cellular uptake of pantothenic acid. To test this hypothesis, we measured the uptake of pantothenic acid in the presence of pharmacologic concentrations of biotin. Biotin at a pharmacologic concentration (10 nmol/L) did not significantly affect cellular uptake of pantothenic acid. It is likely that substrate-binding sites of the multivitamin transporter are not saturated even at pharmacologic concentrations of biotin. Indeed, the combined total of biotin, pantothenic acid and lipoic acid in plasma is ~1 μmol/L (Banno et al. 1990, Mock et al. 1995, Teichert and Preiβ 1995), whereas the Michaelis-Menten constant (indicating half-saturation) of the multivitamin transporter is ~2 μmol/L for pantothenic acid (Prasad et al. 1997).

At present, we can only speculate with regard to the mechanism by which biotin supplementation causes decreased proliferation rates and cytokine synthesis. One possible explanation is that biotin affects the expression of genes. For example, biotin increases expression of some genes such as glucokinase (Borboni et al. 1996, Chauhan and Dakshinamurti 1991, Dakshinamurti and Cheah-Tan 1968) and decreases expression of other genes such as ornithine transcarbamylase (Maeda et al. 1996). In addition, biotin may affect gene expression at a post-transcriptional step as described for the asialoglycoprotein receptor in HepG2 cells (Collins et al. 1988). On the basis of these previous studies, we cannot exclude the following possibilities: *1*) the decreased proliferation rates of PBMC and cytokine release after biotin supplementation might be caused by decreased expression of genes encoding cytokines; and *2*) the effects of biotin supplementation on cytokine release might not be specific for cytokines but might also include other proteins.

Previous studies suggested that the biotin concentration in culture medium does not affect the proliferation rate of mitogen-stimulated PBMC (Zempleni, J. and Mock, D. M., unpublished observations). In those previous studies, normal human PBMC were cultured for 3 d in media containing various concentrations of biotin (0–5000 nmol/L) plus con A (20 mg/L). Proliferation rates as judged by [^{3}H]thymidine uptake were not significantly different among groups. Similarly, biotin concentration in culture medium did not affect proliferation rates of con A–stimulated mouse splenocytes (Báez-Saldaña et al. 1998). The mechanisms by which biotin concentration in vitro did not affect proliferation rates of PBMC, whereas biotin supplementation of subjects in vivo decreased proliferation rates of PBMC, are not clear. Possible factors include timing differences between the two studies (exposure time to biotin was 3 d in vitro vs. 14 d in vivo) and environmental milieu (in vitro vs. in vivo).

In this study, the biotin concentration in culture medium was adjusted to 10 nmol/L for postsupplementation PBMC. It is likely that the average concentration of biotin in plasma during the 14-d supplementation was also ~10 nmol/L, based on the following line of reasoning: *1*) 1 h after ingestion of the last biotin supplement, plasma biotin was ~10 nmol/L (see Materials and Methods); *2*) the plasma concentration of biotin decreased to 2.8 nmol/L 24 h after ingestion of the last biotin supplement (see Results); and *3*) peak concentrations of biotin in plasma occurred within 1 h of supplementation and exceeded 10 nmol/L, on the basis of the following evidence from pharmacokinetic studies. The absorption half-life of biotin in humans is 0.3 h (Bitsch et al. 1989), suggesting that the major fraction (~88%) of bioavailable biotin is absorbed within <1 h. A significant fraction of absorbed biotin is excreted within 1 h postsupplementation whenever excretion of biotin is a rapid process. Indeed, studies in humans and animals have provided evidence that biotin is excreted rapidly. The half-lives of biotin in plasma from cattle and pigs during the initial fast phase of disposition is ~0.5 h (Frigg et al. 1994) and 0.1 h (Wang et al. 1998), respectively. This is similar to the half-life of biotin in human PBMC (0.2 h) during this fast phase of elimination (Zempleni and Mock 1999b).

In summary, this study provides evidence of an inhibitory effect of pharmacologic doses of biotin on PBMC proliferation and cytokine release. The mechanism is currently not known and the physiologic significance, if any, remains unclear.

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