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Am J Clin Nutr. Author manuscript; available in PMC 2006 April 28.

Published in final edited form as:
Am J Clin Nutr. 2004 October ; 80(4): 932–935.

Smoking accelerates biotin catabolism in women[1,2,3]

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Abstract

Background: Smoking accelerates the degradation of many nutrients, including lipids, antioxidants, and certain B vitamins. Accelerated biotin catabolism is of concern in women because marginal biotin deficiency is teratogenic in mammals.

Objective: The objective was to assess the effect of smoking on the biotin status of women.

Design: A preliminary study of 7 women and 3 men examined the urinary concentrations of biotin and its metabolites biotin sulfoxide and bisnorbiotin in smokers. The interpretation of the results of this study was limited by the lack of a contemporaneous control group; consequently, we conducted a cohort-controlled study. Smoking women ($n = 8$) and nonsmoking control subjects ($n = 15$) provided 24-h urine samples; excretion rates of biotin, the biotin metabolites, and 3-hydroxyisovaleric acid were determined. Increased urinary excretion of 3-hydroxyisovaleric acid, which reflects a reduced activity of the biotin-dependent enzyme 3-methylcrotonyl-Co A carboxylase, is a sensitive indicator of biotin depletion at the tissue level.

Results: Compared with control subjects from previous studies, the smoking women in the preliminary study excreted significantly less urinary biotin ($P = 0.02$). Moreover, the ratio of urinary biotin sulfoxide to biotin increased ($P = 0.04$) in these women. In the cohort-controlled study, the urinary excretion of biotin decreased by 30% ($P = 0.04$), and the ratios of urinary bisnorbiotin and biotin sulfoxide to biotin increased significantly, which indicated accelerated catabolism in smokers. Moreover, the urinary excretion of 3-hydroxyisovaleric acid was greater in the smokers than in the control subjects ($P = 0.04$), which indicated biotin depletion in the smokers at the tissue level.

Conclusion: These data provide evidence of accelerated biotin metabolism in smoking women, which results in marginal biotin deficiency.

Keywords

Biotin; biotin catabolism; smoking; 3-hydroxy-isovaleric acid; bisnorbiotin; biotin sulfoxide

INTRODUCTION

Biotin is a water-soluble vitamin generally classified in the B vitamin complex. Using a sensitive and chemically specific assay for biotin and its metabolites, we observed that the ratio of the biotin catabolites bisnorbiotin and biotin sulfoxide to biotin remains constant or decreases with progressive biotin deficiency; this probably reflects appropriate down-regulation of catabolism and thus conservation of biotin (1). However, in certain clinical circumstances, biotin catabolism is accelerated despite biotin deficiency. Indeed, this

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[2]Supported by an Arkansas Tobacco Settlement Funds grant (to WMS and DMM), grant RO1 DK36823 from NIH/DDK (to DMM), and grant MO RR 14288 from NIH/NCRR.

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accelerated breakdown to inactive metabolites likely contributes to marginal biotin deficiency in those situations, eg, therapy with anticonvulsants (2) and pregnancy (3,4). The increased degradation rates seen in pregnancy are sufficiently rapid in comparison with biotin intake that degradation could be the major cause of biotin deficiency (4).

In the 2 studies reported here, we examined the effect of smoking on biotin status using validated indexes of biotin status, ie, the urinary excretion of biotin and biotin sulfoxide (5) and of 3-hydroxyisovaleric acid (6). Increased urinary excretion of the leucine catabolite 3-hydroxyisovaleric acid results from reduced activity of the biotin-dependent enzyme 3-methylcrotonyl-Co A carboxylase; increased 3-hydroxyisovaleric acid excretion is a sensitive indicator of biotin deficiency at the tissue level (6). In the first study, we examined whether biotin status is affected by smoking as judged by comparison with noncontemporaneous control subjects. In the second study, a case-control design was used to test the following hypothesis: Smoking accelerates biotin metabolism in women, which results in reduced biotin status.

SUBJECTS AND METHODS

The human research protocols were approved by the University of Arkansas for Medical Science Committee on Human Research. Informed consent was obtained from each subject.

Study 1

Ten untimed urine samples were obtained from healthy adult smokers: 7 women (mean age: 38 y; range: 25-45 y) and 3 men (mean age: 40 y; range: 35-45 y). Subjects were interviewed regarding tobacco, vitamin, and prescription drug usage. Biotin and biotin metabolites were determined with an HPLC-avidin-binding assay (5). Biotin concentration was normalized by creatinine to approximate excretion rates; we previously showed that the ratio of biotin to creatinine is a valid indicator of marginal biotin status (7). Although statistically significant sex differences have not been shown, for interpretation, smokers were sex-matched to existing nonsmoking control subjects from previous studies (1,8,9).

Study 2

To test the hypothesis that smoking accelerates biotin metabolism in women, resulting in diminished biotin status, the subjects were matched for sex, age, and factors known to affect biotin status; biotin catabolism and biotin status were assessed by urinary excretion rates of biotin, biotin sulfoxide, bisnorbiotin, and 3-hydroxyisovaleric acid.

This study examined urinary indicators of biotin status in smoking women and nonsmoking women who served as control subjects. These healthy, premenopausal adult women were between the ages of 20 and 50 y (mean age: 31.8 y). Racial and ethnic heritage were not criteria for selection or stratification; no data indicate that these characteristics affect biotin status. Potential subjects were interviewed regarding smoking status, vitamin usage, and prescription drug usage before sample collection. Women taking biotin-containing vitamins or certain prescription medications known to alter biotin metabolism, including hormones, were excluded from the study. Because we have shown that pregnancy per se affects biotin status (4), pregnant women were also excluded. Subjects were designated as smoking (n = 8) or nonsmoking controls (n = 15) on the basis of clinical history. Each subject provided a 24-h urine sample. Two subjects whose urinary excretion of biotin was greater than the upper limit of a normal reference population (1,8,10) were excluded. Increased biotin excretion was taken as evidence of biotin supplementation not identified by a medical history.

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Urine processing and storage

Particulate-free urine was obtained by centrifugation at 1500 × *g* for 15 min; the supernatant fluid was stored at -20 ° C. Samples were thawed at 37 ° C for 30 min before use.

Chemicals and reagents

Biotin (*d*-biotin) was obtained from Sigma Chemical Company (St Louis).D-8, 9-H-Biotin (Perkin-Elmer, New England Nuclear, Boston) and D-carbonyl-C-biotin (Amersham, Arlington, IL) were used to synthesize [H]biotin sulfoxide and [C]bisnorbiotin as previously reported (10,11). the purity of biotin was determined by HPLC as described previously (5) and was ≥95% for all radiolabeled compounds used in this study.

Creatinine

Urinary concentrations of creatinine were determined by the picric acid method of Jaffe (12). Excretion rates were expressed as the concentration ratios of total avidin-binding substances, biotin, bisnorbiotin, and biotin sulfoxide to creatinine.

HPLC-avidin-binding assay for biotin and biotin metabolites

Biotin, bisnorbiotin and biotin sulfoxide were determined by avidin-binding assay against authentic standards after HPLC separation as previously described (5). By separating biotin from its inactive metabolites, each can be accurately quantitated (5). The detection limit of the HPLC-avidin-binding assay for biotin is ≈10 pmol/L. Chromatographic fractions were initially assayed in triplicate for avidin-binding activity. Previous studies have validated the HPLC-avidin-binding assay for quantitating total avidin-binding substances, biotin, and the biotin metabolites bisnorbiotin and biotin sulfoxide (5).

3-Hydroxyisovaleric acid

Urinary concentrations of 3-hydroxyisovaleric acid were determined by gas chromatography-mass spectrophotometry as described previously (13). To obtain high precision and accuracy, authentic unlabeled 3-hydroxyisovaleric acid and deuterated 3-hydroxyisovaleric acid were used as external and internal standards, respectively (13). Excretion rates were expressed as μmol/24-h urine volume.

Statistical analysis

The significance of differences between means was tested by an unpaired, one-tailed *t* test. $P < 0.05$ was chosen as the level of significance. All statistical analyses were conducted with STATVIEW 5.0 for Macintosh (SAS Institute Inc, Cary, NC).

RESULTS

Study 1

In the first study, urinary biotin excretion for the 7 women in the smoking group was lower than that in the 5 nonsmoking control women ($P = 0.02$; Table 1). Despite this evidence of biotin depletion, the ratio of urinary biotin sulfoxide to biotin was higher in smoking women ($P = 0.04$), which suggested accelerated catabolism. Although the ratio of urinary bisnorbiotin to biotin tended to be higher in the smoking women (Table 1), the difference was not statistically significant ($P = 0.11$). The urinary excretion of biotin and biotin metabolites in the 3 smoking men was not significantly different from that of the 3 nonsmoking control subjects (Table 1).

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Study 2

The data from study 1 are consistent with biotin depletion and accelerated biotin catabolism. However, interpretation was limited because subject numbers were small and the study lacked a contemporaneous control group. Consequently, we conducted a second case-control study that incorporated a contemporaneous control group. Because study 1 detected effects on biotin metabolism and biotin status in women, study 2 included only women.

Study 2 detected similar effects on biotin metabolism and status (Table 2). The urinary excretion of biotin was ≈30% lower in smoking women than in nonsmoking control women ($P = 0.04$). The ratios of bisnorbiotin and biotin sulfoxide to biotin were significantly higher ($P = 0.003$ and $P = 0.02$, respectively) in smokers. The urinary excretion of 3-hydroxyisovaleric acid was significantly higher in the smokers than in the control subjects ($P = 0.04$; Table 2).

DISCUSSION

Smoking has been shown to accelerate the degradation of a variety of nutrients including lipids, antioxidants, and some B vitamins. Smoking has been shown to alter lipid metabolism (14, 15) and impair insulin action (15,16), probably because of the concomitant increase in levels of counter-regulatory hormones such as catecholamines and growth hormone. Smoking has also been associated with a diminished status of riboflavin (17), vitamin B-12 (17,18), ascorbic acid (17,19), α-tocopherol (17,18), folic acid (17,18), carotenoid (20,21), retinol (17), vitamin B-6 (17), and biotin (17). However, the mechanisms by which smoking alters vitamin status are unknown.

In healthy subjects made marginally biotin deficient by egg-white feeding, urinary excretion of biotin and its 2 major metabolites, bisnorbiotin and biotin sulfoxide, are decreased, which indicates the down-regulation of biotin catabolism (22). Data from the 2 studies presented here provide evidence that biotin catabolism is accelerated in smoking women. The demonstration of increased excretion of 3-hydroxyisovaleric acid despite the deceleration that would normally accompany biotin depletion indicates that biotin status is decreased by this accelerated conversion of biotin to the inactive metabolites, biotin sulfoxide and bisnorbiotin.

Methylcrotonyl-CoA carboxylase catalyzes an essential step in the degradation of the branch-chained amino acid leucine. Reduced activity of this biotin-dependent enzyme leads to the metabolism of its substrate 3-methylcrotonyl CoA by an alternate pathway to form 3-hydroxyisovaleric acid, 3-methylcrotonylglycine, or both (23). Thus, the increased excretion of 3-hydroxyisovaleric acid observed in the current study reflects marginal biotin depletion at the tissue level in smoking women.

Our results appear to differ from those of Benton et al (17), who reported no significant effect of smoking on plasma biotin concentrations in women; Benton et al (17) did detect reduced plasma biotin concentrations in men who smoked. There are several possible explanations for the apparent conflict. Plasma biotin is not a particularly sensitive index of biotin status (1). Thus, the inability of Benton et al (17) to detect differences in plasma biotin in women may reflect the insensitivity of this method. Our failure to detect effects of smoking on the biotin status of men likely reflects the small number (3) of male subjects in study 1. In contrast, the accurate determination of the validated biotin indexes (urinary biotin, bisnorbiotin, biotin sulfoxide, and 3-hydroxyisovaleric acid) in study 2 likely allowed us to detect differences between smoking women and nonsmoking control women that Benton et al (17) were unable to detect. Biotin metabolites constitute ≈40% of the plasma biotin fraction (6). If plasma biotin metabolites had been increased in the smoking women as they were in the urine of women in study 1 and study 2, the biotin assay used by Benton et al (17) would have failed to detect decreased biotin. The microbiological assay used by Benton et al (17) to quantitate biotin

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content is based on the growth of *Lactobacillus plantarum*. Bioassays characteristically suffer from interference by unrelated substances and from the lack of specificity for biotin compared with its metabolites (24). Because the biotin assay used by Benton et al (17) did not discriminate biotin from its metabolites, we speculated that plasma biotin concentrations may have been overestimated and thus failed to detect reduced biotin concentrations.

Although the degree of biotin deficiency detected in smoking women was marginal, marginal biotin deficiency is of concern. Marginal biotin deficiency is teratogenic in some animal species (25-28). Tobacco use has also been linked to teratogenesis (29-31). A meta-analysis of studies published from 1966 to 1996 indicated an increased risk of a child with both a cleft lip and palate or a cleft palate being born to mothers who smoke during their first trimester of pregnancy (32). The authors acknowledged that the meta-analysis did not address whether smoking is a direct teratogen or whether it is a marker for exposure to some other environmental risk factor, but point out that any such confounder must be a potent cause of nonsyndromic oral cleft in its own right (32). We speculate that the effect of smoking on biotin status at conception, on biotin intake during pregnancy (especially in the first trimester), or on individual responses to the acceleration of biotin breakdown by gestational hormones might be such an indirect mechanism.

Interpretation of this study is limited. Theoretically, the effects of smoking on biotin status observed in the current study may be due to factors associated with smoking rather than to smoking per se. Such factors could include reduced dietary intake of biotin, unreported consumption of medications that accelerated biotin catabolism, or occult intake of alcohol (33,34) or other xenobiotics that increase biotin catabolism (35).

These studies detected a significant effect of smoking on biotin metabolism. Although the degree of biotin deficiency was marginal, marginal biotin deficiency is of concern because of the link between biotin deficiency and teratogenesis in animal models (25,26,28). Accelerated biotin metabolism in smoking women could predispose these women to marginal biotin deficiency during pregnancy, especially if smoking and pregnancy act additively to accelerate biotin catabolism. Additional research using these accurate and sensitive indexes is needed. Although care must be taken in applying the results of such small studies to general recommendations, the results of this study could reasonably be added to the substantial body of evidence that smoking should be avoided during pregnancy.

Acknowledgements

WMS designed the study and drafted the manuscript. DMM helped with the study design and the interpretation of the results. AMT and SLS recruited and interviewed the subjects. None of the authors had any personal or financial conflicts of interest with regard to the study.

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TABLE 1

Urinary biotin and its metabolites in smokers and nonsmokers[1]

	Women			Men		
	Smokers (n = 7)	**Nonsmokers (n = 5)**	**P[2]**	**Smokers (n = 3)**	**Nonsmokers (n = 3)**	**P[2]**
Biotin (pmol/mg creatinine)	16 ± 8[3]	39 ± 26	0.02	21 ± 9	22 ± 2	0.40
BSO (pmol/mg creatinine)	8 ± 2	7 ± 3	0.29	10 ± 3	7 ± 2	0.07
BNB (pmol/mg creatinine)	14 ± 5	20 ± 10	0.10	15 ± 5	18 ± 5	0.20
BSO:biotin[4]	0.62 ± 0.4	0.26 ± 0.2	0.04	0.54 ± 0.2	0.30 ± 0.1	0.05
BNB:biotin[4]	1.2 ± 0.8	0.68 ± 0.4	0.11	0.74 ± 0.1	0.83 ± 0.2	0.25

[1] Smokers were sex-matched to existing nonsmoking control subjects from previous studies (1, 8, 9). BSO, biotin sulfoxide; BNB, bisnorbiotin.

[2] Unpaired, one-tailed t test.

[3] $\bar{x}$ ± SD (all such values).

[4] Obtained by dividing the metabolite values (BSO and BNB) by urinary biotin.

TABLE 2

Urinary biotin and its metabolites in smoking and nonsmoking women[1]

	Smokers (n = 7)	Nonsmokers (n = 14)	P[2]
Biotin (pmol/mg creatinine)	42 ± 26[3]	59 ± 16	0.04
BSO (pmol/mg creatinine)	11 ± 4	12 ± 6	0.58
BNB (pmol/mg creatinine)	29 ± 9	24 ± 13	0.20
BSO:biotin[4]	0.31 ± 0.1	0.21 ± 0.1	0.02
BNB:biotin[4]	0.89 ± 0.5	0.41 ± 0.2	0.003
3-HIA (μmol/24 h)	92 ± 25	70 ± 27	0.04

[1] BSO, biotin sulfoxide; BNB, bisnorbiotin; 3-HIA, 3-hydroxy-isovaleric acid.

[2] Unpaired, one-tailed *t* test.

[3] $\bar{x}$ ± SD (all such values).

[4] Obtained by dividing the metabolite values (BSO and BNB) by urinary biotin.

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