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High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference

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Abstract

Background: High-dose biotin therapy is beneficial in progressive multiple sclerosis (MS) and is expected to be adopted by a large number of patients. Biotin therapy leads to analytical interference in many immunoassays that utilize streptavidin-biotin capture techniques, yielding skewed results that can mimic various endocrine disorders. We aimed at exploring this interference, to be able to remove biotin and avoid misleading results.
Methods: We measured free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), parathyroid homrone (PTH), 25-hydroxyvitamin D (25OHD), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, C-peptide, cortisol (Roche Diagnostics assays), biotin and its main metabolites (liquid chromatography tandem mass spectrometry) in 23 plasmas from MS patients and healthy volunteers receiving high-dose biotin, and in 39 biotin-unsupplemented patients, before and after a simple procedure (designated N5) designed to remove biotin by means of streptavidin-coated microparticles. We also assayed fT4, TSH and PTH in the 23 high-biotin plasmas using assays not employing streptavidin-biotin binding.
Results: The biotin concentration ranged from 31.7 to 1160 µg/L in the 23 high-biotin plasmas samples. After the N5 protocol, the biotin concentration was below the detection limit in all but two samples (8.3 and 27.6 µg/L). Most hormones results were abnormal, but normalized after N5. All results with the alternative methods were normal except two slight PTH elevations. In the 39 biotin-unsupplemented patients, the N5 protocol did not affect the results for any of the hormones, apart from an 8.4% decrease in PTH.
Conclusions: We confirm that most streptavidin-biotin hormone immunoassays are affected by high biotin concentrations, leading to a risk of misdiagnosis. Our simple neutralization method efficiently suppresses biotin interference.

Keywords: biotin; hyperthyroidism; immunoassay; interference; multiple sclerosis.

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Introduction

Biotin is a water-soluble vitamin, that functions as a coenzyme for carboxyl transfer by five carboxylases involved in many metabolic processes. Recommended intake for adults is 30–75 µg/day [1]. Beneficial effects of high-dose biotin have been recently demonstrated in progressive multiple sclerosis (MS) [2, 3]. MS is the most common disabling neurological disease in young adults. While some approved treatments aiming to reduce the number of relapses in relapsing-remitting MS exist, there are no efficient treatment options in progressive MS. The above-mentioned encouraging results for high-dose biotin in patients with progressive MS [2, 3] has already led to spreading adoption of high dose biotin therapy in some countries like France, where this prescription is legally possible under early access program. Although not yet recommended and not controlled by the Food and Drug Administration (FDA) in the USA, it was recently reported that, during 1 month in early 2016, a local compounding pharmacy of the Boston area dispensed 370 1-month prescriptions of high dose biotin [4]. Once FDA approved, high doses biotin therapy is expected to become widespread therapy in patients with progressive MS. In

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addition, high biotin doses are also prescribed in several rare inherited metabolic diseases (biotinidase deficiency: 5–10 mg/day; holocarboxylase synthetase deficiency: 30–40 mg/day; biotin-thiamine-responsive basal ganglia disease: 100–300 mg/day). Moreover, supraphysiological doses of biotin (up to 30 mg/day) are now widely used for self-medication aimed at reducing hair loss or improving nail or skin condition.

These supraphysiologic biotin intakes have produced emerging problems with immunoassays that utilize the streptavidin-biotin interaction. These assays account for about half of all current immunoassays. The artifact is falsely increased hormone concentrations when measured with competitive assays (e.g. T3, T4, steroid hormones, 25OH vitamin D), and falsely decreased hormones concentrations when measured with sandwich assays (e.g. glycoprotein regulating hormones) [5]. These opposite effects may lead to results mimicking endocrine disorders such as hyperthyroidism [4, 6–11] with apparently elevated free thyroxine (fT4) and free triiodothyronine (fT3) concentrations and apparently lowered thyroid-stimulating hormone (TSH) concentration. Anti-TSH receptor antibody titers may also be falsely elevated, suggesting Graves' disease. This interference has led to cases of inappropriate therapy [10]. Vitamin D intoxication may also be erroneously evoked (associating seemingly high 25OH D level with low parathyroid hormone [PTH] level). There is a concern that the results of many other hormonal and non-hormonal immunoassays (troponin, natriuretic peptides, ferritin, tumor markers, therapeutic drug assays, serology tests) may be misleading because of biotin interference.

The first aim of the study was to examine the extent of assay interference in subjects receiving moderate to very high doses of biotin, in order to study the relationship between the daily biotin dose, the plasma biotin concentration, and the magnitude of assay errors. We measured a panel of hormonal parameters frequently used to monitor MS, as well as other hormones, using assays known to suffer from biotin interference. The second goal of our work was to validate a method to remove biotin and thereby avoid this interference.

Materials and methods

Patients and controls subjects

Twenty-seven samples from 20 subjects receiving various high dose of biotin were studied, as follows:

- Nine MS patients receiving 300 mg of biotin daily (100 mg ter in die [t.i.d.]) for 12 months (study MS-ON, EudraCT no:2013-002112-27). The last biotin intake was 1 h 15 min to 14 h 50 min before sampling (referred to hereafter as P1–P9).
- Eight healthy controls participating in a pharmacokinetic study of biotin therapy (MD1003-PK, EudraCT no: 2014-000766-22) received a single biotin dose of 100, 200 or 300 mg. They were sampled once or twice, within 1–2 h after biotin administration. Eleven samples were collected from these eight controls (referred to hereafter as T1–T11).
- Three of the authors (MP, CH, JCS) volunteered to receive moderately high biotin doses (15 or 30 mg; 5 or 10 mg t.i.d., respectively) for 3 days, and were sampled 2–3 h after the morning dose (referred to hereafter as V1–V3). Two of these three subjects were also sampled before biotin exposure and 48 h after the last biotin dose.

To validate the neutralization of the interference (see below) we also tested samples from 39 biotin-unsupplemented patients with various hormonal disorders recruited in our endocrinology unit, in order to determine whether or not the neutralization process modified their assay results (thyroid function, n = 12; cortisol, n = 6; PTH and 25-hydroxyvitamin D [25OHD], n = 11; FSH, LH and prolactin, n = 10).

All the subjects gave their informed consent to sampling and assays, and all procedures complied with the Helsinki declaration.

Hormone immunoassays

All blood samples were collected in lithium heparin tubes after an overnight fast and promptly centrifuged. Plasma was stored at – 40 °C until assayed. fT3, fT4, TSH, PTH, 25OHD, cortisol, follice-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and C-peptide concentrations were measured in the high-biotin samples and in the 39 biotin-unexposed samples by using Cobas e411 assays (Roche Diagnostics, Meylan, France), before and after the biotin neutralization protocol (see below), in the same run. The reference values adopted were those indicated by Roche, excepting for 25OHD, for which the recommended values adopted were: 30–60 ng/mL.

Concentrations of fT4, TSH, and PTH were also measured in the high-biotin samples by using alternative methods not utilizing streptavidin-biotin interaction: fT4 and PTH in the Liaison XL® platform (Diasorin, Saluggia, Italy) and TSH in the Access2® platform (Beckman Coulter, Brea, CA, USA). Samples from seven MS patients, three healthy controls and volunteers V1-V3 were tested with all three alternative assays. At least one thyroid parameter was tested (fT4 and/or TSH) except in P9 and PTH was assayed except in T10.

Measurement of biotin and biotin metabolites

Biotin (either free or non-covalently bound to proteins) and biotin main metabolites (bisnorbiotin and biotin sulfoxide) were measured by a liquid chromatography tandem mass spectrometry method (LC-MS-MS), before and after the neutralization procedure. The measuring range is 5.0–3000 μg/L for biotin, 5.0–2000 μg/L for biotin metabolites. The lower limit of quantification (LOQ) is 5 μg/L and the interassay CV is 5%–10% (liquid chromatography separation on a C18 phase column, Shimadzu LC System [Noisiel, France]; Mass spectrometer, API5500 in electrospray positive mode, Sciex [Villebon sur Yvette, France]). The reference range in healthy controls without biotin supplementation is < 5 μg/L.

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Biotin neutralization method

We tested the adsorption of biotin to magnetic microparticles coated with streptavidin. This reagent is included in the Cobas® assays kits supplied by Roche and is also available from Sigma-Aldrich (Saint Louis, MO, USA, ref: 11641778001). The streptavidin microparticles suspension has a binding capacity for free biotin of 1.34 µmol/L, and the streptavidin concentration is 0.72 mg/mL in Hepes–bovine serum albumin buffer, pH 7.4.

We first calculated the optimal ratio of plasma to streptavidin reagent. In a recent pharmacokinetic study of very high biotin doses, the maximum biotin plasma concentration was observed 1.5 h after a single 300-mg dose. It was 823 ± 303 µg/L (mean ± SD) corresponding to 3374 ± 1243 nmol/L of biotin [12]. The mean + 2 SD is therefore 5860 nmol/L. Assuming a biotin binding capacity of the streptavidin reagent of 1340 nmol/L, a ratio of five streptavidin reagent volumes to one plasma volume would be appropriate, because the binding capacity of the collected streptavidin microparticles would be multiplied by five, corresponding to approximately 6700 nmol/L of biotin (1340 × 5), a concentration exceeding the reported maximum concentration. This 5 : 1 ratio was used in a case-report of biotin interference in a patient with end-stage renal disease (ESRD) [13]. The following procedure was adopted: centrifuge five volumes of streptavidin reagent for 10 min (3000 *g*); discard carefully the supernatant; put one volume of serum/plasma sample on the microparticles pellet and let 1 h shaking at room temperature (100 rpm); centrifuge for 10 min (3000 *g*); collect carefully the supernatant and assay. The neutralization "N5" procedure is depicted in Figure 1 as a specification sheet.

We also tested various amounts of streptavidin microparticles in one patient and one healthy control, both of whom were receiving high biotin dose.

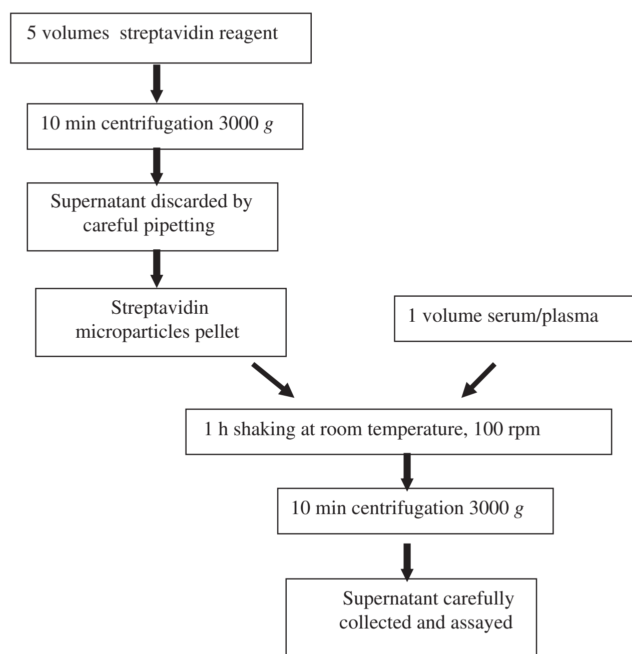

Figure 1: Biotin removal procedure (neutralization protocol N5).

Statistics

Data are presented as raw values. Correlations were assessed with the spearman rank test. Comparison of paired values (e.g. before vs. after neutralization procedure) was assessed with the Wilcoxon test. Unpaired data (e.g. biotin concentrations in patients receiving 100 mg biotin t.i.d. vs. those receiving 300 mg in one dose) were compared with the Mann-Whitney test. A p-Value < 0.05 was considered significant.

Results

Biotin and biotin metabolite concentrations in patients and controls

Table 1 shows the daily biotin doses and the observed biotin and biotin metabolite concentrations. After biotin supplementation, biotin concentrations ranged from 7 µg/L (V1, 48 h after 15 mg biotin) to 1160 µg/L in T5, after daily dose of 300 mg. The highest biotin concentration was observed in a healthy control who received a single dose of 300 mg and was sampled 1 h later. The highest biotin concentration recorded in a MS patient (P4) was 694 µg/L. This sample was obtained 105 min after the intake of the recommended dosage of 100 mg t.i.d. The metabolite concentrations were significantly higher in patients who had been receiving 100 mg t.i.d. for months than in controls who received a single 300-mg dose ($p < 0.001$ for bisnorbiotin, $p < 0.05$ for biotin sulfoxide), whereas biotin concentrations were higher in the controls ($p < 0.001$). In two patients (P5 and P7), the bisnorbiotin concentration was similar to the biotin concentration. Thus, the type of intake (acute vs. chronic) and the time elapsed since the last intake are major determinants of biotin and metabolite concentrations.

Hormone concentrations before and after the neutralization procedure in patients and healthy subjects receiving biotin

Table 2 shows the results of the 10 hormone assays before and after biotin neutralization with the N5 protocol in samples from the patients and healthy subjects. As expected, biotin had a major impact (>10%) on hormone levels at biotin concentrations of 30 µg/L or more. There was a strong relationship between the plasma biotin concentration and the degree of interference (evaluated as the % of change after neutralization) (Table 3). The susceptibility of the assays to the biotin interference was highly variable: prolactin assay

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Table 1: Biotin, biotin metabolite plasma concentrations (µg/L), and biotin daily dose (mg/day) in the 27 samples from MS patients (P1–P9), healthy controls in a pharmacokinetic study (T1–T11), and healthy volunteers (V1–V3). Concentrations are shown before and after neutralization using streptavidin microparticles (N5 protocol).

Samples	Biotin daily dose	Time after last intake, h:min	Biotin Before (after)	Bisnorbiotin Before (after)	Biotin sulfoxide Before (after)
P1	3×100	5:10	507 (<LOQ)	149 (108)	17 (<LOQ)
P2	3×100	13:20	690 (<LOQ)	87.7 (68.8)	25.6 (<LOQ)
P3	3×100	1:15	487 (<LOQ)	221 (196)	20 (<LOQ)
P4	3×100	1:45	694 (<LOQ)	233 (215)	30.3 (<LOQ)
P5	3×100	12:25	180 (<LOQ)	188 (49.3)	17.8 (<LOQ)
P6	3×100	13:29	363 (<LOQ)	123 (106)	28 (<LOQ)
P7	3×100	11:08	169 (<LOQ)	152 (87.9)	12.5 (<LOQ)
P8	3×100	14:50	233 (<LOQ)	106 (52.3)	17.9 (<LOQ)
P9	3×100	13:14	176 (<LOQ)	85.2 (46.7)	12.8 (<LOQ)
T1	200	1:30	827 (<LOQ)	71.8 (45.5)	16.5 (<LOQ)
T2	200	2:00	758 (<LOQ)	108 (28.8)	17.0 (<LOQ)
T3	200	2:00	1000 (<LOQ)	62.2 (50.6)	17.2 (<LOQ)
T4	300	1:30	1110 (27.6)	59.6 (56.1)	13.0 (<LOQ)
T5	300	1:00	1160 (<LOQ)	32.7 (25.2)	16.3 (<LOQ)
T6	300	1:00	669 (<LOQ)	30.7 (24.3)	10.1 (<LOQ)
T7	300	1:30	1010 (<LOQ)	49.2 (40.1)	17.2 (<LOQ)
T8	300	2:00	959 (<LOQ)	71.4 (58.2)	20.8 (<LOQ)
T9	100	2:00	407 (<LOQ)	18.7 (10.2)	6.34 (<LOQ)
T10	300	1:00	914 (8.3)	20.4 (17.9)	15.6 (<LOQ)
T11	200	2:00	814 (<LOQ)	45.0 (38.2)	12.1 (<LOQ)
V1	0	/	<LOQ	<LOQ	<LOQ
V1	3×5	2:00	43.9 (<LOQ)	15.1 (8.2)	<LOQ (<LOQ)
V1	3×5	48 h	7.1	<LOQ	<LOQ
V2	0	/	<LOQ	<LOQ	<LOQ
V2	3×10	2:00	56.8 (<LOQ)	14.6 (8.2)	<LOQ
V2	3×10	48 h	10.6	<LOQ	<LOQ
V3	3×5	3:30	31.7 (<LOQ)	10.5 (5.8)	<LOQ (<LOQ)

<LOQ: below the lower limit of quantification.

was least affected (no misleading result), while 25OHD assay was most affected. Clinically misleading results were systematically observed for 25OHD and PTH (biotin concentration ≥169 µg/L), FSH, LH and TSH (≥180 µg/L), fT4 (≥233 µg/L), fT3 and cortisol (≥363 µg/L), and C-peptide (≥487 µg/L). Clinically misleading results were obtained for cortisol in 43% of samples (falsely elevated, or falsely normal because of artifactual overestimation. The alternative assays for fT4, TSH, and PTH gave results within their reference intervals, except for two PTH results slightly above the reference range with the Liaison method, and at the upper limit of the reference range with the Roche assay after neutralization (Supplemental Material, Table 1).

Validation of the neutralization method

The N5 neutralization protocol effectively removed biotin, based on the observation that all MS patients had a biotin concentration below the LOQ after this pre-treatment (Table 1). Only two healthy controls receiving 300 mg in a single intake who were sampled within 2 h had detectable biotin concentrations after N5 neutralization (27.6 and 8.3 µg/L, samples T4 and T10, respectively, see Table 1).

To validate the optimal plasma to streptavidin reagent ratio, various amounts of streptavidin microparticles were tested in one sample from a patient and one sample from a healthy control receiving high-dose biotin. The 25OHD assay being the most susceptible to biotin interference, was used to evaluate the efficacy of the neutralization process. The following ratios of streptavidin reagent to plasma were studied: two, three, four, five and ten streptavidin reagent volumes per volume of plasma (protocols N2–N10). Table 4 shows the neutralization results. While protocol N3 neutralized a biotin concentration up to 694 µg/L (P4), protocol N5 was better suited for the higher biotin concentrations (T10).

Lastly, we verified that the hormonal profile was unchanged by the neutralization process in biotin-unsupplemented patients. The N5 neutralization protocol was

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Table 2: Hormone results observed before and after biotin neutralization (protocol N5, shaded in grey) in the 23 samples. Data listed according to biotin plasma concentrations.

Samples	Biotin	FT3	FT4	TSH	PTH	25OH D	FSH	LH	Prolactine	Cortisol	C peptide
Units	µg/L	pmol/L	pmol/L	mUI/L	ng/L	µg/L	UI/L	UI/L	µg/L	µg/dL	nmol/L
Reference values		3.1–6.8	12–22	0.27–4.2	15–65	30–60				6.2–19	0.37–1.47
V3	31.7	4.8	17.9	2.42	58.0	52	3.7	5.2	6.4	11.5	0.96
	<LOQ	4.6	16.9	2.87	56.6	45	3.7	5.4	6.3	11.1	0.94
V1[a]	43.9	5.0	19.3	1.16	40.1	46	117	62.3	16.6	10.8	0.98
	<LOQ	4.4	17.6	1.54	41.9	35	132	68	16.4	10.3	0.98
V2	56.8	5.4	20.3	0.76	48.9	41	3.0	4.4	10.4	11.1	1.07
	<LOQ	4.8	18.5	1.07	54.5	33	3.4	4.8	10.2	10.8	1.11
P7	169	5.7	16.7	0.66	**13.8**	**113[b]**	**0.99**	5.75	**30.6**	15.9	**0.34**
	<LOQ	4.9	13.0	3.90	26.3	**>70**	2.7	8.9	**32.6**	13.6	0.42
P9	176	6.5	18.9	0.41	20.6	**90.7[b]**	2.09	2.25	11.3	18.2	0.61
	<LOQ	5.8	15.3	2.40	39.9	54.5	5.8	3.4	11.3	16.1	0.74
P5[a]	180	5.21	21.4	**0.098**	**10.9**	**75.6[b]**	**11.1**	**11.2**	19.0	13.5	0.41
	<LOQ	4.6	15.8	1.05	28.7	**20.7**	41.6	19.9	20.3	11.8	0.54
P8	233	5.6	**25.6**	**0.062**	**14.5**	**115[b]**	**0.84**	**0.96**	11.8	11.5	0.57
	<LOQ	4.8	17.7	1.46	34.7	48.4	3.76	1.74	12.3	10.1	0.80
P6[a]	363	**7.75**	**49.5**	**0.037**	**7.67**	**>140[b]**	**13.8**	**16.5**	9.8	**22.0**	**0.29**
	<LOQ	4.5	18.6	1.87	20.3	**68.5**	132	59.2	11.8	17.9	0.67
T9	407	**8.68**	**56.4**	**0.02**	**7.98**	**>140[b]**	**0.22**	**0.56**	**24.2**	**20.7**	0.43
	<LOQ	4.7	16.3	1.21	22.5	**26.5**	2.0	2.4	**28.2**	17.6	1.44
P3[a]	487	**13.0**	**95.8**	**0.016**	**7.31**	**>140[b]**	**3.5**	**3.2**	4.4	11.4	**0.11**
	<LOQ	5.2	14.6	1.49	30.5	**65.1**	48.9	24.3	6.9	7.5	0.491
P1	507	**11.7**	**>100**	**0.008**	**6.76**	**>140[b]**	**0.40**	**0.84**	2.1	8.8	**0.14**
	<LOQ	4.9	17.6	0.83	22.9	**17.3**	4.9	5.4	3.0	6.0	0.62
T6	669	**13.28**	**>100**	**0.021**	**8.63**	**>140[b]**	**0.28**	**0.47**	9.4	17.7	**0.09**
	<LOQ	5.1	16.4	2.29	19.0	40.9	3.6	3.8	16.5	10.2	0.45
P2	690	**14.6**	**>100**	**0.011**	**9.13**	**>140[b]**	**0.18**	**0.27**	3.7	**22.7**	**0.20**
	<LOQ	5.2	17.5	1.23	25.0	**17.0**	2.4	1.9	7.1	12.8	1.11
P4[a]	694	**15.3**	**>100**	**0.015**	**9.89**	**>140[b]**	**3.23**	**3.4**	3.1	**31.4**	**0.14**
	<LOQ	5.1	16.2	1.75	62.1	38.8	63.8	33.3	7.2	16.0	0.81
T2	758	**16.8**	**>100**	**0.007**	**7.91**	**>140[b]**	**0.12**	**0.27**	4.4	17.7	**0.07**
	<LOQ	5.1	15.1	1.31	31.5	**14.3**	1.7	3.0	11.8	8.2	0.41
T11	814	**17.8**	**>100**	**<0.005**	**5.93**	**>140[b]**	**0.12**	**0.43**	5.4	16.7	**0.11**
	<LOQ	6.0	14.1	0.73	18.8	**10.4**	2.2	4.0	11.5	8.1	0.78
T1	827	**18.4**	**>100**	**0.006**	**7.02**	**>140[b]**	**0.11**	**0.31**	4.1	16.2	**0.07**
	<LOQ	5.0	15.0	1.40	33.5	**14.9**	1.68	3.6	12.2	6.9	0.44
T10	914	**14.2**	**>100**	**0.005**	**5.21**	**>140[b]**	**0.10**	**0.10**	3.7	**>63.4**	**0.08**
	8.3	4.4	16.7	1.32	18.2	**29.0**	2.0	1.4	9.1	**28.0**	0.59
T8	959	**17.8**	**>100**	**0.005**	**8.6**	**>140[b]**	**0.16**	**<0.1**	2.2	**24.7**	**0.11**
	<LOQ	4.8	15.6	1.13	29.9	**18.9**	3.9	1.5	6.8	9.2	0.84
T3	1000	**17.2**	**>100**	**0.005**	**8.79**	**>140[b]**	**0.12**	**0.44**	1.7	7.6	**0.07**
	<LOQ	5.1	18.8	0.97	26.3	**12.0**	2.2	4.8	4.4	**3.0**	0.50
T7	1010	**17.3**	**>100**	**0.007**	**9.79**	**>140[b]**	**0.20**	**0.13**	2.5	**29.2**	**0.10**
	<LOQ	4.8	15.8	1.30	26.1	**17.4**	3.9	1.7	7.3	11.6	0.82
T4	1110	**18.1**	**>100**	**0.012**	**8.15**	**>140[b]**	**0.10**	**0.61**	2.3	12.0	**0.09**
	27.6	5.1	16.6	2.00	23.1	**12.9**	2.3	8.2	6.6	**4.3**	0.71
T5	1160	**20.0**	**>100**	**0.010**	**7.47**	**>140[b]**	**0.14**	**0.27**	3.0	**52.8**	**0.04**
	<LOQ	5.7	13.3	2.39	26.0	**19.0**	3.3	3.9	10.1	17.8	0.36

Values in bold are outside of the method, gender and age – related reference ranges. <LOQ: below the limit of quantification. $p < 10^{-4}$ for all assays. [a]Post-menopausal woman. [b]Obtained after a ½ dilution.

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Table 3: Spearman's correlation coefficient (ρ) between the biotin or bisnorbiotin concentration and the percentage difference in hormone results before and after the neutralization procedure in 23 samples (P1–P9, T1–T11, and V1–V3). This correlation was not tested for fT4, 25OHD, or TSH, as many values before neutralization were out of the range of quantification.

	Biotin	Bisnorbiotin
PTH	$\rho = 0.70$; $p = 0.001$	$\rho = 0.38$; NS
FSH	$\rho = 0.96$; $p < 10^{-4}$	$\rho = 0.11$; NS
LH	$\rho = 0.97$; $p < 10^{-4}$	$\rho = 0.18$; NS
Prolactin	$\rho = 0.96$; $p < 10^{-4}$	$\rho = 0.44$; NS
C-peptide	$\rho = 0.99$; $p < 10^{-4}$	$\rho = 0.004$; NS
fT3	$\rho = 0.95$; $p < 10^{-4}$	$\rho = 0.03$; NS
Cortisol	$\rho = 0.98$; $p < 10^{-4}$	$\rho = 0.06$; NS

NS, non-significant.

Table 4: Results of different neutralization ratios: streptavidin microparticles corresponding to two, three, four, five and ten streptavidin reagent volumes per plasma volume (protocol N2–N10).

	Net plasma	N2	N3	N4	N5	N10
Patient P4						
Biotin	694	180	<LOQ		<LOQ	
Bisnorbiotin	233	257	237		215	
Biotin sulfoxide	30.3	26.8	17.9		<LOQ	
25OH D	>140	>70	37.6	36.1	38.8	
Subject T10						
Biotin	914				8.3	<LOQ
Bisnorbiotin	20.4				17.9	9.3
Biotin sulfoxide	15.6				<LOQ	<LOQ
25OH D	>140				29.0	28.1

Biotin, biotin metabolites and 25OH vitamin D in μg/L. <LOQ: below the lower limit of quantification.

applied to 39 samples from unsupplemented subjects with hyper- or hypothyroid biochemical profiles, hyper- or hypocortisolism, elevated or low PTH concentrations, and various FSH, LH and prolactin profiles (Supplemental Material, Table 2). No change in clinical interpretation was observed after the N5 protocol. The changes recorded after neutralization were always smaller than ±8.9%, except for PTH, for which an average change of −8.4% was observed (maximum recorded difference −15.7%).

Time-response study in two healthy volunteers

In volunteers V1 and V2, cessation of biotin supplementation for 48 h led to complete restoration of pre-biotin hormonal levels (data not shown).

Discussion

We show that high biotin doses in MS patients and healthy controls interfere with many hormone immunoassays, falsely mimicking pathological conditions. We therefore developed and validated a simple method to overcome this analytical interference, that is suitable for use in any clinical laboratory.

As recently indicated by several case-reports [4, 6–11], patients receiving high-dose biotin are at risk for a false diagnosis of hyperthyroidism. In our series, 15/17 subjects receiving high doses of biotin (>100 mg) had at least one result suggestive of hyperthyroidism. Anti-TSHR antibody titers were not measured in our study but are also reported to be highly erroneous [6, 7, 9, 10] even after moderate (10 mg) biotin intake [9]. Thyroid testing is very regularly prescribed to MS patients to detect adverse effect of MS treatment (e.g. 3-monthly during the first 48 months of alemtuzumab treatment, and yearly with β-interferon therapy). Therefore this interference has driven much attention on false biochemical diagnosis of hyperthyroidism.

Several MS specialists recommend routine vitamin D supplementation for MS patients; adequacy of therapy is most commonly monitored by 25OHD serum levels [14, 15]. All our MS patients and controls receiving high dose biotin (>100 mg) had a very high 25OHD concentration when measured without biotin neutralization. A logical response to such results would be to stop vitamin D treatment, to ask the patient to avoid sunlight, and to prescribe additional laboratory tests such as serum and urinary calcium and serum PTH to detect biochemical features of vitamin D intoxication. Indeed, all but one of our patients and controls receiving high-dose biotin had low PTH levels suggestive of vitamin D overdosing, potentially confirming the erroneous diagnosis of vitamin D intoxication.

Although abnormal cortisol and testosterone secretion have been reported in MS [16–20], the corticotropic and gonadotropic axes are not routinely tested in MS patients. If measured, artifactually high cortisol, low FSH, and low LH might be found based on observations presented here and those of others [9], again leading to further useless and costly explorations. Based on mechanism of assay, artifactually low ACTH (not tested in the present study), and high testosterone or estradiol levels might support the erroneous need for further workup.

One way to avoid biotin interference is to withdraw biotin supplementation. Indeed, the impact of biotin on immunoassay results is maximal 2 h after a single intake of 30 mg, and remains significant up to 25 h thereafter [21]. Biotin withdrawal can be efficient in biotin

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auto-medication with moderately high dose (as evidenced in V1 and V2 subjects). As expected, the time interval is longer for biotin 300 mg dose, because of a calculated half-life varying between 7.8 and 18.8 h (no accumulation of biotin in blood is expected in case of repeated administration [12]). Two to three days off biotin has been reported to be sufficient to recover normal fT4 and TSH assays values in patients receiving 100 mg t.i.d. [4, 7, 9], but a 7-day washout period was needed before anti-TSH receptor antibody assay normalized [10]. Several factors are likely to influence how long to discontinue biotin therapy before obtaining sample for assay by a method that uses streptavidin-biotin technique. Because biotin is excreted primarily by the kidneys [1], moderately impaired renal function is likely to reduce clearance and extend the duration of interrupted therapy; ESRD even more so. For example, PTH underestimation has been reported up to 15 days in a patient with ESRD receiving 10 mg/day of biotin [13]. Moreover, biotin withdrawal may be deleterious in some cases of biotin-responsive inherited metabolic disease (such as biotinidase deficiency), and the effects of interrupting biotin therapy in MS are unknown. To circumvent the potential problems caused by biotin treatment interruption, the use of assays not based on streptavidin-biotin interaction could be considered. However, high volume analytical platforms measuring several hormones are widespread in Europe and North America; persuading the laboratory to employ an alternative method for a relatively small number of samples may be difficult.

Accordingly, we validated a simple method to remove biotin; the method can be widely implemented and is unlikely to affect any of the current assay methods. This method efficiently removed biotin even at the highest biotin concentrations. In the current study, we observed no effects on any hormone assays excepting for a slight PTH underestimation. This appears to be related to a time-dependent loss for this fragile hormone [22]. A similar effect was also observed by comparing the results obtained after N5 neutralization and those obtained with the alternative method (Supplemental Material, Table 1, subjects P4, V3).

In this study, we used heparin plasma from MS patients and healthy controls available from the clinical studies of high-biotin doses. The question of whether the results in serum or EDTA plasma might be comparable can be raised. We had recently encountered in our routine practice a MS patient who was taking biotin 100 mg t.i.d. He presented a biochemical profile of hyperthyroidism, elevated 25OHD and low PTH levels in serum, and a low ACTH concentration in EDTA plasma. All these abnormal results became normalized after the N5 procedure was performed. Therefore we conclude that this interference also exists in other matrix such as EDTA plasma and serum, as also reported [6, 9, 11, 13, 21, 23, 24]. Furthermore, the removal of biotin using the N5 protocol is most likely efficient in these alternative assay matrix.

Biotin metabolites bind to avidin, although less tightly than biotin itself [1, 25]. Streptavidin is less efficient than avidin for binding bisnorbiotin and biotin sulfoxide [25]. The streptavidin microparticles that we used do not have the same affinity for bisnorbiotin as for biotin itself (Tables 1 and 4), suggesting that this metabolite accounts for only a small part of the interference. Other metabolites or in vivo-biotinylated compounds may, however, have slight influence on some assays, depending on the reagent source.

The critical limitation in avoiding biotin interference is the need to identify supraphysiologic biotin intake. Clinicians should specifically ask their patients about intake of biotin, which is sometimes included as an unnamed supplement in hair, nail and skin products. As a vitamin, the patient may not even consider biotin worth-mentioning [9, 26]. Furthermore, MS patients frequently obtain high-dose biotin directly from compounding pharmacies and this may not be recorded in their medical chart [4]. Laboratories need appropriate clinical and therapeutic information to be able to overcome this problem and should also provide routine warnings about biotin interference when reporting results obtained with methods based on streptavidin-biotin interaction. Suppliers of analytical platforms using the streptavidin-biotin methodology should be encouraged to offer an “interference testing” (it is unlikely that the assay of biotin, which is analytically very demanding, could be routinely performed). The reagent companies could also provide modified assay protocols designed to avoid inhibition of the streptavidin-biotin interaction by biotin.

Our study has several strengths, including the large number of subjects receiving high-dose biotin, measurement of biotin and biotin metabolites with LC-MSMS, the large panel of hormone assays tested, and validation of a simple method to overcome biotin interference. One limitation is that we only tested assays manufactured by Roche, while other immunoassays from other manufacturers also use the streptavidin-biotin methodology and are similarly concerned by the biotin interference [5]. The susceptibility of each assay to this artifact is highly variable within a manufacturer’s tests panel (as demonstrated here) and between manufacturers. Our results cannot be extrapolated to other streptavidin-biotin based reagents, as assay design and sample volume are strong determinants of assay errors. In theory, the results obtained after

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removal of biotin might not be reportable although the strong evidence indicates that the initial value is unreliable. Nevertheless, because our neutralization protocol introduced no bias (except for PTH: max – 15.7%) and confirmed the diagnoses in all 39 patients with various endocrine disorders, results after biotin removal are quite likely to be reliable. Finally, the precise impact of biotin metabolites or in vivo-biotinylated compounds that may contribute to interference in some assays deserves further studies.

Conclusions

Our study confirms that high plasma biotin (>30 μg/L) can lead to clinically misleading interferences with streptavidin-biotin immunoassays. This interference has been known since 1996 [23], but high-dose biotin therapy for MS is an emerging treatment that will lead to frequent false biochemical endocrine profiles. The particular behavior of this interference specially concerns hormonal profiles because of both positive and negative errors, mimicking coherent endocrine pathologies. Because of the high frequency of thyroid testing in MS patients, false profiles of hyperthyroidism are likely to be the most frequent consequence of biotin interference in routine practice. Endocrinologists must also be aware that many assays in other endocrines axes may be impacted: hypercortisolism associated with falsely low ACTH level, hypertestosteronemia or hyperestrogenism and falsely low FSH, LH levels [9], and high 25OH vitamin D levels with low PTH levels, may also be observed in patients treated with high dose biotin. Many non-hormonal immunoassays may also be inaccurate in biotin-treated patients, showing falsely low tumor markers and ferritin levels, falsely high vitamin B12 and folate levels [7, 24], and falsely negative serologies. Our method of biotin neutralization is highly efficient and may be easily performed in any clinical laboratory, either to investigate unexpected hormonal profile, or to prevent artifact for known biotin supplementation. This highlights the paramount importance of communication between prescribing physicians and clinical laboratory staff.

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