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Further assessment of the prevalence of biotin supplementation and its impact on risk

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1. Introduction

Heightened awareness of biotin interference is prompting laboratories to evaluate biotin risk and its attenuation, as two recent publications in this Journal show [1,2]. The determinants of risk include the frequency of the risk event, and the capacity to detect and mitigate adverse consequences. Thus far, the prevalence of biotin usage has been a major gap in understanding the scope of biotin interference, but a recent study is redressing this, providing the first published report of biotin prevalence [1]. We have also investigated biotin prevalence in an Emergency Department (ED) population and now report our findings.

2. Materials and methods

2.1. Prevalence

As a quality control initiative, we measured the biotin concentration of routine samples presenting to our laboratory for immunoassay testing in order to flag samples at risk of clinically significant interference. At three time points between May and August 2018, we specifically sub-sampled our ED immunoassay requests (primarily troponin T). Samples that had been stored at 4 °C were sent to our main laboratory for biotin measurement, which was carried out within 10 days of initial blood collection.

2.2. Biotin measurement by LC-MS/MS

We developed a fully automated LC-tandem mass spectrometry method using the Shimadzu clinical laboratory automation module (CLAM-2000). Samples underwent acetonitrile precipitation prior to chromatography using a biphenyl column (Cat. No. 9309A52, Restek) and gradient elution with sequential mobile phase. Biotin was detected using a Shimadzu LCMS-8060 and quantitated relative to deuterated internal standard (Cat. No. 5023, IsoSciences). Our method was calibrated using Sigma secondary reference material (Cat. No. PHR1233, Sigma-Aldrich) traceable to a European Pharmacopoeia Reference Standard. The limit of quantitation was 1 μg/L (20% CV), and inter-run precision 7.5% CV at 3 μg/L, 5.8% CV at 30 μg/L and 4% CV at 75 μg/L.

3. Results

During the study, 490 serum samples from ED patients were collected for biotin measurement, 52% of which were from female patients. The patient characteristics are given in Table 1. The concentration of biotin was < 1 μg/L in 98% of samples, and < 5 μg/L in 99% (Fig. 1). Only 4 samples contained biotin concentrations exceeding 10 μg/L, a threshold which may cause 10% analytical bias in the most biotin-sensitive Roche immunoassays used in our laboratory.

4. Discussion

The increased availability of over the counter mg-strength biotin supplements has raised concerns over the increased risk of biotin interference, but true prevalence rates have until recently been undefined. Katzman and colleagues' report on biotin supplement usage in patients presenting to the ED is a welcome and important addition to the biotin interference literature [1]. The concentrations of biotin reported in their study are striking, with approximately 7% of 1442 plasma samples containing ≥ 10 μg/L biotin. At least superficially, this corresponds with their outpatient survey data, with just over 7% of the 1944 respondents self-reporting biotin use. This data fits with emerging description of the pharmacokinetics of mg-range biotin supplementation in individuals with normal renal function [3]. Together, both the biotin concentrations measured by the Mayo Clinic and their survey data point to a surprisingly high frequency of mg-strength biotin use in their population. This contrasts with our study, where < 1% of ED samples contained biotin exceeding 10 μg/L. Such differences in

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Table 1
Age (quartiles), gender and eGFR (quartiles) of the cohort of ED patients in which biotin was measured.

	Total (n = 490)	Males (n = 237)	Females (n = 253)
Age	72 (58, 83)	71 (60, 80)	74 (56, 86)
eGFR	77 (49, > 90)	74 (48, 89)	79 (50, > 90)

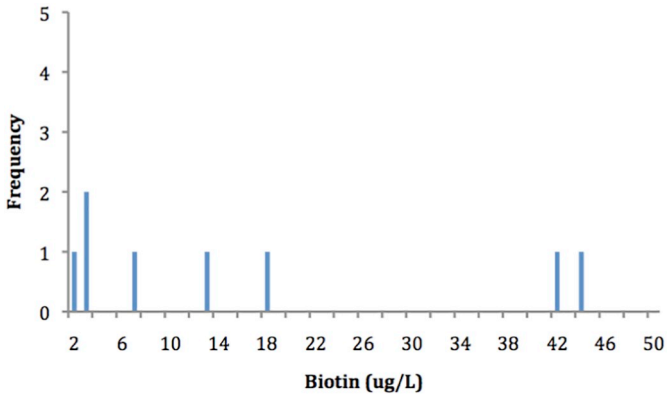

Fig. 1. Distribution of biotin in 8 samples with biotin > 2 μg/L. Of 490 ED samples assessed, 482 (98%) contained biotin concentrations < 1 μg/L.

prevalence are perhaps not surprising, as heterogeneity in over-the-counter mg-strength biotin supplementation is likely across populations and demographics.

We are surprised, however, at the low-end biotin concentrations reported in each study. Although there are no large-scale reference interval studies for biotin in healthy (unsupplemented) populations, there are numerous smaller studies providing estimates of "normal" biotin concentrations that range from under 0.1 to approximately 1 μg/L [4–9]. Such studies have measured biotin by various means, including microbial bioassays, RIA, ELISA and HPLC-coupled avidin binding assays. Biotin concentrations measured in our cohort were consistent with these published values, with 98% of samples containing biotin concentrations < 1 μg/L. Interestingly, 42% of the routine Mayo ED presenters demonstrated biotin concentrations between 5 and 9 μg/L. From indications in the literature, this far exceeds what can reasonably be expected as a "normal" concentration of biotin. It also stands out against the authors' cited LC-MS/MS derived biotin reference interval of 0.05–0.83 μg/L [10]: almost half of the Mayo cohort had biotin concentrations between 6 and 11 fold greater than the upper limit of this interval.

Such marked difference in biotin concentrations across the two studies is, of course, provocative, and of more than academic interest, particularly with regard to potential impact on susceptible assays. Whilst the 5–9 μg/L concentration bracket does not affect Roche Elecsys immunoassays, it is expected to exert clinically significant analytical bias on assays with lower biotin thresholds, such as Vitros Troponin I and DiaSorin Liaison XL murex HIV Ab/Ag, each of which demonstrate 10% analytical bias at approximately 2.5 μg/L biotin [11,12].

Does this reflect a major difference in multivitamin use or other modes of biotin fortification between the Minnesota and Melbourne cohorts? It is notable that approximately 42% of the Mayo survey respondents reported multivitamin use. Yet most multivitamins contain amounts of biotin ranging from the adequate dietary intake level of 30 μg [13] to 300 μg [1], and should not cause biotin concentrations of this order [4]. Extrapolating from Grimsey and colleagues' pharmacokinetic data, biotin doses exceeding 500 μg are required to cause serum concentrations in the 5–9 μg/L bracket [3]. Such consumption does not fit neatly with available evidence, and we remain curious as to alternative sources of biotin fortification. The impact of renal function is also a relevant consideration in explaining differences in serum biotin concentrations, given the renal clearance of biotin and its metabolites. Although our cohort is older than the studied Mayo population (median age 72 vs. 58, respectively), our median eGFR was 77 mL/min, and perhaps relatively preserved renal function has contributed to the lower measured biotin concentrations in our study. However we must also question whether method differences between the respective LC-MS/MS protocols may have skewed apparent biotin prevalence across our study groups. Notwithstanding the superior molecular specificity afforded by LC/MS-MS technology, variation in chromatographic conditions, calibration and standardisation can cause significant differences in measured concentrations of analytes [14].

In the current climate of concern over biotin interference, over-the-counter biotin use has loomed as an unquantified threat to laboratories [15]. Assessment of the prevalence of biotin use is an important step forward for risk analysis, though there is still more to learn of the granularity of biotin prevalence within different populations and demographics, and indeed of biotin measurement and its normal reference intervals. Answers to these questions will ultimately help laboratories understand which of the many risk mitigation possibilities [2] are best calibrated to a given setting.

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