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J Food Compost Anal. Author manuscript; available in PMC 2006 April 28.

Published in final edited form as:
J Food Compost Anal. 2004 December ; 17(6): 767–776.

Determination of the biotin content of select foods using accurate and sensitive HPLC/avidin binding

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Abstract

Assessing dietary biotin content, biotin bioavailability, and resulting biotin status are crucial in determining whether biotin deficiency is teratogenic in humans. Accuracy in estimating dietary biotin is limited both by data gaps in food composition tables and by inaccuracies in published data. The present study applied sensitive and specific analytical techniques to determine values for biotin content in a select group of foods. Total biotin content of 87 foods was determined using acid hydrolysis and the HPLC/avidin-binding assay. These values are consistent with published values in that meat, fish, poultry, egg, dairy, and some vegetables are relatively rich sources of biotin. However, these biotin values disagreed substantially with published values for many foods. Assay values varied between 247 times greater than published values for a given food to as much as 36% less than the published biotin value. Among 51 foods assayed for which published values were available, only seven agreed within analytical variability (720%). We conclude that published values for biotin content of foods are likely to be inaccurate.

Keywords

Biotin; Water-soluble vitamins; Biotin content in foods

1. Introduction

Biotin is a water-soluble vitamin and an essential co-factor for five biotin-dependent carboxylases: acetyl-CoA carboxylase-a and acetyl-CoA carboxylase-b, propionyl-CoA carboxylase, pyruvate carboxylase, and b-methylcrotonyl-CoA carboxylase (Mock, 1999). Clinical findings of frank biotin deficiency include periorificial scaly dermatitis, conjunctivitis, loss of hair, loss of appetite, hallucinations, depression, and developmental delay. Recent evidence indicates that pregnant women develop marginal biotin deficiency during normal pregnancy (Mock, 1999; Zempleni and Mock, 1999). The marginal biotin deficiency observed in pregnancy does not cause these pathognomonic clinical manifestations because the deficiency is mild; however, deleterious health consequences for the fetus are a concern because marginal biotin deficiency is teratogenic in several animal species (Zempleni and Mock, 2000). Assessing dietary biotin content, biotin bioavailability, and the resulting biotin status is crucial to determining whether biotin deficiency is teratogenic in humans. Estimation

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of dietary biotin intake may be limited both by data gaps in food composition tables and by inaccuracies in published values. The present study determined biotin content for a group of foods thought to be good sources of biotin and for a group for which no published biotin content values are available but which are commonly consumed in Arkansas.

2. Materials and methods

2.1. Analysis of foods

Protein-bound biotin was released by acid hydrolysis (Mock et al., 1992) with subsequent biotin quantitation by the HPLC/avidin-binding assay (Mock, 1997). This method for release of bound biotin has been optimized for human milk o and rat liver tissue to obtain maximum (>95%) release from protein while minimizing (10%) biotin oxidation to biotin sulfoxide (Mock et al., 1992). To evaluate whether this acid hydrolysis procedure would release biotin from foods without destroying it, we determined biotin release and recovery in three foods. These foods were chosen because they differed substantially in protein content. Beef liver was chosen for high protein content (267 mg/g), cooked sweet potato was chosen for the low amount of protein (16.5 mg/g), and whole wheat bread was chosen for the moderate amount of protein (97 mg/g). In order to assess destruction, biotin content was quantitated in the presence of enough supplemental protein (bovine serum albumin (BSA) 40 mg) to prevent biotin destruction even during hydrolysis of the lowest protein food samples (o40 mg protein/g); control contained no added protein. The effect of acid hydrolysis on free biotin was also determined. Total biotin content of each sample was determined using acid hydrolysis as previously described (Staggs, 2002). The biotin content of beef liver, sweet potato, and whole wheat bread did not differ significantly whether supplemental protein was added or not. Free biotin was not degraded during acid hydrolysis for any of the three foods. These data provide evidence that this acid hydrolysis method does release biotin from foods with a variety of protein levels, while minimizing degradation.

Sampling: Foods were selected for analysis based on three criteria: (1) substantial biotin content as judged by published values, (2) absence of published information for the biotin content, and (3) high frequency of appearance in the 24-h diet record of Arkansas children participating in a study of nutritional status in the Mississippi Delta (Staggs, 2002).

Food items were purchased from local restaurants and local chain grocery stores supplied by large-scale producers, local producers, and wholesalers. Food samples (Table 1) were obtained by a single collection of representative products available locally. Beef liver, chicken liver, pork chop, and eggs were purchased fresh and cooked by microwave prior to homogenization. All other meats, fish and poultry were purchased fully cooked from local restaurants. Vegetables (fresh, frozen, or canned) were analyzed without cooking with the exception of sweet potato, which was purchased fresh and cooked by microwave prior to analysis. Fruits were purchased as fresh or frozen depending on availability and peeled where applicable prior to homogenization. Breads and grains, sweets, entrees, condiments and sauces, beverages, nuts, and ‘miscellaneous’ food were purchased from commercial sources and homogenized without further preparation. All food samples (approximately one serving by weight per food) were weighed and homogenized in water maintaining a weight ratio of 1 part food to 4 parts water. Homogenates were stored at -20°C until acid hydrolysis.

Acid hydrolysis: Acid (1.5 mL of 3 mol/L HCl) was added to 0.5 mL of the aqueous homogenate. The mixture was vortexed and incubated at 100°C for 120 min; samples were vortexed again after the first 10 min and every 30 min thereafter. After incubation, samples were cooled to room temperature and centrifuged at 550g for 10 min. A 1-mL aliquot of the supernatant was adjusted to a pH 2.5 with 10 mol/L NaOH and filtered through a 0.45 mm filter.

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Separation of biotin: Biotin was separated from its inactive metabolites by HPLC using a C18 reversed-phase column as previously described (Mock, 1997). A 0.5-mL aliquot of the filtrate was fractionated by reversed-phase HPLC. The HPLC fractions were collected at specific retention times determined by prior injection of radiolabeled biotin. To ensure maximum recovery of the biotin peak, four fractions (1.0 ml each) were collected. Injection and collection of radioactive standards utilizing this HPLC protocol produced recovery rates of approximately 95% (Mock, 1997). The HPLC eluate fractions containing biotin were dried to remove chromatography solvents and then rehydrated with 1 mL of distilled deionized water followed by an equal volume of 0.2 mol/L HEPES 2 mol/L NaCl, 0.05% Tween 20, pH=7 buffer. Rehydrated fractions were directly transferred into 96-well round-bottom plates and assayed as described below.

HRP-avidin assay for biotin: Previous studies have validated the HRP-avidin-binding assay for quantitating biotin (Mock, 1997). The HPLC/avidin-binding assay is very sensitive with detection limits of approximately 0.001 ng biotin and has the capability to accurately separate biotin from its inactive metabolites (Mock et al., 1992).

Initially, eluate fractions were incubated with avidin coupled to horseradish peroxidase and then transferred to a microtiter well coated with biotinylated BSA. Those HRP-avidin molecules with unoccupied biotin-binding sites bound to the biotinyl-BSA adhering to the well. The more biotin (or biotin metabolite) present in the sample (or standard), the fewer avidin molecules bound to the well. The amount of bound avidin is quantitated optically by bound HRP-avidin using a timed oxidation of O-phenylenediamine. Optical density was determined using a multiwell spectrophotometer at 490 nm with background correction at 630 nm. The concentration of biotin was determined by comparing the change in the optical densities to a standard curve constructed by diluting a known concentration of biotin.

Biotin (d-biotin) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Biotin stock was prepared as previously described by Mock (1997). Assay standards were prepared by diluting a 100 nmol/L biotin solution to a final concentration of 3000 pmol/L. Biotin standard concentrations were confirmed by comparison with radiolabeled biotin of a known specific activity.

2.2. Published values for biotin content in foods

Food biotin content values determined by this method were compared to values obtained from the Bowes and Church's Food Values of Portions Commonly Used (Pennington and Church, 1985; Pennington, 1989; Hands, 2000), and from individual reports of biotin content in food data (http://lpi.orst.edu/infocenter/vitamins/biotin/biotin.html).

2.3. Calculations of biotin and the determination of relative difference

Biotin content in foods is presented as ng of biotin per g of food. The relative differences between published values and values directly assayed were calculated as follows: relative difference=(published biotin values-assay value)/assay value.

3. Results and discussion

We sought to use more modern sensitive and specific analytical techniques to provide more accurate and precise values for biotin content in a select group of foods. Previously, the available data indicated that foods such as liver, egg yolks, and green vegetables are good sources of biotin (Pennington and Church, 1985; Pennington, 1989). Relative to values available for other micronutrients, few biotin values have been published. We determined the biotin content for foods previously reported to have substantial biotin content as well as a

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number of foods present in a typical Arkansas diet for which no biotin value has been published (87 foods; Table 1).

Our results confirm previous conclusions that meat, fish, poultry, egg, dairy, and some vegetables are rich dietary sources of biotin. Biotin values determined here, however, frequently did not agree with published values (Table 2). Differences in assay values ranged from 247 times greater to 0.85 times less than previously published biotin values for select foods. Of the 51 foods for which values had been published, only seven (14%) agreed within an analytical error of 20%.

The differences between published values and values determined here may arise from one or more of the following: (1) Heterogeneity in foods assayed might have arisen from differences in growing conditions, biotin content of animal feeds or fertilizers, fortification, processing, season, and geographic location. Food samples presented here were not pooled from more than one source. (2) Analytical problems are likely to have contributed to the differences with respect to published values. Published biotin values likely both over- and underestimate true biotin content of particular foods because the majority of these studies used bioassays. Bioassays characteristically suffer from interference by unrelated substances, nonspecificity for biotin versus its metabolites, incomplete release of biotin from its protein-bound state, destruction of biotin during release from protein binding, and release of substances that cause assay artifacts during acid hydrolysis (Mock et al., 1992). Bioassays may measure ‘sparing factors’ and thereby overestimate biotin. Bioassays do not consistently discriminate biotin from its inactive metabolites. Biotin in food is largely protein bound to both endogenous and exogenous biotinyl proteins. The proportion of free (water extractable) biotin versus protein-bound (released by acidic or enzymatic hydrolysis) biotin varies among foods; the majority of biotin in meats and cereals appears to be protein bound (Zempleni and Mock, 1999). Furthermore, published biotin values rely heavily on values for compound foods reported by the food manufacturer (Pennington and Church, 1985; Pennington, 1989). These manufacturers do not necessarily specify methods for biotin analysis and may over- or underestimate biotin content in food values due to the same factors listed previously.

4. Conclusions

In summary, the data presented here support our assertion that estimation of dietary biotin is confounded both by data gaps in food composition tables and by inaccurate published values. We contend that assessing dietary biotin intake for a given population is likely fraught with error, making formulation of recommendation for dietary biotin intake difficult. The possibility that marginal biotin deficiency is teratogenic in humans mandates accurate determination of biotin content in foods. Subsequent research also must address the bioavailability of biotin from foods and the effects on biotin status, which remains a largely unexplored area.

Acknowledgements

This study was funded in part by NIH, RO1 grant DK36823 (DMM) and the USDA grant #9304550 (DMM). The authors thank Tiffany Womack and Ashlee Metcalf for their help in obtaining food samples and laboratory analysis.

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Table 1

Biotin content of select foods determined by HPLC/avidin binding assay[a]

Foods	ng biotin/g food	Serving size (g)	μg biotin/serving
Meat, fish, poultry, egg			
Beef liver, cooked	416	74	30.8
Chicken nuggets, breaded, fried	13.4	75	1.00
Chicken strips, breaded, fried	4.30	85	0.37
Chicken liver, cooked	1872	74	138
Egg, whole, cooked	214	47	10.0
Egg, white, cooked	58	35	2.02
Egg, yolk, cooked	272	15	4.08
Catfish, breaded, fried	7.44	93	0.69
Fish sticks, minced, breaded, fried	10.0	87	0.87
Hamburger patty, cooked	45	37	1.65
Hot dog, chicken and pork, cooked	37	56	2.06
Pork chop, cooked	45	80	3.57
Salmon, pink, canned in water	59	63	3.69
Sliced turkey, processed deli	7.30	21	0.15
Tuna, canned in water	6.82	63	0.43
Dairy			
2% milk	1.13	236	0.27
American cheese	31	19	0.59
Cheddar cheese, mild	14	28	0.40
Chocolate milk, low-fat	3.81	236	0.90
Plain yogurt	0.84	170	0.14
Provolone cheese	1.17	24	0.03
Skim milk	1.31	236	0.31
Whole milk	0.91	236	0.22
Cereals			
Cheerios®	1.08	30	0.03
Frosted Flakes®	1.38	31	0.04
Golden Grahams®	1.46	31	0.05
Kix®	0.95	30	0.03
Vegetables			
Broccoli, fresh	9.43	113	1.07
Carrots, canned	6.22	29	0.18
Cauliflower, fresh	1.61	32	0.05
Green beans, canned	0.07	120	0.01
Mushrooms, canned	21.6	120	2.59
Spinach, frozen	7.05	83	0.58
Sweet potato, cooked	14.5	80	1.16
Whole kernel corn, canned	0.47	125	0.06
Fruits and berries			
Apple, fresh	0.20	185	0.04
Apple juice, canned, from concentrate	0.52	250	0.13
Avocado, fresh	9.61	37	0.36
Banana, fresh	1.33	103	0.14
Orange, fresh	0.49	258	0.13
Orange juice, canned, from concentrate	4.13	296	1.22
Raisins	3.91	43	0.17
Raspberries, fresh	1.78	140	0.25
Strawberries, fresh	15.0	111	1.67
Tomatoes, fresh	7.01	43	0.30
Bread and grains			
Crackers, saltine	2.90	17	0.05
Grilled toast	12.3	84	1.03
Grits	0.51	190	0.10
Hamburger bun	2.89	58	0.17
Noodles	1.81	180	0.32
Oatmeal	1.91	190	0.36
Roll, dinner	0.48	28	0.01
Whole wheat bread	0.74	33	0.02
Sweets			
Banana pudding	10.2	170	1.73
Chocolate sandwich cookie	1.43	31	0.04
Oatmeal cream pie	0.91	40	0.04
Poptart, blueberry	0.33	53	0.02
Sugar cookie	2.79	37	0.10
Vanilla cake with frosting	0.34	43	0.01
Entrees			
Beef vegetable soup	1.18	126	0.15
Cheese pizza	1.09	175	0.19
Corn chip chili pie	0.59	191	0.11
Chili	5.20	441	2.29
French fries	3.18	104	0.33

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Foods	ng biotin/g food	Serving size (g)	µg biotin/serving
Hush puppies	2.03	81	0.16
Macaroni and cheese	1.30	147	0.19
Mashed potatoes, with brown gravy	1.33	136	0.18
Pepperoni pizza	2.12	112	0.24
Ramen noodles, oriental	1.01	43	0.04
Salad, mixed garden	2.85	155	0.44
Tator tots	0.62	57	0.04
Condiments and sauces			
Ketchup	0.74	9	0.01
Mayonnaise	1.85	12	0.02
Ranch dressing	2.35	35	0.08
Spaghetti sauce, with beef	0.60	123	0.07
Beverages			
Coke®	0.81	113	0.09
Red fruit punch	1.55	200	0.31
Tea, sweet	1.42	200	0.28
White wine	1.17	212	0.25
Beer	1.14	280	0.32
Nuts			
Almonds, roasted, salted	44.07	30	1.32
Peanuts, roasted, salted	175	28	4.91
Pecans, fresh	20.0	30	0.60
Sunflower seeds, roasted, salted	78.0	31	2.42
Walnuts, fresh	25.9	30	0.78
Miscellaneous			
Potato chips, baked, barbecue flavor	0.50	28	0.01
Yeast	202	1	0.20

[a] Foods were analyzed by acid hydrolysis and HPLC/HRP-avidin binding assay for biotin (Mock, 1997); n = 1 homogenate analyzed per food.

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Table 2

Comparison of assay values to published values for biotin in foods (ng/g of fresh weight)

Foods	Assay values	Published values	Relative differences[a]
Meat, fish, poultry, egg			
Beef liver	416	365; 960[bc]	-0.12; 1.30
Chicken strips	4.30	40.5[b]	8.42
Egg, whole	214	538; 220[bde]	1.51; 0.03; -0.23
Egg, white	57.6	78.8[d]	0.37
Egg, yolk	272	494[d]	0.82
Pork chop	44.6	27.0; 30.0[be]	-0.39; -0.32
Salmon, pink, in water	58.6	54.1; 50.0[be]	-0.08; -0.15
Tuna, in water	6.80	20.0[e]	1.94
Dairy			
American cheese	30.9	35.7; 20.0[de]	0.15; -0.20
Cheddar cheese, mild	14.3	71.4; 21.4[bd]	3.99; 0.50
Provolone cheese	1.17	18.0; 38.7[fe]	14.3; 31.9
Skim milk	1.31	20.0; 20.0[fe]	14.3; 14.3
Whole milk	0.91	31.0; 18.9[fe]	33.1; 19.8
Yogurt, plain	0.85	22.0[g]	24.9
Vegetables			
Broccoli, fresh	9.43	19.0[h]	1.01
Carrots, canned	6.22	15.2; 50.0[ceh]	1.44; 7.04; 3.34
Cauliflower	1.61	12.3; 15.0[be]	6.66; 8.32
Green beans	0.07	10.1; 7.70[ceh]	143; 109; 70.4
Mushrooms	21.6	72.5; 84.6[ce]	2.36; 2.92
Spinach	7.05	69.0; 11.0[ce]	8.79; -0.85
Sweet potato	14.5	43.0; 59.0[eh]	1.96; 3.07
Whole kernel corn	0.47	21.6; 62.0[ch]	45.0; 131
Fruits and berries			
Apple	0.20	9.2; 12.3[ce]	45.0; 60.5
Apple juice	0.50	8.2[e]	15.4
Avocado	9.60	80.4; 36.0[be]	7.38; 2.75
Banana	1.30	44.0; 26.3[ce]	32.9; 19.2
Orange	0.50	19.0; 10.4[ce]	37.0; 19.8
Orange juice	4.10	8.3; 7.6[ce]	1.02; 0.88
Raisins	3.91	46.7; 19.4[ce]	10.9; 3.96
Raspberries	1.78	14.3; 19.4[be]	7.03; 9.90
Strawberries	15.0	40.0; 10.8[ce]	1.67; -0.28
Tomatoes	7.00	40.0; 40.0[cei]	4.71; 4.71; 1.00
Bread and grains			
Crackers, saltine	1.96	486[d]	247
Grits	0.51	43.5[b]	84.3
Noodles	1.81	25.0[b]	12.8
Oatmeal	1.91	250[d]	130
Roll	0.48	19.0[f]	38.6
Whole wheat bread	0.74	182.4; 60.7[bej]	245; 81.0; 59.8
Cereals			
Cheerios®	1.08	107[f]	98.0
Entrees			
Cheese pizza	1.09	38.0[d]	33.9
Macaroni and cheese	1.30	23.1[d]	16.8
Pepperoni pizza	2.12	52.4[d]	23.7
Mashed potatoes with gravy	1.33	4.00[f]	2.01
Chili	5.20	22.0[d]	3.24
Miscellaneous			
Yeast	202	2000; 850[bdc]	8.88; 3.19; 3.94
Nuts			
Almonds, roasted, salted	44.0	821; 436[deh]	17.64; 8.89; 9.91
Peanuts, roasted, salted	175	1014; 820[ehi]	4.78; 3.68; 7.56
Walnuts, fresh	25.9	183; 173[eh]	6.07; 5.67
Pecans, fresh	20.0	206[h]	9.31

[a] Relative difference=(published biotin value analyzed values)/analyzed values); n = 1 homogenate analyzed per food.

[b] Biotin (2000).

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[c] Hardinge and Crooks (1960).

[d] Pennington (1989).

[e] Hands (2000).

[f] Pennington and Church (1985).

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