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Spectrophotometric Analysis of Urinary Iodine in 18-22 Year-Old Women in Central Minnesota

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Spectrophotometric Analysis of Urinary Iodine of 18-22 Year-Old Women in Central Minnesota

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Abstract

Iodine is an overlooked but incredibly important micronutrient, especially with regard to early fetus development. The United States does not mandate salt iodization, so widely consumed processed foods are not likely to contain iodized salt, which may potentially put the population at risk for developing iodine deficiencies. Therefore, the purpose of this study is to correspond iodine intake to iodine status and establish effective methods for determining a relationship between the two. Subjects (n=23) completed a food survey and supplied 50mL urine samples to compare an estimated average daily iodine intake based on foods over the course of a week and urinary iodine content determined via spectrophotometric analysis of the Sandell-Kolthoff reaction. Most subjects fell into the optimal intake and urinary iodine categories, but the survey inconsistently correlated intake and urine categories, most likely due to the use of a single spot urine sample. Future studies should investigate more representative intake surveillance, potentially through the use of 24-hour recalls or food diaries, and more representative urine sample collection methods, as with a 24-hour collection or a control for hydration.

Introduction

Iodine is an essential micronutrient that plays a vital role in human growth and development. Thyroxine and triiodothyronine are thyroid hormones that require iodine for their synthesis and regulate the body's metabolism, particularly protein synthesis and enzyme activity.[1] Iodine may also play a role in immune function and in preventing gastric cancer but is primarily associated with metabolism regulation.[1]

Iodine deficiency disorders (IDDs), such as cretinism and goiter, involve growth and developmental dysfunction because thyroid hormones are crucial for brain myelination during perinatal development.[1] IDDs typically first present as goiter but also involve "impaired

reproductive outcome, increased childhood mortality, decreased educability, and economic stagnation."[1] Because IDDs are so closely tied with proper growth and development, women of reproductive age must consume adequate amounts of iodine.

The Recommended Dietary Allowance (RDA) for nonpregnant adults is 150μg per day and 220μg per day for pregnant women.[1,2] Iodine occurs in seafood, some dairy products and breads, and very few processed foods containing iodized salt.[1,3] However, salt iodization, perhaps the most accessible method for ensuring sufficient iodine intake, is not mandatory in the United States; therefore, many processed foods do not contain iodized salt.[4] Salt iodization initially contributed to preventing thyroid disorders and was introduced in the 1920's. However, the only FDA-approved iodization vectors are KI and CuI, which are less stable than the World Health Organization (WHO)-preferred KIO_3.[4] The lack of mandated, stable iodization, compounded by a growing push for lower salt intake, is cause for growing concern regarding a possible resurgence of iodine deficiency in the United States.[4] As a result, some studies have recommended coordinating salt reduction and iodine deficiency control programs by increasing salt iodization and using iodized salt in the food industry while decreasing the total amount of salt consumed.[3,5]

The National Health and Nutrition Examination Survey (NHANES) III reports that, on average, Americans have adequate urinary iodine levels.[6] Iodine, surprisingly, remains a health problem, especially in vulnerable populations.[3] Women aged 20-39 years had the lowest iodine levels of those surveyed.[6] Hollowell, in particular, has traced iodine status since 1971 and noticed a more than 50% decrease in urinary iodine levels for pregnant women from 1971 to 1988 that has leveled-off to present day.[7]

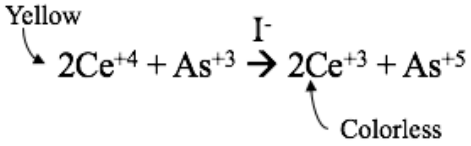
Pregnant women and children in an iodine-deficient region in China were supplemented with 400mg and 50mg of iodine, respectively. The probability of abortion, low head circumference babies, and mild or severe abnormalities in babies decreased for women who started iodine supplementation earlier in the pregnancy, particularly before the third trimester.[8] Half of the women aged 20-39 years in NHANES III had iodine levels below the median of approximately 130μg/L,[2,6] which may put them at risk for developing similar iodine deficiency-related problems in pregnancy. Therefore, there is some support for the use of supplemental iodine, as well as promotion of the importance of adequate iodine intake in pregnant women and women of reproductive age.[7]

Iodine levels are typically measured via urinary iodine, because 90% of iodine is excreted in the urine.[10] Urinary iodine is determined through spectrophotometric analysis of the Sandell-Kolthoff reaction.[10] Iodide catalyzes the redox reaction in which excess arsenious acid reduces ceric ions from yellow (Ce^{+4}) to a colorless (Ce^{+3}) (Scheme 1).[11] The yellow color and potential impurities are removed during a digestion step with ammonium persulfate before the analysis.[11] Any iodate in solution—such as in the calibration standards—is reduced to iodide during an incubation period with arsenious acid before initiating the reaction by adding the cerium.[12] The absorbance is read at a fixed time for each sample, so any change in absorbance is due to differences in iodine concentration.[10,11] The more iodine in a sample, the more ceric ion reduction and the lower the absorbance value.[10,11,12] Because the reaction is first-order[12] and is stopped at a consistent time, the natural log of the absorbance—a proxy for the product concentration—is plotted as a function of iodine concentration to give a linear calibration curve.

Numerous variations of this method—involving different digestion acids, specialized reaction chambers, and different instrumentation[10]—have been reported. The present method

involves ammonium persulfate digestion and a Spec-20 spectrophotometer[13,14] for safety and ease of collection. Ammonium persulfate is less hazardous than the typically used chloric acid digestion,[10,14] and using a spectrophotometer is more accessible than the microplate or Inductively Coupled-Plasma Mass Spectrometry methods.[13]

Few studies have linked iodine intake values with urinary iodine determination.[15] Therefore, the purpose of this study was to investigate how the iodine intake of women of reproductive age in the Midwest (a region with a history of iodine deficiency[4]) corresponded to actual urinary iodine levels and to develop effective methods for continued study.



Scheme 1. Sandell-Kolthoff reaction, adapted from Ref. 11.

Methods

Sampling

Upon receipt of Institutional Review Board approval, students in foundational chemistry lab courses and athletes from the College of St. Benedict Swimming and Diving Team were asked to provide urine samples and to complete an iodine intake survey. A total of 23 willing participants signed an informed consent form (Appendix A), supplied a 50mL aliquot of non-first void urine (first-void samples may underestimate iodine levels[16]), and completed the online survey (Appendix B). All participation was voluntary and was in no way linked with students' or athletes' relationships with the Chemistry or Athletic Departments.

Iodine Intake Survey

A survey[17] was provided via Internet link on the CSB/SJU Forms Manager (Appendix B). Participants provided information regarding the type of salt owned, supplement use, and a food survey involving iodine-rich foods consumed in a typical week. The approximate amount of iodine in 100g of each food product was determined using *Bowes and Church's Food Values*[18] and the Food and Drug Administration's "Total Diet Study Statistics on Element Results"[19] and was adjusted for the portion size given in the survey. The sum of each participant's estimated iodine from multivitamins (if necessary) and typical foods eaten during the week was divided by seven to approximate the amount of iodine consumed each day. Iodine intake levels were then categorized as severely deficient (<0.03mg/day), moderately deficient (0.02-0.74mg/day), mildly deficient (0.75-0.149mg/day), or optimal (>0.150mg/day) according to cut-off values determined by the American Thyroid Association.[20] The average of all subjects' intakes was also determined, excluding a single outlying value.

Solution Preparation

Solution preparation and urine analysis are based on one of the methods recommended by the Centers of Disease Control Ensuring the Quality of Urinary Iodine Procedures (EQUIP) Program. We focused on the spectrophotometric detection of the Sandell-Kolthoff reaction using an ammonium persulfate digestion.[13]

All solutions used 18.2mΩxcm water and were stored in acid-washed brown glass bottles.

Iodine Standards

Iodine standards were made from a 1L (1000mg/L) stock solution of potassium iodate (1.68g) in water. An intermediate stock solution (0.01L, 100mg/L) was used to dispense 50, 100, 150, 200, 250, 300μL of iodine solution into 100mL volumetric flasks to give 50, 100, 150, 200, 250, and 300μg/L standard solutions.

Ammonium Persulfate

Ammonium sulfate (22.82g) was dissolved in 100mL of water.

Arsenious Acid

Arsenic trioxide (1.25g) and sodium chloride (6.251g) were dissolved with heat in 5N sulfuric acid (50mL) and water up to the calibration line of a 250mL volumetric flask.

Ceric Ammonium Sulfate

Ceric ammonium sulfate (1.200g) was dissolved in 3.5N sulfuric acid (50mL).

Urine Analysis

Aliquot bottles containing urine samples were stored at -20°C until the time of analysis. All analyses were carried out using a Spectronic 20D+ digital spectrophotometer (Spectronic Instruments, Serial Number 3DU9232049).

Samples were allowed to reach ambient temperature and were vortexed to release particulates. Ammonium persulfate (1.00mL) was added to each of 20 test tubes, followed by the standards and samples (0.250mL). Each test tube was quickly vortexed, then all were placed in the heating block (Thermolyne Type 17600 Dri-Bath, Serial Number 17613714) atop a shaker table (Gyrotory Shaker-Model G2, Serial Number 880407244) at 75 RPM and were heated and shaked at 91-95°C for one hour.

Room-temperature test tube contents were transferred to corresponding cuvettes containing arsenious acid (3.5mL) and vortexed. After a 15-minute incubation, ceric ammonium

sulfate (0.400mL) was added to each cuvette and vortexed at 30 second intervals, recording the time of addition with a stopwatch. After exactly 30 minutes, sample absorbances were read on the spectrophotometer blanked with water.

Participants' urinary iodine concentration was interpolated from calibration curves made with the iodine standards from each run. Iodine levels were then categorized as insufficient (<100μg/L), sufficient (100-200μg/L), or excessive (>200μg/L) according to cut-off values recommended by the American Thyroid Association.[20]

Standard Additions

A sample was randomly selected for standard addition. The 50μg/L solution was added in 0.5, 1, and 1.5mL amounts to 2mL of sample. Absorbances for each were recorded at the end of three runs. The calculated concentration was then compared with the sample concentration determined in the assay using a one-sample, two-tailed t-test in Microsoft Excel.

Reproducibility

Three randomly selected samples were run in triplicate over three different sample runs. The relative standard deviations were then calculated in Microsoft Excel.

Time Sensitivity

The urine analysis method was repeated using only the 150μg/L standard solution. Absorbance readings were obtained after 15, 20, and 25 minutes to demonstrate the constant negative trend in absorbance over time. Readings were taken every ten and every five seconds within minutes 29-32 to determine how much variation in absorbance occurs during the time window around the 30-minute collection time. The absorbance values were then interpreted according to the calibration curve from the second sample run to determine a hypothetical standard deviation of iodine concentration values in Microsoft Excel.

Results

Intake Sufficiency

The average iodine intake was 184±89μg (n=22). Optimal (>150μg), mildly deficient (75-150μg), moderately deficient (30-75μg), and severely deficient (<30μg) iodine intake levels were determined using the Recommended Dietary Allowance for Iodine[1] and cut-off values from the American Thyroid Association.[20] The majority of subjects fell into the optimal iodine intake category (67%)—one subject even exceeded the Upper Intake level of 1.1mg per day and was not included in calculating the average intake. No subjects were in the severely deficient intake category, 12.5% were in the moderately deficient, and 21% were in the mildly deficient intake categories (Figure 1, Table 1). Only 28% of the subjects reported owning iodized salt (Figure 2), and five subjects received the RDA for iodine from multivitamins, which was added to their total iodine intake from food.

Table 1. Classification of subjects' urinary iodine sufficiency and iodine intake sufficiency (n=24).

	Urine				
Intake	Optimal	Mild	Moderate	Severe	Total
Optimal	13	3	0	0	16
Mild	3	2	0	0	5
Moderate	2	1	0	0	3
Severe	0	0	0	0	0
Total	18	6	0	0	24

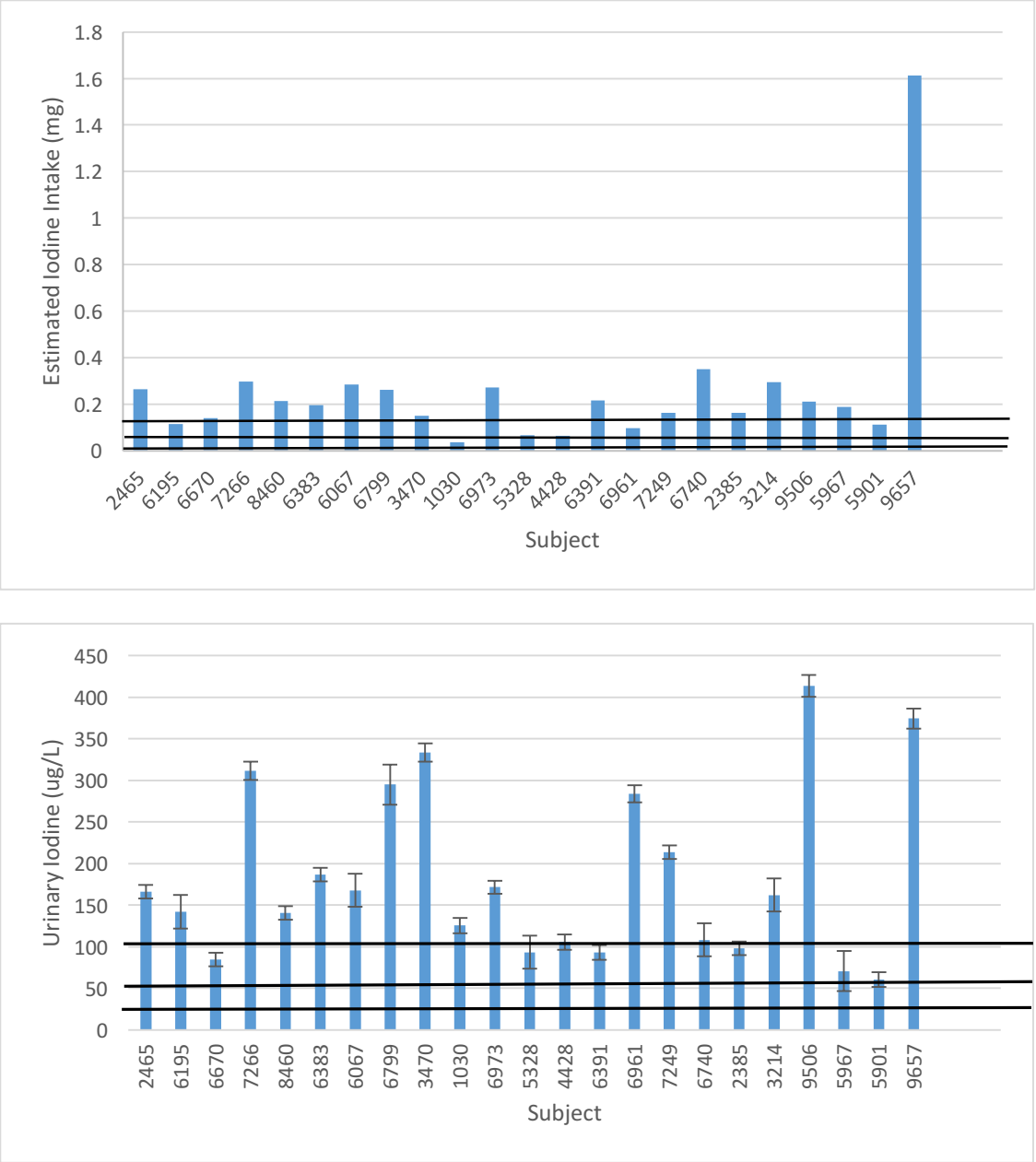


Figure 1. (a) Estimated iodine intake (mg) for each subject. Black lines indicate cut-off values for optimal intake (0.15mg), mild deficiency (0.075mg), and moderate deficiency (0.03mg).[19] (b) Urinary iodine concentration (μg/L) with an average percent error of 9.5%. Black lines indicate cut-off values for optimal nutrition (100μg/L), mild deficiency (50μg/L), and moderate deficiency (20μg/L).[20]

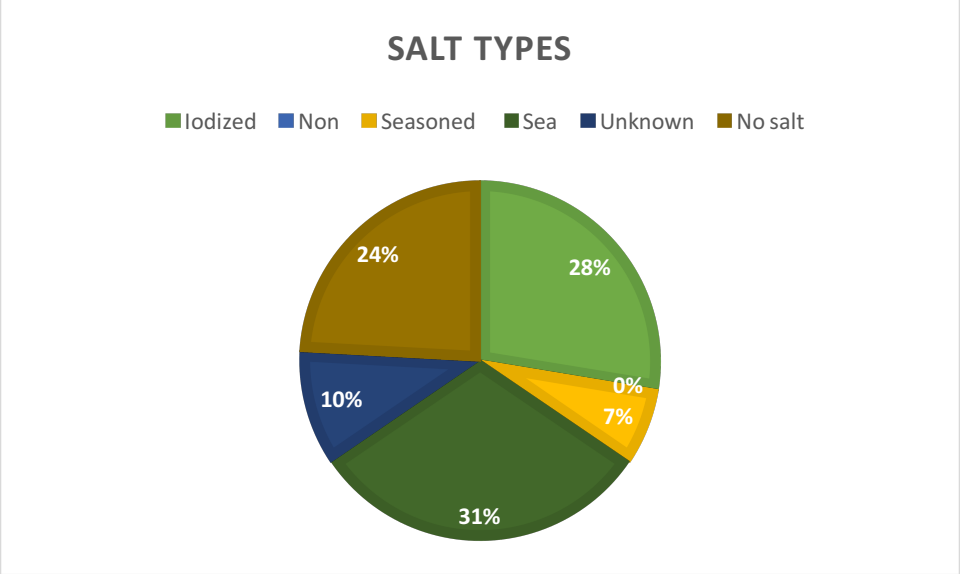


Figure 2. Proportion of types of salt owned by subjects.

Urinary Analysis Method Reliability

Calibration curves for every sample run resulted in R^2 values above 0.99, except the third run ($R^2 = 0.976$) (Figure 3). The average error associated with values determined from the calibration curves was 9.5%. Three samples were run in triplicate to test method reproducibility and had relative standard deviation values ranging from 16.6% to 24.3% (Table 2). Also, method accuracy was assessed using standard addition to a sample analyzed at the end of each run. Values calculated from the standard addition were not statistically different from the values determined for the original sample during the run (p=0.75) (Table 3).

The time sensitivity of the method was also determined by analyzing how iodine concentration varies during the two minutes around the 30-minute reading time. There is a gradual but constant negative relationship between absorbance and time (Figure 4, slope: -

0.0118, R^2: 0.990). The standard deviation of hypothetical iodine concentrations was only 3.8µg/L (Table 4).

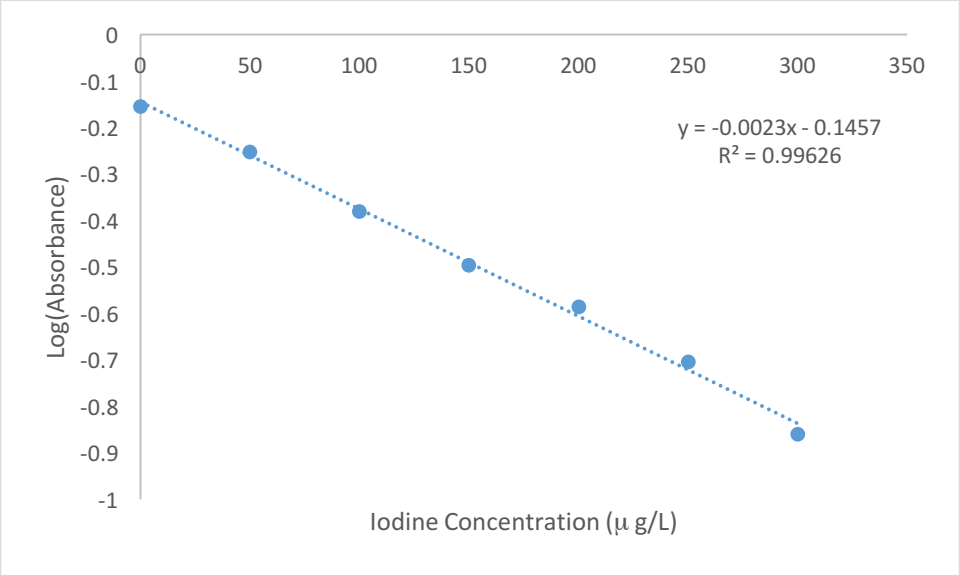


Figure 3. Sample calibration curve from second sample run.

Table 2. Method precision for three samples run in triplicate over three sample runs.

Sample	Average (µg/L)	Standard Deviation	Relative Standard Deviation (%)
3324	92.682	23	24
6973	202.35	34	17
6799	249.05	45	18

Table 3. Method accuracy as determined by iodine concentration calculated by standard addition. P-value indicates probability that the mean iodine value calculated from standard addition is the same as that determined during the run.

	Calculated I Concentration (µg/L)	**Spec-20 I Concentration (µg/L)**	**p-value**
Trial 1	71.08	83.46	
Trial 2	89.17	118.36	
Trial 3	155.43	76.22	
Average	105.23	92.68	0.75

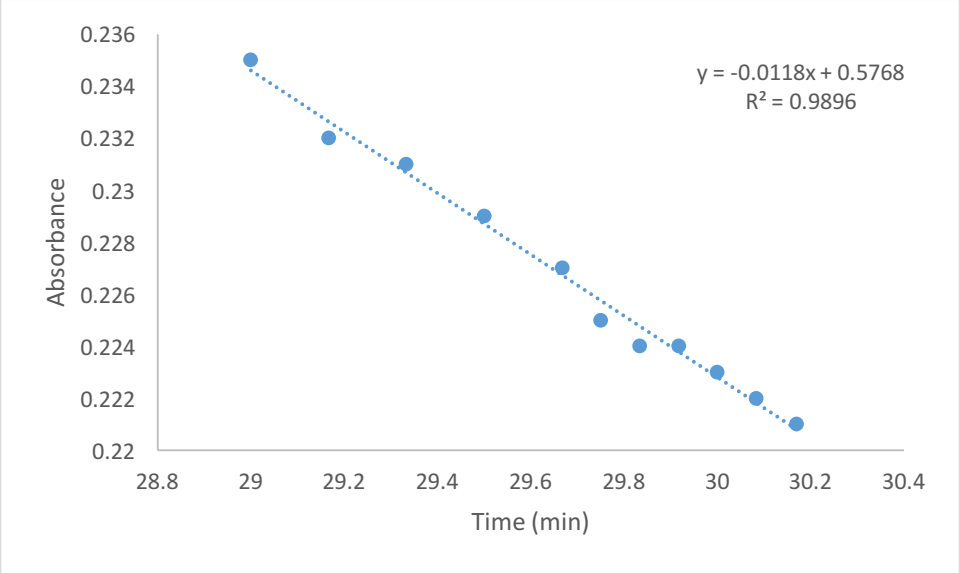


Figure 4. Absorbance readings as a function of time within a two-minute time window around the 30-minute reading time.

Table 4. Hypothetical iodine concentrations of a 150μg/L standard over time within a two-minute time window around the 30-minute reading time.

Time (min)	Hypothetical Iodine concentration (μg/L)
29	210.1
29.17	212.5
29.333	213.3
29.5	215.0
29.667	216.6
29.75	218.3
29.833	219.2
29.917	219.2
30.00	220.0
30.083	220.8
30.17	221.7
Standard Deviation	**3.8**

Urine Sufficiency

The median urinary iodine concentration for all participants was 151.99μg/L, and the average was 173.81±96μg/L (n=22). Optimal (>100μg/L), mildly deficient (50-100μg/L), moderately deficient (20-50μg/L), and severely deficient (<20μg/L) urinary iodine levels were determined according to cut-off values from the American Thyroid Association.[20] The majority of subjects fell into the optimal urinary iodine category (75%), the same subject with excessive intake also had excessive urinary iodine levels (>300μg/L) and was, again, excluded from the average and median calculations. No subjects fell into the severely or moderately deficient categories, and 25% were in the mildly deficient category (Figure 1, Table 1).

Intake and Urine Comparison

There was some discrepancy between iodine sufficiency as determined by intake and that calculated by urinary iodine. Of the subjects with optimal iodine intake, 81.3% also had optimal urinary iodine levels. The remaining 18.7% had mildly deficient urinary iodine levels. As iodine intake level decreased, however, the percentage of subjects with corresponding urine levels decreased. There was some agreement for subjects with mildly deficient iodine intakes who also had mildly deficient urinary iodine levels (40%); 60% of subjects with mildly deficient intakes had optimal urinary iodine levels. Subjects with moderately deficient iodine intakes had the worst agreement with urinary iodine levels, none of the subjects had moderately deficient urinary iodine levels; instead, 33% of the subjects had mild deficiency, and 67% had optimal urinary iodine levels (Table 1).

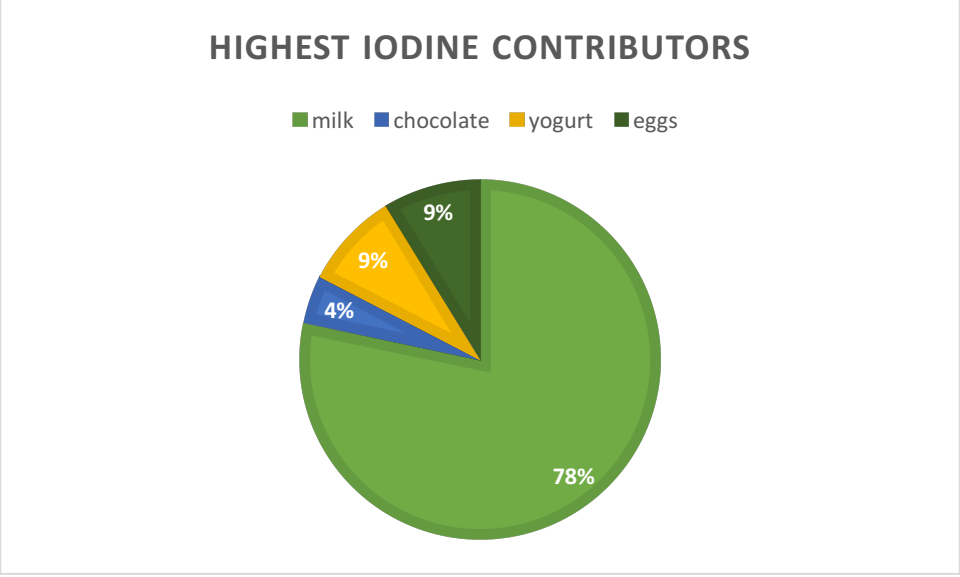


Figure 5. Highest iodine source-foods on the average intake survey. The majority of subjects had milk as their number one iodine source, followed by yogurt, eggs, and chocolate.

Discussion

Iodine Intake

The average iodine intake was 184±89μg (n=22), and the majority of subjects had optimal iodine intake levels (>150μg/day), considering that most of the United States population consumes sufficient iodine.[1-7] However, a previous study investigating iodine intake of students at the College of Saint Benedict reported that less than 50% of women consumed sufficient iodine, which is more in-line with the expected low iodine levels for women in the Midwest.[4,17] Differences in the number of subjects, sampling method, and interpretation of survey questions by subjects may have contributed to discrepancies in estimation of iodine intake sufficiency between the two surveys.

The majority of iodine intake came from dairy products (Figure 5), in which iodine is typically used as a disinfectant and feed supplement.[4] Although iodine is commonly available in seafood products, the low consumption of products like tuna, seaweed, sushi, and other marine products[1] prevents significant contributions of dietary iodine from these sources (Figure 5). It should also be noted that 10g of 0.001% iodized salt can provide up to 770μg of iodine.[1] An American's average sodium consumption of 3.4g per day would correspond to approximately 8.21g salt if all sodium were consumed as salt. Iodized salt accounted for approximately 42% of salt sales in 2009, and shaker-added salt accounts for only 11-12% of all salt consumption.[26] Therefore, an average American may expect to consume up to 29.2μg of iodine from salt, which may be enough to promote someone up to a more sufficient intake category. Because the intake survey did not directly calculate iodine contribution from salt intake due to difficulties in estimating quantity and iodization of salt, even those individuals now labeled as mildly deficient could, potentially, have sufficient iodine levels. Also, only 28% of subjects owned iodized salt

(Figure 2). The variability in use of table salt and in iodization level of the salt prevents an accurate estimation of the contribution of iodized salt to individual iodine intake. Future studies should determine the relative contribution of salt intake to total iodine intake to see if it plays a significant role in individual consumption.

Additionally, any iodine intake survey has limited reliability due to the variability of calculated iodine in foods.[22] The calculated iodine content of most foods in the FDA Total Dietary Study Statistics on Elemental Results had relatively low standard deviations of 0.1 or lower.[19] However, white bread and bagels had noticeably higher standard deviations at 1.611 and 1.042, respectively, which may indicate increased variability in iodine content in more highly processed foods or in foods that may come in different varieties, such as flavored or cultural breads. It is also particularly difficult to determine the iodine content of an individual's total diet because iodine is not assessed in most foods. Therefore, many foods may have unknown iodine values, preventing an accurate estimate of iodine intake.[22]

The applications of the present findings are limited. First, it is difficult for subjects to estimate their weekly food consumption. Second, as previously mentioned, the iodine content of foods is either unknown or variable in the foods for which it has been assessed. Third, iodine intake needs to be assessed daily to make connections between daily urine values. Finally, the limited sample size (n=23) prevents extrapolation to even the population at the College of Saint Benedict. Therefore, these data should not be used to assess the general iodine sufficiency of the tested population. These data were, instead, intended for comparison with individual urinary iodine categorization. However, errors in sampling prevented significant conclusions from being drawn from the comparison.

Urinary Analysis Method Reliability

The calibration curves for the iodine standards were the main metric used to determine the reliability of the method for predicting urinary iodine levels. Original calibration curves had R^2 values of 0.96 when separate cuvettes were used for analysis. The R^2 value was improved to 0.99 after using a single cuvette for analysis of all samples. Although an R^2 of 0.999 would generate more reliable urinary iodine results, the method was not further adjusted because of time and equipment constraints. Also, the percent error associated with the calculated urinary iodine values was only 9.5% and only accounted for potential categorization errors in five of the subjects (Figure 1).

The method reproducibility was determined by running three samples in triplicate and calculating the relative standard deviation. The high relative standard deviation values (Table 1) indicated a potential problem with consistency in the method. A variety of factors could have contributed to the lack of precision. Samples may have been inconsistently vortexed throughout the process, inconsistent heating may have resulted in varying tube volumes,[14] solutions (particularly the arsenious acid[12]) or urine samples may have settled-out over time, or the low R^2 value of the third run could have prevented consistent results across sample runs.

Method accuracy was assessed by running three standard additions to a randomly selected sample at the end of each sample run. There was no significant difference between the values calculated by the standard addition and those determined using the original sample in the spectrophotometer (Table 2). This indicates that there are no significant matrix effects, so the calibration curves are valid for determining iodine concentrations from the samples. There is some concern with the large difference between the values for Trial 3, which may have resulted

from the lower R^2 value of the calibration curve and increased error associated with calculated iodine concentrations. The accuracy of the method at the end of the run was of particular concern due to the time-sensitive nature of the method and negative correlation involved with the Sandell-Kolthoff reaction. However, the present data suggest that end-of-run accuracy is not as much of a concern as originally thought.

Also, the low standard deviation of hypothetical iodine concentrations (Table 4) indicates that there will not be much error associated with collecting absorbance readings in a two-minute time window around the suggested 30-minute reading time. Future studies should investigate the time-sensitive nature of the assay, however, because the present experiment was unable to conduct multiple trials or use a calibration curve from the same sample run as the analysis.

The present method is one of three common methods recognized by the CDC EQUIP Program for use in national labs.[13] However, the potential errors in reproducibility reported here indicate that more exact sample preparation methods and, perhaps, use of a more reliable instrument of analysis should be used to improve the reliability of the method.

Urine Iodine Content

The majority of subjects had sufficient iodine levels. Most people in the United States have sufficient iodine, but women aged 20 to 39 years had the lowest median urinary iodine concentrations at approximately 140μg/L,[2] which is lower than the median concentration of the subjects in this study (151.99μg/L). Optimal iodine nutrition is between 100 and 200μg/L.[21]

However, the results of this study cannot be used to definitively state an individual's iodine sufficiency, because too few samples were collected, and they were collected at various times throughout the day. There is some diurnal and day-to-day variations in urinary iodine concentration due to daily intake changes or hydration status.[16,23,24] Therefore, the most reliable

urinary iodine calculations come from multiple 24-hour urine collections.[16,19] Spot samples, such as those used in the present study, may be used, but the potential fluctuations in iodine with time mean that ten or more samples, collected at the same time, are required.[16] The accuracy of urinary iodine calculations can be further improved by using age- and sex-adjusted iodine:creatinine ratios to control for variations in hydration status among individuals.[16,23] Alternatively, a parallel urine specific gravity analysis could have indicated samples that did not fall within appropriate hydration parameters. The present study did not have the means to collect either a 24-hour sample or the number of recommended spot samples needed to determine subjects' definitive iodine status; therefore, all conclusions regarding subjects' urine iodine content are simple categorizations, not status statements. However, the purpose of the study was not to make definitive statements regarding individual status but rather to examine how the reported iodine intake categories corresponded with calculated urinary iodine categories.

Intake and Urine Comparison

The ability to predict urinary iodine sufficiency based on iodine sufficiency from intake decreased as iodine intake decreased. Subjects with mildly or moderately deficient iodine intakes were more likely to have higher urinary iodine levels. At first sight, this might imply that the survey could have underestimated iodine intake, especially considering it was unable to account for salt intake, and the reported iodine levels in different foods are variable.[22] However, it is more likely that the inability to draw significant conclusions arises from the use of non-representative sampling methods.

The survey estimated daily iodine intake based on food frequency over the course of a week. Urinary iodine can fluctuate with daily intake.[16,23,24] The seven-day average iodine intake, based on the survey, may not reflect a subject's iodine intake on the day of urine collection. The

half-life for organic iodine in tissues other than the thyroid is 12 days, but organic iodine in the thyroid has a half-life of 120 days.[25] The relatively short half-life of ingested iodine, therefore, requires either a daily account of iodine intake or a larger number of spot urine samples to better reflect a comparison between a subject's urinary iodine level and reported intake. It may be more feasible to accomplish this by conducting more urine samples, because most foods in food diaries could either have unknown or unreliable iodine amounts.[15,22]

Previous studies relating iodine intake based on food diaries and questionnaires to urinary iodine content have had similar difficulties establishing relationships between iodine intake and urinary output.[15] The authors attributed the lack of relationship to an uncomprehensive questionnaire; however, as in the present study, the problem may be more likely due to only one spot sample being collected in both studies. Ideally, the iodine content of all foods could be known and calculated from reference values and reported intake. However, it is more important to obtain adequate urine samples, because it is impossible to establish any sort of relationship between estimated iodine intakes and urinary iodine if the calculated urinary iodine content is not representative of an individual's typical iodine status.

The use of a single spot sample, the potential for errors in method precision, and the analysis of only three samples in triplicate prevents significant conclusions from being drawn regarding individual urinary iodine status. Furthermore, the iodine intake survey could leave out potentially unknown iodine-rich foods, does not account for amounts of iodized salt consumed, relies on self-reported intake amounts, and may not be representative of an individual's diet on the day of urine sample collection. Therefore, there is much room for error in establishing a relationship between iodine intake and urinary iodine content.

Much of the difficulty in establishing the relationship lies in the difficulty of finding subjects to provide a 24-hour urine sample and availability of food iodine data. Therefore, future studies should involve either a simple method for 24-hour collection or a control for hydration status (such as iodine:creatinine ratios or urine specific gravity), a control for urine collection time, and repeat spectrophotometric iodine concentration calculations. More comprehensive food reference values and a method for iodized salt intake estimation should also be created for more reliable estimation of iodine intake, and a 24-hour food log should correspond with the day of the urine sample collection. Finally, a controlled experiment in which iodine intake is standardized and compared with urine collection should be considered to assess the predictability of iodine intake and urinary iodine status.

Conclusion

The majority of subjects had optimal iodine intake and urinary iodine levels. However, it was more likely that a subject would have optimal urinary iodine content regardless of her iodine intake. Therefore, the present data did not result in a strong relationship between a subject's iodine intake and urinary iodine content. Numerous variables prevented drawing significant conclusions from the data, namely the lack of sufficient urine samples and the potential for unreliable estimation of iodine intake. Future studies may avoid this problem with a controlled iodine intake experiment or 24-hour urine and intake analyses.

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Appendix A: Consent Form

COLLEGE OF ST. BENEDICT/ST. JOHN'S UNIVERSITY
Spectrophotometric Analysis of Urinary Iodine of College-Aged Women in Central Minnesota

INTRODUCTION

You are invited to be in a research study about iodine sufficiency in the St. Ben's student population. This study is being conducted by Haley Chatelaine, under the supervision of Dr. Brian Johnson. You were selected as a possible participant because you are a college-aged woman at the College of Saint Benedict. We ask that you read this form and ask any questions you may have before agreeing to be in the study. You will be offered a copy of this form to keep for your records.

BACKGROUND and PROCEDURES

The purpose of this study is to identify the link between iodine status and iodine intake.
If you agree to be in this study, we would ask you to do the following things: complete a brief survey regarding iodine intake, then provide a urine sample, coded with the identification number you supply on the survey. The survey should take approximately 10 minutes, and the urine collection should take approximately 5 minutes. There is no further need for your participation once the urine samples are collected.

RISKS/BENEFITS

Participants may benefit by learning their personal iodine status—if they choose to inquire and forsake anonymity—after the urine analysis takes place but before ID numbers are deleted from the data. Society may also benefit from increased awareness of iodine nutrition within the St. Ben's community. This could spur dietary interventions and/or increased attention to iodine and other micronutrient nutrition.

There are no known physical risks of this study. However, the urine collection location in a public restroom could cause some psychological stress.

CONFIDENTIALITY

The records of this study will be kept private. Research records will be kept in a password-secured document; only the researchers will have access to the records. In any reports or public presentations, no information will be included that would make it possible to identify a participant.

VOLUNTARY NATURE OF THE STUDY

Your participation in this research study is completely voluntary. You may stop participating at any time without penalty or costs of any kind.

Your decision whether or not to participate will not affect your current or future relations with the College of Saint Benedict or Saint John's University. Additionally, your decision to

participate or not will not in any way affect your relations with the Swim and Dive Team or the Athletics Department.

CONTACTS AND QUESTIONS

The researcher conducting this study is Haley Chatelaine. You may ask any questions you have now. If you have questions later, you may contact her at (952) 738-2061 or hachatelaine@csbsju.edu; you may also contact her advisor, Dr. Brian Johnson at (320) 363-5314 or bjohnson@csbsju.edu. If you have additional questions you may also contact the CSB/SJU Institutional Review board chair, Bob Kachelski at irb@csbsju.edu.

STATEMENT OF CONSENT

I have read the above information. I have asked questions and have received answers. I consent to participate in the research.

Signature _____ **Date** _____

Printed name _____

Last 4 Digits of Student ID number _____

Appendix B: Iodine Intake Survey

Date

Major

Last 4 Digits of Student ID Number

My diet is high in iodine
- True
- False
- I don't know

Has your physician ever talked to you about limiting your iodine intake?
- Yes
- No

If yes, please indicate why:

Do you currently own table salt?
- Yes
- No

If yes, what kind of salt do you own?
- Iodized
- Non-Iodized
- Seasoned Salt
- Sea Salt
- I don't know

Where do you store your salt?

What type of container do you keep your salt in?
- Original container
- Glass salt shaker
- Metal salt shaker
- Other

When did you buy your salt?

How often do you add table salt to your food?

How often do you use salt in cooking or preparing foods?
- Never
- Rarely

Occasionally
Very Often
Always

Do you take a multivitamin?
Yes
No
If yes, please answer the following:
What brand is the multivitamin? (Example picture: One A Day)

What is the name of the multivitamin? (Example picture: Women's Formula)

Do you take any supplements?
Yes
No
If yes, please answer the following:
What is the brand of the supplement? (Example picture: Nature Made)

What is the type of supplement? (Example picture: iron)

In a typical week, how many of the indicated servings do you consume of each product?
Cow's milk (1 cup - equal to a milk carton)
Cheese (1 oz - size of a tube of lipstick)
Yogurt (1 cup - 1 container)
Ice cream (1/2 cup - size of a tennis ball)
Frozen yogurt (1/2 cup)
Sherbet (1/2 cup)
Cream cheese (1 tbsp - a spoonful)
Sour cream (1 tbsp)
Cottage cheese (1 cup - the size of a tennis ball)
White bread (1 oz - equal to one slice)
Wheat bread (1 oz)
Bagels (1 oz - equal to one large bagel half)
Pepperoni (1 oz - 5 pieces)
Fruit cocktail in syrup (1 cup)
Chocolate (2 oz - large bar)
Salami (1 oz - 3 slices)
Bologna (1 oz - 1 slice)
Beef (3 oz - size of a deck of cards)
Whole eggs (2 oz - equal to a large egg)

Seaweed (sometimes labeled as nori, wakame, kombu, or dulse) (2 tbsp - equal to 4 sheets)
Kelp (2 tbsp - equal to 4 sheets)
Sushi (3 oz)
Tuna (3 oz - the size of a checkbook)
Cod (3 oz)
Haddock (3 oz)
Halibut (3 oz)
Salmon (3 oz)
Tilapia (3 oz)
Lobster (3 oz)
Scallops (3 oz)
Shrimp (3 oz)
Crabmeat (3 oz)
Fish sticks (3 oz)

Signature Page

Project Title: Spectrophotometric Analysis of Urinary Iodine in 18-22 year-old Women in Central Minnesota

By Haley Chatelaine

_____
Brian Johnson, Professor of Chemistry

_____ _____
Amy Olson, Professor of Nutrition Richard White, Professor of Chemistry

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Kate Graham, Co-Chair: Department of Chemistry

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Emily Esch, Director, All College Thesis Program