ASSOCIATIONS BETWEEN OYSTER CONSUMPTION AND IRON, ZINC, AND CADMIUM CONCENTRATIONS IN GHANAIAN WOMEN

Alyssa Abreu

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ASSOCIATIONS BETWEEN OYSTER CONSUMPTION AND IRON, ZINC, AND CADMIUM CONCENTRATIONS IN GHANAIAN WOMEN

BY

ALYSSA MARIA ABREU

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

HEALTH SCIENCES

UNIVERSITY OF RHODE ISLAND

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DOCTOR OF PHILOSOPHY IN HEALTH SCIENCES DISSERTATION

OF

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UNIVERSITY OF RHODE ISLAND
2023
ABSTRACT

**Background:** Oysters are an excellent source of iron and zinc but may be contaminated by heavy metals such as cadmium. The micronutrient and metal content of smoked, dried oysters as well as the impact of oyster consumption on iron, zinc, and cadmium concentrations in Ghana is unknown.

**Objectives:** Our objectives were to 1) determine the micronutrient and metal content and health risk of dried, smoked oysters, 2) determine the associations between dietary iron intake with hemoglobin or ferritin concentrations and anemia, 3) determine the associations between oyster consumption and iron, zinc and cadmium concentrations in Ghanaian women living near the Densu Estuary.

**Methods:** We purchased one package of dried smoked oysters from the Densu estuary in the Bortianor community in the Greater Accra Region of Ghana. Dried oysters selected from the package were analyzed in bulk by inductively coupled plasma-mass spectrometry for 12 micronutrients and 5 heavy metals. We calculated a total hazard quotient and hazard index (>1 may indicate potential health risks). We also enrolled women in the Bortianor/Tsokomey area in Ghana in a 6-month, longitudinal pilot study, with 66 women who consumed oysters during the open season (oyster group) and 70 women did not consume oysters (non-oyster group). We collected data at two time points: 1) March 2020 (end of closed season) and 2) August 2020 (5 months into the open season). We collected the amount and frequency of dietary iron intake and oyster consumption using a 30-day food frequency questionnaire. We analyzed hemoglobin (to determine anemia prevalence), ferritin (to determine iron deficiency), zinc, and cadmium (ug/g creatinine) concentrations and used paired t-tests to analyze the difference between
seasons and used independent t-tests between groups. We also used a pathway analysis to understand the relationships between dietary iron intake or oyster consumption and zinc, cadmium, and serum ferritin or hemoglobin concentrations.

**Results:** The heavy metal contents of the dried oysters (mg/kg) were: Hg (8.96 ± 3.59), Cd (0.96 ± 0.26), As (4.46 ± 0.67), V (1.08 ± 0.32), and Pb (0.59 ± 0.11). The hazard index of dried oyster consumption was 44.62. At enrollment, mean±SD age was 34±8 y and BMI was 26±5 kg/m2. For women in the oyster group, Hb did not differ by season. The prevalence of anemia and iron deficiency (ID) in the oyster group was 78% and 54% in the closed season and 70% and 70% in the open season (anemia p=0.64, ID p=0.008). The prevalence of anemia and ID in the non-oyster group during the closed season was 83% and 44%, and 78% and 56% during the open season. For women in both the oyster and non-oyster group, zinc and ferritin concentrations were lower during the closed season compared to the open season and cadmium concentrations were higher during the open season compared to the closed season. There was no difference between groups in Hb, ferritin, zinc, cadmium and anemia prevalence during the closed or open season.

Oyster consumption was not associated with zinc or cadmium concentrations in continuous models. Cadmium and zinc did not mediate the association between dietary iron intake or oyster consumption and ferritin or hemoglobin concentrations.

**Conclusions:** The dried oysters are an excellent source of iron and zinc but contaminated with cadmium, lead, arsenic, vanadium, and mercury, which may increase health risks for consumers. We observed that during the open season women in the oyster and non-oyster group had lower ferritin and zinc and higher cadmium concentrations, indicating that there may be factors outside of oyster consumption and cadmium exposure
contributing to iron deficiency in this population. The prevalence of anemia and iron
deficiency anemia was high during both seasons in both groups of women. Iron
deficiency anemia is of great concern in this population and it is necessary to identify and
implement effective interventions to address anemia and iron deficiency at a community
level. Based on low cadmium concentrations among women who consumed oysters,
cadmium contamination of oysters may not be of great concern in this community and
oysters may continue to serve as a valuable nutrition source for women of reproductive
age in Ghana. However, future research should evaluate whether oyster consumption
increases the risk of exposure to other heavy metals such as mercury, and the association
with iron deficiency, which was high in this population.
ACKNOWLEDGMENTS

First, I want to extend my deepest appreciation to my advisor, Dr. Brietta Oaks. Your expertise, patience, and mentorship have shaped who I am as a researcher and what I have been able to accomplish. I am grateful for all of the hours you have spent providing feedback, sharing your experiences, and pushing me to reach my full potential.

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I also would like to express my sincere appreciation to all the participants who volunteered their time and contributed to this research. Without your willingness to participate and share your experiences, this study would not have been possible. Thank you to the collaborators, enumerators, and study staff who worked tirelessly to make this research possible. Also, thank you to my research assistants. Your dedication, commitment, and attention to detail have been extremely helpful in collecting and analyzing data.

Thank you to all of my peers and friends from the graduate program who have encouraged me and provided endless support and resources during the last six years of graduate school. Special thank you to Dr. Haley Parker, Andrea Ramirez, Dr. Katelyn Fox, Dr. Noereem Mena, Dr. Bridget Owens, and Dr. Helena Bentil. This would not have
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PREFACE

This dissertation follows a manuscript format. The manuscript #1 was prepared following the guidelines for submission to the *Journal of Food Composition and Analysis* and manuscripts #2-3 to the *Journal of Nutrition*. These manuscripts are in preparation and have yet to be submitted for review.
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MANUSCRIPT #1

This manuscript was prepared in accordance with author guidelines for the Journal of Food Composition and Analysis. It is currently in preparation for submission.

Title: Health risk assessment of heavy metals in dried smoked oysters from the Densu Estuary in Ghana

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Keywords:** Oysters; Heavy metals; Health Risk; Micronutrients; Mercury; Ghana

**Highlights:**

- Dried oysters from the Densu estuary in Ghana are an excellent source of zinc and iron
- Dried oysters were contaminated with cadmium, lead, arsenic, vanadium, and mercury
- Dried oysters from the Densu estuary in Ghana may be a health concern for consumers
- Mercury was the primary driver for increased health risk
ABSTRACT

Oysters are a good nutrition source of iron and zinc, but risk being contaminated with heavy metals. Micronutrient and heavy metal levels in dried oysters from the Densu estuary in Ghana are currently unknown. Our objectives were to 1) determine the micronutrient and heavy metal content of dried oysters collected from the Densu estuary in Ghana, and 2) determine the health risk of daily dried oyster consumption. We purchased one package of dried smoked oysters from the Densu estuary in the Bortianor community in the Greater Accra Region of Ghana. Dried oysters (n = 10) selected from the package were analyzed in bulk by inductively coupled plasma-mass spectrometry for 12 micronutrients and 5 heavy metals. We calculated a total hazard quotient and hazard index (>1 may indicate potential health risks). The heavy metal contents of the dried oysters (mg/kg) were: Hg (8.96 ± 3.59), Cd (0.96 ± 0.26), As (4.46 ± 0.67), V (1.08 ± 0.32), and Pb (0.59 ± 0.11). The hazard index of dried oyster consumption was 44.62. The dried oysters are an excellent source of iron and zinc but contaminated with cadmium, lead, arsenic, vanadium, and mercury, which may be increase health risks for consumers.
1. INTRODUCTION

Oysters are known to be a rich source of iron and zinc.\textsuperscript{1} Based on U.S. Pacific oysters, one medium-sized oyster (\~50 grams) is estimated to provide 14\% (2.56 mg) of the iron recommended dietary allowance (RDA) and 104\% (8.3 mg) of the zinc RDA for non-pregnant women.\textsuperscript{2} Oysters are also recommended as one of the best seafood choices by the FDA due to the low methyl-mercury content of Pacific oysters in the U.S.\textsuperscript{3}

Oysters are filter feeders that contribute to improved biodiversity and decreased water contamination.\textsuperscript{4,5} They accumulate nutrients, sediment, and pollutants from surrounding environments as they filter large amounts of water.\textsuperscript{4} Oyster filtration rates depend on environmental factors such as temperature and food and pollution levels. Bioaccumulation of toxic metals is likely in environments with high pollution, potentially leading to adverse health effects when oysters are harvested for consumption. Two metals of particular concern are cadmium and lead. Acute and chronic oral exposure to cadmium has numerous negative health effects including renal damage, increased risk for cardiovascular disease, and adverse fetal development.\textsuperscript{6,7} During pregnancy, high doses of cadmium (1–20 mg/kg/day) may cross and damage the placenta and negatively impact fetal development leading to lower birth weight.\textsuperscript{6} Lower doses of cadmium during pregnancy have also been associated with neurodevelopmental effects, although more research in human studies is needed.\textsuperscript{6} Lead accumulates throughout the lifetime and acute and chronic lead exposure is a significant health risk during pregnancy.\textsuperscript{8} During pregnancy, accumulated lead is released in the blood and negatively impact fetal development. Lead exposure may inhibit brain development and increase the risk of anemia, neurological function, and cancer.\textsuperscript{9}
Ghana is a low-middle income country in West Africa. In Ghana, oysters (Crassostrea Tulipa) have been harvested, processed, and consumed by community members along several Densu River estuary and other coastal areas. Fresh oysters are sold in various sized packets ranging in price from 1-5 GH₵ (USD $0.13 – $0.67) making them an affordable and available food that could be consumed during pregnancy when nutritional demands and risk of micronutrient deficiencies are increased. Oyster consumption, however, could also lead to negative health outcomes due to potential exposure to heavy metals. Cadmium and lead are often found in the soil and water near battery manufacturing and recycling plants. Due to manufacturing practices, several areas in Ghana are at risk for cadmium and lead as environmental exposures. The Hazardous and E-Waste act was passed in Ghana in 2016, which requires companies to pay an electronic waste levy. The levy provides funds to manage electronic waste and mitigate the effects of electronic waste on health and the environment. However, while site remediation is possible, there are currently no regulations in Ghana to require companies to address environmental contamination directly. In 2017, a study by Gottesfeld et al. determined that environmental contamination was still high in Ghana around manufacturing facilities.

Because oyster harvesting communities include oyster consumption as a regular part of their diet, research is needed to determine the micronutrient and heavy metal concentrations of oysters in Ghana and the risk for adverse health outcomes. The objectives of this study are to 1) determine the micronutrient and heavy metal content of dried oysters collected in the Densu estuary, Ghana, and 2) determine the health risk of daily consumption of dried oysters.
2. METHODS

2.1 Sample Collection

We purchased one package of dried smoked oysters that was packed by oyster harvesters on June 13, 2019, near the Densu estuary in the Bortianor community in the Ga South municipality in the Greater Accra Region of Ghana. An individual, dried oyster weighed 0.59 g (Graphic 1) on average and one package contained a total of 63 g of dried oysters.

2.2 Sample Preparation and Analysis

A sample of 10 oysters was selected from the package for analysis in February 2020. Oysters were individually powdered using a SPEX 8000 Mixer/Mill using a tungsten carbide vessel. The powdering vessel was cleaned with quartz sand in between each oyster powdering to ensure no contamination between oysters. Approximately 50 mg of each powdered oyster was placed into individual Teflon vials for acid digestion. 1 mL of trace metal grade 70% nitric acid was pipetted into each vial and then each vial was capped and warmed on a hot plate at ~90°C for 3 days to allow the reaction to come to completion. Vials were then cooled to room temperature and 1.5 mL of hydrogen peroxide was added to each vial. The vials were left uncapped on a hot plate at ~50°C for 24 hours. Approximately 37.5 mL of milli-Q H₂O was added to each sample for a total solution volume of 40mL to achieve a dilution factor of ~800. One of the ten samples was split into four 10 mL aliquots, three of which were spiked with a solution of known trace element concentration to produce a set of standards.

The elements arsenic (As), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), mercury (Hg), lead (Pb), magnesium (Mg), manganese
(Mn), nickel (Ni), phosphorus (P), potassium (K), selenium (Se), vanadium (V), and zinc (Zn) were analyzed by solution inductively-coupled plasma mass spectrometry (Thermo X-Series II quadrupole). The elements As and Se were analyzed using a collision cell to remove ArCl and ArAr interferences at the masses of the analytes. Abundances of these elements in the spiked oyster sample were determined by the method of standard additions, using the spiked and unspiked aliquots, which then were used as the calibration standard suite for the other unknown oyster solutions. For both sessions, sample one was analyzed periodically throughout the session as a monitor of instrumental drift, and drift was corrected using an external solution drift correction method. Samples were analyzed in duplicate, and average reproducibility of duplicate analyses following drift correction was ≤3% relative standard deviation for all elements except Ni and Cd, which averaged 6% relative standard deviation.

We calculated the means and percentages of micronutrients and heavy metals per gram of oyster tissue, and then expressed values in one serving (30 g, consistent with the Reference Amounts Customarily Consumed for dried fish products published by the FDA) and 100 g of dried oysters. We analyzed the percent of the recommended dietary allowance (RDA) provided by 1 serving of dried oysters (30 g) for non-pregnant, pregnant, and breastfeeding women. We calculated the percentage of oyster samples that were above the EU/FAO maximum concentrations for cadmium, lead, and mercury.

2.2 Health Risk Assessment

We assessed the health risks of consuming dried smoked oysters from the Densu Estuary in Ghana based on the Estimated Daily Intake (EDI) of metals, Target Hazard Quotient (THQ), and Hazard Index (HI). Dried, smoked oysters are a new method of
processing and consuming oysters. One serving of dried oysters is likely to be recommended and included on packaging as it becomes a more common form of consumption. Therefore, we calculated estimated daily intake using one serving of dried oysters (30 g). Estimated daily intake was calculated as follows:

\[ \text{Estimated Daily Intakes, } EDI = \frac{\text{Metal conc in dried oyster} \times \text{Average daily oyster consumption}}{\text{Average body weight}} \]

To assess the non-carcinogenic, chronic health risk of consuming the dried oysters, the target hazard quotient was calculated using the estimated daily intake and oral reference dose (RfD). A THQ > 1.00 for an individual metal is considered a hazard to health because the level of metal exposure from the estimated daily intake of dried oysters exceeds the oral reference dose for that metal. The RfD (mg/kg/day) is derived from the U.S. Environmental Protection Agency (USEPA) and indicates the exposure dose that will not result in increased chronic health risks. The RfD values used to calculate the THQ are shown below (Table 1).

\[ \text{Target Hazard Quotient, } THQ = \frac{EDI}{RfD} \]

A hazard index (HI) was calculated to present the cumulative potential health risk of daily dried oyster consumption. An HI > 1 is indicative of a possibility of chronic health risks related to oyster consumption.\(^{18}\)

\[ HI = THQ_{\text{cadmium}} + THQ_{\text{cadmium}} + THQ_{\text{cadmium}} \]

3. RESULTS

3.1 Heavy Metal and Micronutrient Concentrations
All micronutrient and heavy metal concentrations were above the limit of detection. The mean micronutrient content ranged from 0.170 mg/100 g for nickel to 881.12 mg/100 g for phosphorous (Table 2).

Notably, one serving of dried oysters contained 741% of the RDA and 148% of the upper limit of zinc for non-pregnant women, 247% of the RDA for copper, and 339% of the RDA for chromium. One serving of dried oysters was also an excellent source (>20% DV) of phosphorous (38%), magnesium (51%), iron (67%), manganese (89%), and selenium (125%) (Figure 1).

The mean heavy metal content (mg/kg) were: lead (0.59 ± 0.11), cadmium (0.96 ± 0.26), arsenic (4.46 ± 0.67), mercury (8.96 ± 3.59), and vanadium (1.08 ± 0.32) (Table 3).

3.2 Health Risk Assessment

One serving of dried oysters contained 280% of the provisional tolerable intake (PTI) per week of mercury, and 482% of the minimal risk level for each heavy metal for a 60 kg person for cadmium, 743% for arsenic, and 541% for vanadium.19,20 The EPA and Joint FAO/WHO Expert Committee on Food Additives (JEFCA) determined that there is no safe level of lead exposure and it is recommended to avoid foods or beverages contaminated with lead, however, the FDA established an interim reference level (IRL) of 12.5 μg/day (Table 4) and the dried oysters contained 17.61 μg/serving (141% IRL).21

All of the 10 oyster samples collected from the Densu Estuary in Ghana exceeded the EU/FAO regulatory maximum limit for mercury, 40% for cadmium, and none of the oysters sampled exceeded the limit for lead (Table 5). The EDI (mg/kg per bodyweight/day) of mercury was highest (44.1 x 10⁻⁴) compared to cadmium (4.7 x 10⁻⁴) or lead (2.9 x 10⁻⁴). The total HI was 44.62, after accounting for average body weight and
consumption. This is much greater than 1.0, indicating that dried oyster consumption may present a significant health risk. The THQ value for cadmium and lead contributed < 2% of the HI, while the THQ for mercury was high (44.06) and accounted for nearly 99% of the HI (Table 6).

4. DISCUSSION

Our aim for this study was to determine the micronutrient and heavy metal content of dried oysters from the Densu estuary, Ghana. We found that one serving of dried oysters was an excellent source (>20% DV) of zinc, copper, chromium, selenium, manganese, and iron. However, dried oysters exceeded the upper limit for zinc and were contaminated with cadmium, lead, arsenic, mercury, and vanadium. Daily oyster consumption may also present a significant health hazard, primarily due to the excessive mercury exposure.

Dried oysters are high in zinc and iron may help in alleviating zinc and iron deficiencies in communities where oyster harvesting and consumption is common. Adequate zinc intake during pregnancy decreases the risk for stunting, which is positively associated with child mortality. Adequate iron intake may reduce the risk of anemia during pregnancy, which has been associated with adverse birth outcomes including preterm birth and low birth weight. However, our findings show that one serving of dried oysters was 741% of the zinc RDA for women. It is unlikely that high intake of zinc from food sources will have significant negative health impacts. Supplemental zinc intake of 50-150 mg/day has been associated with GI distress and acute zinc toxicity may lead to nausea, vomiting, GI distress, or diarrhea. It is possible that consistent intake of high levels of zinc, including from food sources, may contribute to copper deficiency, altered
iron function, and anemia. In communities where oyster consumption is consistent and high, copper levels and iron status should be regularly monitored.

We found that this sample of dried oysters from Ghana was high in cadmium, lead, arsenic, mercury, and vanadium. This is of great concern for women of child-bearing age who may become pregnant. These toxic metals have been associated with adverse birth outcomes and delayed childhood cognitive development. The lack of mercury in oysters in the U.S. has been used by the FDA and EPA to categorize oysters as a best seafood choice, including for those who are pregnant. However, our findings show that dried oysters from the Densu estuary in Ghana do contain high levels of mercury, in addition to cadmium, lead, arsenic, and vanadium. Mercury was the primary driver of the high hazard index, which is consistent with previous reports. Our findings oppose the FDA and EPA categorization of oysters within the best seafood choice category due to potential mercury exposure. Recommendations for oyster consumption should be designed to better reflect health risks for specific populations and geographic locations. Further monitoring of heavy metal concentrations is essential due to the variability of oyster content globally.

It is possible that the heavy metals may not be completely bioavailable during consumption. Metal concentrations in foods are present in soluble and insoluble forms, influencing bioavailability, with soluble forms being more bioavailable to consumers. Solubility can be influenced by pH, various enzymes, and food processing. A study conducted in oysters from France found that 44-75% and 50-80% cadmium and zinc were bioavailable to consumers due to the insoluble form of the metals present in the oysters. Our findings show that in only one serving of dried oysters, cadmium
concentrations provided 452% of the minimal risk level. Even with decreased bioavailability, women who consume one serving of dried oysters are still likely to exceed the minimal risk level. It is possible that the process of smoking oysters contributes to heavy metal contamination. In Ghana, there are currently no regulations for the smoking industry despite 95% of fish products undergoing the smoking process and the use of a variety of ovens, fuels, and inconsistent smoked fish quality. Further research is needed on the effect of the smoking process on heavy metal contamination in oysters.

Limitations of this study include the low sample size; the sample may not be representative of the risk through the seasons or in different parts of the estuary. However, this is the first study to analyze dried oysters from the Densu estuary in Ghana. We also were limited to analyzing dried smoked oysters, which are less commonly consumed than fresh oysters in these areas. However, the micronutrient and heavy metal content per individual dried oyster is likely underestimated due to nutrient loss experienced during the drying process. A limitation of this research is that there are no known estimates of daily intake of dried oysters. Our hazard index may be an underestimate of risk, as women may consume greater than one serving in a sitting. Future research should determine usual intake of dried oysters over time and risk for adverse health outcomes. While the serving size may overestimate the concentrations of fresh oysters, women may be exposed to increased amounts of micronutrients and heavy metals with increased frequency of consumption. A strength of our study was the use of ICP-MS for analysis, increasing the accuracy of the micronutrient and heavy metal concentration analyzed and considered the gold standard for analysis. Research is needed
to determine the effect of oyster consumption in Ghanaian communities on micronutrient and heavy metal concentrations and adverse health outcomes. This research also supports further sampling on dried and fresh oysters in Ghana, specifically during different seasons, temperatures, and in additional geographical locations. Oyster consumption recommendations may need to be adjusted by geographical location and environmental exposure. More importantly, regulations in Ghana should be implemented to reduce and remove the environmental pollution that contaminates the oysters. After addressing environmental pollution, oysters may serve as a highly valuable food source that can provide critical micronutrients for women of reproductive age.
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### Table 1. RfD values for selected metals.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Reference Dose (RfD) values, mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.001</td>
</tr>
<tr>
<td>Lead¹</td>
<td>0.0035</td>
</tr>
<tr>
<td>Mercury¹</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

¹RfD values for lead and mercury were not available from the IRIS (USEPA, 2018).

### Table 2. Micronutrient Content of Oysters (n=10) from the Densu Estuary in Ghana

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Amount per kg of sample</th>
<th>1 dried oyster (per 0.59 g)</th>
<th>1 serving (per 30 g) dried oysters</th>
<th>100 g dried oyster (per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron, mg</td>
<td>402.20 ± 106.98</td>
<td>0.24</td>
<td>12.06</td>
<td>40.22</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>1976.20 ± 757.26</td>
<td>1.17</td>
<td>59.29</td>
<td>197.62</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>5307.30 ± 654.68</td>
<td>3.13</td>
<td>159.22</td>
<td>530.73</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>2025.20 ± 868.13</td>
<td>1.19</td>
<td>60.76</td>
<td>202.52</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>8811.30 ± 1887.60</td>
<td>5.20</td>
<td>264.34</td>
<td>881.13</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>2548.20 ± 513.20</td>
<td>1.50</td>
<td>76.45</td>
<td>254.82</td>
</tr>
<tr>
<td>Manganese, mg</td>
<td>53.57 ± 17.30</td>
<td>0.03</td>
<td>1.61</td>
<td>5.36</td>
</tr>
<tr>
<td>Cobalt, mg</td>
<td>88.69 ± 32.59</td>
<td>0.05</td>
<td>2.66</td>
<td>8.87</td>
</tr>
<tr>
<td>Nickel, mg</td>
<td>1.70 ± 0.42</td>
<td>0.001</td>
<td>0.051</td>
<td>0.170</td>
</tr>
<tr>
<td>Copper, μg</td>
<td>74.21 ± 20.90</td>
<td>43.78</td>
<td>2226.30</td>
<td>7421.00</td>
</tr>
<tr>
<td>Chromium, μg</td>
<td>2.83 ± 0.50</td>
<td>1.67</td>
<td>84.90</td>
<td>283.00</td>
</tr>
<tr>
<td>Selenium, μg</td>
<td>2.29 ± 0.31</td>
<td>1.35</td>
<td>68.55</td>
<td>228.50</td>
</tr>
</tbody>
</table>

### Table 3. Heavy Metal Content of Oysters (n=10) from the Densu Estuary in Ghana

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>mg/kg</th>
<th>Mean ± SD</th>
<th>1 dried oyster (per 0.59 g)</th>
<th>1 serving (per 30 g) dried oyster</th>
<th>100g dried oyster (per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium, μg</td>
<td>0.96</td>
<td>0.26</td>
<td>0.57</td>
<td>28.91</td>
<td>96.37</td>
</tr>
<tr>
<td>Lead, μg</td>
<td>0.59</td>
<td>0.11</td>
<td>0.35</td>
<td>17.61</td>
<td>58.70</td>
</tr>
<tr>
<td>Mercury, μg</td>
<td>8.96</td>
<td>3.59</td>
<td>5.29</td>
<td>268.77</td>
<td>895.90</td>
</tr>
<tr>
<td>Arsenic, μg</td>
<td>4.46</td>
<td>0.67</td>
<td>2.63</td>
<td>133.65</td>
<td>445.50</td>
</tr>
<tr>
<td>Vanadium, mg</td>
<td>1.08</td>
<td>0.32</td>
<td>0.001</td>
<td>32.48</td>
<td>0.11</td>
</tr>
</tbody>
</table>
### Table 4. Exposure limits for Arsenic, Cadmium, Lead, Mercury, and Vanadium by Agency

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Organization</th>
<th>Year</th>
<th>Oral Exposure Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>JEFCA</td>
<td>2011</td>
<td>PTI withdrawn, no longer considered health protective</td>
</tr>
</tbody>
</table>
|             | ATSDR        | 2007 | Acute MRL: 5 μg/kg/day  
|             |              |      | Chronic MRL: 0.3 μg/kg/day |
| Cadmium     | JEFCA        | 2011 | PTI: 25 μg/kg/bw per month |
|             | ATSDR        | 2012 | Acute-duration MRL: 0.5 μg/kg/day  
|             |              |      | Chronic-duration MRL: 0.1 μg/kg/day |
| Lead        | JEFCA        | 2011 | PTI withdrawn, no longer considered health protective |
|             | ATSDR        | 2022 | MRL Not derived due to serious adverse effects at low levels |
|             | FDA          | 2020 | Maximum intake from food: 12.5 μg/day |
| Methylmercury| JEFCA   | 2004 | PTI: 1.6 μg/kg/bw per week |
|             | ATSDR        | 2022 | Insufficient data for MRL derivation |
| Vanadium    | ATSDR        | 2012 | Intermediate-duration MRL: 0.01 mg/kg/day |
|             | IOM          | 2001 | UL: 1.8 mg/day |

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; FDA, U.S. Food and Drug Administration; IOM, Institute of Medicine; JEFCA, Joint FAO/WHO Expert Committee on Food Additives; MRL, Minimal Risk Level; PTI, Provisional tolerable intake; UL, Upper Tolerable Limit

### Table 5. Percentage of Oyster Samples (n=10) from the Densu Estuary that exceeded European Union/Food and Agricultural Organization Regulatory Limits for Cadmium, Lead, and Mercury

<table>
<thead>
<tr>
<th>Regulatory limit</th>
<th>Oyster samples with heavy metals above maximum levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium 1.0 mg/kg wet weight</td>
<td>40%</td>
</tr>
<tr>
<td>Lead 1.5 mg/kg wet weight</td>
<td>0%</td>
</tr>
<tr>
<td>Mercury 0.5 mg/kg wet weight</td>
<td>100%</td>
</tr>
</tbody>
</table>

\(^1\)EU/FAO regulatory limits for bivalve mollusks were not available for arsenic or vanadium
<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>EDI (mg/kg BW/day)</th>
<th>THQ</th>
<th>HI</th>
<th>% of metal in HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>4.7 x 10^-4</td>
<td>0.47</td>
<td>1.05%</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>2.9 x 10^-4</td>
<td>0.08</td>
<td>0.18%</td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td>44.1 x 10^-4</td>
<td>44.06</td>
<td>98.77%</td>
<td></td>
</tr>
<tr>
<td>Cumulative potential health risk</td>
<td>44.61</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EDI, Estimated daily intake; HI, Hazard Index; THQ, Target Hazard Quotient
GRAPHICS AND FIGURES

Graphic 1. Dried oyster collected near the Densu estuary in the Bortianor community in the Ga South municipality in the Greater Accra Region of Ghana
Figure 1. Percent of Recommended Dietary Allowance (RDA) from 1 Serving of Dried Oysters (30 g)
MANUSCRIPT #2

This manuscript was prepared in accordance with author guidelines for the Journal of Nutrition. It is currently in preparation for submission.
Abstract

**Background:** Oysters are a rich source of iron and consumption may increase markers of iron status and reduce the risk of anemia and iron deficiency. It is unknown how oyster consumption affects iron status among women living near oyster harvesting communities in Ghana.

**Objectives:** To determine the association between dietary iron intake or oyster consumption and serum ferritin and hemoglobin concentrations, and anemia in Ghanaian women.

**Methods:** We enrolled women in the Bortianor/Tsokomey area in Ghana in a 6-month, longitudinal pilot study. A total of 66 women consumed oysters during the open season and were included in the oyster group and 70 women did not consume oysters and were in the non-oyster group. We collected data at two time points: 1) March 2020 (end of closed season) and 2) August 2020 (5 months into the open season). We collected the amount and frequency of dietary iron intake and oyster consumption using a 30-day food frequency questionnaire. We analyzed hemoglobin (Hb) and ferritin concentrations and used paired t-tests to analyze the difference in Hb concentrations and McNemar’s test for anemia (Hb < 12.0 g/dL) and iron deficiency (ID) (ferritin < 15ng/ml) between seasons and used independent t-tests between groups.

**Results:** At enrollment, mean±SD age was 34±8 y and BMI was 26±5 kg/m². For women in the oyster group, Hb did not differ by season and ferritin concentrations were lower during the open season compared to the closed (closed season ferritin mean±SD: 14.6±2.0, open season ferritin: 8.9±1.3, p=0.0001). The prevalence of anemia and ID in the oyster group was 78% and 54% in the closed season and 70% and 70% in the open...
season (anemia p=0.64, ID p=0.008). For women in the non-oyster group, Hb did not differ by season and ferritin concentrations were lower during the open season compared to the closed (closed season ferritin mean±SD: 17.2±2.3, open season ferritin: 12.1±1.7, p=0.0006). The prevalence of anemia and ID in the non-oyster group during the closed season was 83% and 44%, and 78% and 56% during the open season. There was no difference between oyster and non-oyster groups in Hb, ferritin, and anemia prevalence during the closed or open season.

Conclusions: We observed that during the open season, women who consumed oysters as well as women who did not consume oysters had low ferritin concentrations, indicating that oyster consumption may not decrease the risk of iron deficiency and anemia. There may be factors outside of oyster consumption contributing to iron deficiency in this population. The prevalence of anemia and iron deficiency anemia was high during both seasons in both groups of women. Iron deficiency anemia is of great concern in this population and it is necessary to identify and implement effective interventions to address anemia and iron deficiency at a community level.
ASSOCIATIONS BETWEEN DIETARY IRON INTAKE AND OYSTER CONSUMPTION WITH IRON DEFICIENCY ANEMIA AMONG GHANAIAN WOMEN

INTRODUCTION

Iron is a critical micronutrient before and during pregnancy for maternal and child health outcomes.\(^1\) Iron deficiency anemia (IDA; presence of both anemia and iron deficiency) increases the risk for adverse birth outcomes such as low birth weight, stunting, and preterm birth.\(^2,3\) Oysters are considered a good source of iron,\(^4\) which may reduce the risk of iron deficiency anemia during pregnancy. Based on studies conducted in U.S. Pacific oysters, *Crassostrea gigas*, one medium-sized oyster (~50 grams) provides 14% (2.56 mg) of the iron recommended dietary allowance (RDA) for non-pregnant women.\(^5\)

Ghana is a low-middle income country in Sub-Saharan Africa where over 13% of women are iron deficient.\(^6\) Oysters have historically been consumed year-round in Ghanaian communities located near oyster harvesting areas and coastal fisheries.\(^7\) In the Bortianor/Tsokomey area in Ghana, oyster harvesting is common.\(^7\) In Ghana, oysters are consumed in high quantities among oyster harvesters. We previously determined that smoked, dried oysters collected near the Densu Estuary in Ghana were high in iron and zinc, but contaminated with heavy metals such as mercury, lead, and cadmium (manuscript in preparation, Chapter 1 of this dissertation). In November 2017, oyster harvesters (primarily women) living near the Densu estuary in Ghana, with support from stakeholders, instituted an annual, 5-month closed season for oyster harvesting, allowing
Women who harvest oysters from this community have stated in pilot qualitative interviews that their oyster consumption ranges from 20-200 oysters per week during the oyster harvest season. During closed season, oysters are not harvested and consumption is expected to be low or nonexistent. From a research point of view, this means the impact of oysters on iron concentrations in women can be measured with a clear washout period (i.e., the 5-month closed season) in a natural community setting. While the practice of annual closed seasons is important for increasing income and improving biodiversity, it may also negatively impact nutritional status and the food security of women in oyster harvesting communities. A closed season may result in decreased oyster consumption, leading to lower iron concentrations and increased risk for iron deficiency anemia.

Two markers of iron status include hemoglobin and serum ferritin concentrations. Hemoglobin is a marker of anemia (Hb < 12 g/dl), although hemoglobin concentrations are multifactorial and only 40% of people with anemia have IDA. The cutoff for anemia using hemoglobin concentrations is < 12 g/dL for non-pregnant women and < 11g/dL for pregnant women. The WHO also determined that an anemia prevalence of ≥ 40% as a public health emergency. Serum ferritin is used as a reliable clinical marker of IDA. The WHO identified the serum ferritin threshold for iron deficiency as < 15 ng/mL and recommends that inflammation should be assessed along with ferritin concentrations, since the presence of inflammation may overestimate ferritin concentrations.

It is unknown how oyster consumption affects iron status. Currently, there is not enough information to know whether women of reproductive age should be encouraged or discouraged to eat oysters. Our objectives were to 1) determine the association
between dietary iron intake, serum ferritin and hemoglobin concentrations, and anemia in Ghanaian women, and 2) determine the association between oyster consumption, serum ferritin and hemoglobin concentrations, and anemia in Ghanaian women. Our hypotheses are presented in Figure 1.

METHODS

Study Design, Setting, and Participants

We conducted a 6-month, longitudinal pilot study in women of reproductive age and collected data at two timepoints (Figure 2). The first timepoint was in March 2020, near the end of the closed season of oyster harvesting (November-March), and the second timepoint was in August 2020 during the open season of oyster harvesting. We recruited and enrolled women residing near the oyster estuary in the Densu River basin in the Bortianor/Tsokomey area in Ghana, with the support of the Densu Oyster Pickers Association (DOPA). The study was approved by the University of Rhode Island IRB (BI1920-005) and the Ghana Health Service Ethical IRB (GHS-ERC 012/12/19). We obtained informed consent from all study participants and updated our protocol and screening process during COVID-19.

Eligible women were aged between 18 and 49 years, not pregnant, planned to continue living in the area for the next 6 months, and free from recent illness or recent exposure to COVID-19. A power calculation was performed to determine the sample size for this pilot study. Using Cohen's D with a medium effect size of 0.3, 80% power, and a significance level of 0.05, a minimum of 72 participants was determined for a one-sample, paired t-test between two timepoints. The effect size of 0.3 was derived from the
standard deviation (SD) of 1.2 g/dL observed in a previous study on hemoglobin concentrations in pregnant Ghanaian women.²

A total of 139 participants were recruited and 136 enrolled during the closed season of oyster harvesting. Women were classified into two groups based on self-reported intake of oysters during the open season: Group 1, oyster group of women who consumed oysters during the open season (n = 66); and Group 2, non-oyster group of women who did not consume oysters in the last 30 days (n = 70). A small number of women (12) reported eating oysters during the closed season.

Data Collection

Questionnaires and Anthropometry

Data were collected over two days at each timepoint and analyzed using SAS software 9.4 (Cary, NC). Socio-demographic information was collected by trained enumerators using an interviewer-administered questionnaire adapted from the Ghana Demographic Health Survey (DHS).¹⁴ Data on age, ethnicity, marital status, number of children, education, occupation, income, and household assets were recorded, and we used the Household Food Insecurity Access Scale to assess food insecurity scores and food insecurity severity.¹⁵ The Household Food Insecurity Access Scale has been previously validated in Sub-Saharan Africa.¹⁶ Trained enumerators used standard procedures to measure height and weight using a height measuring board (UNICEF S0114540) and a digital weight scale (SECA 874). Height was recorded to the nearest 0.1 cm and weight to the nearest 0.1 kg. Participant body mass index (BMI) was calculated as follows: (weight, kg)/(height, m)².

Food Frequency and Oyster Consumption Questionnaires
Dietary intake data were collected using a 30-day food frequency questionnaire (FFQ). The locally adapted FFQ contained 138 foods commonly consumed foods in the region, with participants reporting the amount and frequency of food consumption over the past 30 days. We included standard portion sizes for each food item, including cups, tablespoons, and bowls and used pictures from the Food Amounts booklet to aid participants and interviewers. We also used locally acceptable portion sizes for certain foods, such as 2-cedi packs of oysters, and collected the weight of those food portions in grams. Nutrient content of the foods was determined using regional food composition tables specific to Ghana and supplemented with the West African Food Composition Table and U.S. Department of Agriculture food composition database when necessary. For the iron content of oysters, we used a recent report on oysters analyzed from the Densu area in Ghana. We calculated monthly iron intake by multiplying the frequency of the portion size consumed in grams times the iron content of that food item.

**Blood Collection and Lab Analysis**

Blood samples were collected from women by a trained phlebotomist during both timepoints of data collection. Blood samples were collected to assess hemoglobin, ferritin, malaria, alpha-1 glycoprotein (AGP), and C-reactive protein (CRP), which are markers of inflammation. Onsite assessments were conducted to determine the malaria status using a Codix SD Malaria Pf/Pan rapid diagnostic test. Hemoglobin concentrations were assessed using a Hemocue Analyzer 201. Serum samples were allowed to clot for 30 minutes and then centrifuged and aliquoted into 2 mL vials. Samples were then stored in a -20°C freezer at the project site. At the end of each data collection day, aliquoted samples were transferred to the University of Ghana on ice, and stored in a -80°C freezer.
until shipment on dry ice to the University of Rhode Island for storage and analysis.

Serum ferritin was measured using an enzyme-linked immunosorbent assay (ELISA) (Thermoshifer). AGP and CRP concentrations were also measured using ELISA kits (Eagle biosciences). All samples were measured in duplicate. We calculated the intra-assay coefficients of variation (CVs) for ferritin (5%), AGP (8%), and CRP (5%). We also calculated the inter-assay CVs for ferritin (8%), AGP (13%), and CRP (6%).

Statistical Analysis

Serum ferritin was adjusted for inflammation using the BRINDA method. We tested the difference in hemoglobin and ferritin concentrations between the oyster and non-oyster groups at both timepoints using independent t-tests and dependent t-tests for within-group comparisons between the open and closed oyster harvesting seasons. McNemar’s test was used to test the difference between groups for anemia (Hb <12 g/dl), severe anemia (< 8 g/dl), iron deficiency (ferritin < 15 ng/ml), and IDA (Hb < 12 g/dl and ferritin < 15 ng/ml). We used a median split to categorize all women into groups by high and low dietary iron intake. The same was done for the high and low dietary iron groups. P-values < 0.05 were considered statistically significant. We used unadjusted and adjusted multiple linear regression models to determine the beta coefficients for the associations between total dietary iron and serum ferritin and hemoglobin concentrations.

Continuous models were checked for normally distributed residuals using the Shapiro-Wilk statistic. Variables that did not follow a normal distribution were log-transformed. Outliers were identified using the median absolute deviation (MAD) method, with a rejection criterion of 3 if they were biologically impossible. For smaller sample sizes, MAD is recommended as it is less sensitive to outliers. Heteroscedasticity
was examined through residual versus fit plots, and weighted least squares were used for correction if necessary. Scatterplots were examined to assess linearity between independent and dependent variables. Because we had 9 different enumerators collecting data, we checked the distribution of all questionnaire variables by enumerator. We noticed that during timepoint 1, one enumerator had highly skewed data towards high dietary consumption, with a majority of food items marked as being consumed on a daily basis with large portion sizes. In addition, of the 16 participants that worked with this enumerator and 9 items on the HFIAS measuring varying levels of food security, there were zero positive responses among any of the participants on any of the items, which is highly unlikely. Therefore, we marked the FFQ and HFIAS responses for those 16 participants as missing during timepoint 1. The participants whose responses were marked as missing were assigned a different enumerator during timepoint 2, and upon review, data were not significantly different by enumerator among all participants.

Confounding variables were included in the adjusted regression models if they had a statistically significant association with the outcome (p<0.1) in bivariate regressions. For each regression model mentioned above, 10 possible prespecified covariates were examined for adjusted analyses based on existing literature. The covariates recorded at enrollment that were examined included age, parity, education, BMI, height, household food insecurity assessment scores, CRP and AGP, malaria, and marital status. Collinearity for continuous predictors was assessed by examining variance inflation factors (VIF) and running models with covariates.
We evaluated variables that may modify the association between oyster consumption and hemoglobin or ferritin concentrations. Interactions that were found to be significant (p<0.1) between the oyster consumption group and baseline covariates were included. Potential effect modifiers selected a priori included parity, BMI, CRP, and AGP.

RESULTS

Background characteristics of study participants

Participant characteristics are presented in Table 1. Two women were not eligible due to illness or sickness and one woman was not enrolled as we had just met the number of women needed for both groups. Only 11 participants were lost to follow-up due to pregnancy (n=5), illness (n=2), or having traveled during the open season (n=4). The mean (SD) age of participants in the oyster and non-oyster group was 35.0 (8.2) and 33.4 (8.3) years. Two participants at each time point were missing blood samples because their blood could not be drawn or they refused a blood draw. A majority of women in both groups attended school, were married or cohabitating, and were food insecure. There was a low prevalence of malaria in both groups during both seasons (< 2%). The mean (SD) BMI was 25.8 (4.5) kg/m² for the oyster group and 27.1 (5.5) kg/m² for the non-oyster group.

Variables that were significantly associated with hemoglobin and included in adjusted regression models included marital status, CRP, and AGP at enrollment. Variables that were significantly associated with ferritin and included in adjusted models were food insecurity, marital status, and parity. None of the covariates presented a VIF greater than 2.
Associations between oyster consumption with hemoglobin and ferritin concentrations

During the open season, there was no association between oyster consumption and hemoglobin in unadjusted ($\beta = 0.147$, SE: $0.135$, p=0.279) or adjusted analyses ($\beta = 0.153$, SE: $0.133$, p=0.255) (Table 2). Oyster consumption was also not associated with ferritin concentrations in unadjusted ($\beta = 0.163$, SE: $0.134$, p=0.230) or adjusted analyses ($\beta = 0.242$, SE: $0.133$, p=0.075). We did not evaluate these associations during the closed season because the number of women who consumed oysters during the closed season was small (n=12).

Comparisons of hemoglobin, ferritin, and anemia prevalence by season and group

Contrary to hypothesis, paired t-tests revealed no differences between seasons in hemoglobin concentrations or the prevalence of anemia or IDA in the oyster or non-oyster group (Table 3). There were also no differences between the oyster and non-oyster groups for hemoglobin, anemia, ferritin concentrations, iron deficiency, or iron deficiency anemia in the closed or open season (Table 3). However, in the oyster group, severe anemia was significantly lower in the closed season compared to the open season, and during the closed season, there was a lower prevalence of severe anemia than the non-oyster group (Table 3). Both the oyster and non-oyster group had higher ferritin concentrations in the closed season compared to the open season. In the oyster group, the prevalence of iron deficiency was lower during the closed season compared to the open season.

Dietary Iron Intake Groups

We found that the food frequency questionnaire systematically overestimated dietary iron intake (Appendix 3). The Cancer Institute of the National Institute of Health
(NIH) recommends against using 30-day FFQs for estimating mean intake without calibration using a 24-hr food recall or alternative method.\textsuperscript{38} Therefore, we cannot accurately present mean dietary iron intake. However, it is acceptable to group women into high or low dietary iron intake groups. We used the median values of dietary iron intake during the closed and open season to group all women, which were 26 and 29 mg/day (likely overestimates of actual intake). During timepoint 1 (closed season), there were 119 participants with dietary intake data. There were 59 participants in the low dietary iron intake group and 60 participants in the high dietary iron intake group. During timepoint 2 (open season), there were 123 participants with dietary iron intake data. There were 61 participants in the low dietary iron intake group and 62 in the high dietary iron intake group.

Associations between dietary iron intake, serum ferritin, and hemoglobin concentrations

During the closed season, there was no association between dietary iron intake and hemoglobin in unadjusted ($\beta = 0.026, \text{SE: } 0.093, p=0.782$) or adjusted analyses ($\beta = 0.026, \text{SE: } 0.094, p=0.783$) among all women (Table 4). Dietary iron intake was also not associated with ferritin concentrations during the closed season in unadjusted ($\beta = 0.019, \text{SE: } 0.094, p=0.843$) or adjusted analyses ($\beta = 0.020, \text{SE: } 0.094, p=0.822$). During the open season, higher dietary iron intake was significantly associated with higher hemoglobin in unadjusted analyses ($\beta = 0.185, \text{SE: } 0.090, p=0.044$), although the relationship was attenuated after adjusting for marital status, CRP, and AGP at enrollment ($\beta = 0.174, \text{SE: } 0.090, p=0.057$). Dietary iron intake was not associated with ferritin concentrations during the open season in unadjusted ($\beta = 0.015, \text{SE: } 0.092, p=0.872$) or adjusted analyses ($\beta = -0.046, \text{SE: } 0.096, p=0.637$).
Comparisons between high and low dietary iron intake and iron status

During the closed season, there were no differences between the high and low dietary iron intake groups for hemoglobin, anemia, severe anemia, ferritin concentrations, iron deficiency, or IDA. Notably, hemoglobin and ferritin concentrations were low for both groups and the prevalence of anemia, severe anemia, iron deficiency, and IDA high for both groups (Table 5).

During the open season, there was a difference between groups in some of the parameters measured, with the high iron intake group showing significantly higher hemoglobin and ferritin concentrations a significantly lower prevalence of severe anemia than the low dietary intake group (Table 5).

Stratification by AGP, CRP, and age

We found that AGP modified the effect of oyster consumption and ferritin concentrations so we then stratified the analyses by high AGP (≥ 1.00 g/L) and normal AGP (< 1.00 g/L). AGP, CRP, and age modified the effect of oyster consumption and iron deficiency and IDA, and so we stratified analyses by high and normal AGP, and high (≥ 5 mg/L) and normal (< 5 mg/L) CRP, and calculated the median age to create two groups (< 35 or ≥35 years).

During the open season, women with high dietary iron intake and normal AGP had significantly higher ferritin concentrations compared to women with low dietary iron intake and normal AGP. There was no difference in ferritin concentrations between groups among women with high AGP (Table 6).

Women in the oyster group with normal AGP had lower ferritin concentrations during the open season compared to the closed season (Table 7). There was no difference
among women with high AGP. Women in the non-oyster group with normal AGP or High AGP had lower ferritin concentrations during the open season compared to the closed season. There was no difference between the groups at either timepoint among women with normal or high AGP (Figure 5). Women in the oyster group with normal AGP also had a higher prevalence of iron deficiency compared to women in the non-oyster group with normal AGP during both the closed and open seasons (Figure 6).

DISCUSSION

This study examined the associations between oyster consumption and dietary iron intake with hemoglobin, ferritin concentrations, and anemia during the open and closed seasons of oyster harvesting. We found that dietary iron intake and oyster consumption were not associated with any outcomes in continuous models. Notably, ferritin concentrations were lower during the open season compared to the closed season for both the oyster and non-oyster groups when we would expect ferritin concentrations to increase in the oyster group during the open harvesting season and remain constant for the non-oyster group. This indicates that oyster consumption was not protective against iron deficiency and anemia, and there may be factors other than oyster consumption influencing markers of iron status in this population.

Our findings also show that the prevalence of anemia, iron deficiency, and iron deficiency anemia were extremely high in both groups during both seasons. In this oyster harvesting community, the prevalence of anemia ranged from 70% in the oyster group during the open season to 83% in the non-oyster group during the closed season. IDA rates were also high at 41% in the non-oyster group to 52% in the oyster group during the closed season. Currently, the WHO reports that the prevalence of anemia in Ghanaian
women of reproductive age is 35.4%. The World Health Organization indicates that an anemia prevalence >40% is a severe public health problem, due to increased risks for maternal and child mortality. Therefore, anemia is a significant public health problem in oyster harvesting communities in Ghana.

One of the main risk factors for iron IDA includes a low dietary iron intake or poor absorption of dietary iron. During the open season, high iron intake was associated with a lower prevalence of severe anemia and higher ferritin concentrations compared to those with low iron intake. In general, however, higher iron intake and oyster consumption was not associated with markers of iron status. It is possible that oysters do not provide a significant amount of bioavailable iron. In Pacific Oysters from the U.S., oysters were found to have only 1 mg of heme iron and 5 mg of non-heme iron. Non-heme iron is less bioavailable with only 5-12% of non-heme iron absorbed compared to 15-35% of heme iron.

Consistent with the Ghanaian diet, we found that a majority of dietary iron intake was sourced from plants, and specifically cereals eaten on a daily basis. Plant sources of iron which provide non-heme iron are less bioavailable than animal sources of iron that provide heme iron. Phytates, polyphenols, and oxalates, which are naturally occurring compounds in whole grains, tea, coffee, and green leafy vegetables, have the ability to bind with iron and create complexes that are less readily absorbed by the body. Additionally, high zinc intake may decrease the absorption and utilization of dietary iron. We previously found that smoked, dried oysters from the Densu estuary had high concentrations of zinc, easily surpassing the zinc RDA, which may significantly
contribute to overall zinc intake and inhibit the absorption of dietary iron (manuscript in preparation, Chapter 1).

We found that during the open season, high dietary iron intake was associated with higher hemoglobin concentrations, although this association was attenuated in adjusted models. This indicates that marital status, CRP, and AGP influenced both dietary intake and hemoglobin, accounting for the significant association. When we stratified analyses by AGP, a chronic marker of inflammation, we found differential outcomes by group. During the open season, women with high dietary iron intake and normal AGP had significantly higher ferritin concentrations compared to women with low dietary iron intake and normal AGP, while there was no difference in ferritin concentrations between groups among women with high AGP. Therefore, in the absence of chronic inflammation, we see a significant and expected association between higher dietary iron intake and higher ferritin concentrations, indicating that increased iron intake in this population may be beneficial for increasing ferritin concentrations and decreasing the risk of iron deficiency. Moreover, chronic inflammation may mask underlying iron deficiencies, as shown in figure 2, and underestimate the need for increased iron intake in this population.

We also found that women in the oyster group with normal AGP had a higher prevalence of iron deficiency compared to women in the non-oyster group with normal AGP during both the closed and open seasons. This indicates that oyster consumption may influence iron status in a negative direction in this population after further consideration for inflammation. Sources of inflammation may include viruses, parasites, and other underlying health conditions. Iron metabolism may also be influenced by inflammation. As the body tries to prevent iron from being used by invading hosts, it
reduces iron reutilization, contributing to an increased risk for anemia and iron deficiency. This emphasizes the need to account for inflammation to more accurately determine iron status. The iron status of women in Ghana may be severely overestimated if inflammation is masking underlying iron deficiencies and inflammation is not taken into account.

Because oysters are filter feeders, it is also possible that the oysters are contaminated with heavy metals that decrease the absorption and utilization of iron and increase the risk for iron deficiency anemia. Specifically, oysters may be contaminated with mercury, cadmium, and lead, which are heavy metals directly associated with iron status. Mercury exposure is of great concern as it is associated with adverse neurological, kidney, cardiovascular, and immunological outcomes. Seafood items can be a significant source of mercury, and our previous analysis found high mercury concentrations in smoked, dried oysters from the Densu estuary in Ghana (manuscript in preparation, Chapter 1). One serving of oysters presented a hazard index ~45, where a hazard index >1 is considered a significant health risk. High mercury exposure may increase the risk of iron deficiency and iron deficiency anemia. A study of children with high mercury exposure in Brazil found that higher hair mercury concentrations were associated with lower hemoglobin concentrations. More research is needed on the associations between mercury exposure and iron deficiency in women of reproductive age.

Cadmium is another toxic metal that competes with iron for absorption in the intestines, so it is possible that exposure to cadmium may decrease ferritin concentrations. Similarly, lead exposure has been associated with increased risk for
anemia. Lead can decrease iron absorption and also negatively impacts enzymatic activity during iron metabolism. So even if iron is absorbed in the intestines, the presence of lead may limit the ability of iron to be utilized in the body. Future research should determine if oyster consumption increases exposure to heavy metals and the associations with iron status.

We found that ferritin concentrations were already low during the closed season, and significantly lower for both groups during the open season. It is possible that seafood items other than oysters are a significant source of heavy metals that negatively impact hemoglobin and ferritin concentrations in both the oyster and non-oyster group. We observed that sardines and anchovies were both commonly consumed by women in this population, which are a significant source of mercury. On a national level, seafood consumption is high in Ghana. The national average of seafood consumption is >300% of recommended intake. Future research is needed to evaluate the associations between seafood consumption in Ghana with heavy metal concentrations and iron status among women of reproductive age.

Limitations to this study include using self-report for dietary intake and oyster consumption. We were not able to present mean dietary iron intake with the 30-day food frequency questionnaire used. We originally did not include dietary intake as a major aim of the study, and therefore included a 30-day food frequency questionnaire that is better at capturing the intake of and frequency of foods less often consumed over a longer period of time. Because food frequency questionnaires also systematically over-report mean intake, we are unable to accurately report daily iron intake without additional adjustments or calibrators. Using FFQs are not recommended to calculate mean,
proportion of individuals above or below a threshold (like adequate dietary iron intake), and percentiles of usual intake. It is also unclear how the COVID-19 pandemic impacted usual intake or oyster harvesting and consumption.

Strengths of this study include the use of multiple markers to measure iron status. It is notable that all biological markers of iron status were low, indicating a significant iron deficiency problem in this population. In addition to questionnaires, we analyzed both hemoglobin and ferritin concentrations at both timepoints with a high intra- and inter-assay CV. A strength of this study is that we adjusted for inflammation using a validated method and highly reliable biomarkers (BRINDA). Inflammation has a significant and direct impact on serum ferritin concentrations, which is an acute phase protein, and we were able to more accurately estimate the prevalence of iron deficiency. The presence of inflammation may mask actual iron deficiency and overestimate the iron status of women of reproductive age. We also were able to conduct within group comparisons, which reduce external variability error. The presence of a control group of women who did not consume oysters helped us evaluate variability due to external factors like COVID-19.

In conclusion, hemoglobin and ferritin concentrations were higher in women with high dietary iron intake compared to low dietary iron intake. We did not find a significant association between oyster consumption and hemoglobin or ferritin concentrations in linear models. Contrary to our hypothesis, we observed that during the open season women who consumed oysters as well as women who did not consume oysters had lower ferritin concentrations, indicating that there may be factors outside of oyster consumption contributing to iron deficiency in this population. The prevalence of anemia and iron
deficiency anemia was high during both seasons in both groups of women. Further research into the causes of anemia and iron deficiency among this population is needed. Previously, we have shown that oysters in Ghana are high in mercury and may present a significant health risk with regular consumption (manuscript in preparation). Future studies should evaluate the impact of mercury exposure from oyster consumption on iron deficiency and anemia. Iron deficiency anemia is of great concern in this population and it is necessary to identify and implement effective interventions to address anemia and iron deficiency at a community level.
TABLES

Table 1. Characteristics of Ghanaian women of reproductive age enrolled in the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Oyster Group</th>
<th>Oyster Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD) or %</td>
<td>Mean (SD) or %</td>
</tr>
<tr>
<td>n</td>
<td>70</td>
<td>66</td>
</tr>
<tr>
<td>Age, years</td>
<td>33.4 (8.3)</td>
<td>35.0 (8.2)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>58%</td>
<td>47%</td>
</tr>
<tr>
<td>Secondary School</td>
<td>42%</td>
<td>53%</td>
</tr>
<tr>
<td>Married or Cohabitating</td>
<td>64%</td>
<td>74%</td>
</tr>
<tr>
<td>Household Food Insecurity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Secure</td>
<td>36%</td>
<td>34%</td>
</tr>
<tr>
<td>Food Insecure</td>
<td>64%</td>
<td>66%</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>14%</td>
<td>5%</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 (5.5)</td>
<td>25.8 (4.5)</td>
</tr>
<tr>
<td>Positive Malaria Test</td>
<td>1.6%</td>
<td>1.8%</td>
</tr>
<tr>
<td>CRP*, mg/L</td>
<td>2.01 (.31)</td>
<td>1.38 (.21)</td>
</tr>
<tr>
<td>AGP*, g/L</td>
<td>1.00 (0.06)</td>
<td>0.79 (0.05)</td>
</tr>
</tbody>
</table>

*CRP and AGP show geometric means and SE

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.

Table 2. Unadjusted and adjusted beta (β) coefficients of hemoglobin and serum ferritin predictors and oyster consumption during the open season in Ghanaian women of reproductive age enrolled in a pilot study.

<table>
<thead>
<tr>
<th>Oyster Consumption</th>
<th>n</th>
<th>β (SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>121</td>
<td>0.147 (0.135)</td>
<td>0.279</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.153 (0.133)</td>
<td>0.255</td>
</tr>
<tr>
<td>Ferritin</td>
<td>120</td>
<td>0.163 (0.134)</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.242 (0.133)</td>
<td>0.075</td>
</tr>
</tbody>
</table>

1Models adjusted for marital status, CRP, and AGP collected at enrollment
2Models adjusted for food insecurity score, parity, and marital status collected at enrollment. Ferritin adjusted for CRP and AGP using the BRINDA method.
Table 3. Comparisons of hemoglobin, serum ferritin concentrations, and anemia by oyster group (n=66) and non-oyster group (n=70) and oyster harvesting seasons among Ghanaian women of reproductive age enrolled in a pilot study.

<table>
<thead>
<tr>
<th></th>
<th>Closed Season</th>
<th>Mean (SE)</th>
<th>Open Season</th>
<th>Mean (SE)</th>
<th>p</th>
<th>Closed Season</th>
<th>Frequency</th>
<th>Open Season</th>
<th>Frequency</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oyster group</td>
<td>n=134</td>
<td>10.4 (0.3)</td>
<td>n=121</td>
<td>9.9 (0.4)</td>
<td>0.151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-oyster group</td>
<td>n=134</td>
<td>9.6 (0.3)</td>
<td>n=121</td>
<td>9.8 (0.3)</td>
<td>0.287</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>0.540</td>
<td></td>
<td>0.135</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia (Hb &lt; 12 g/dL)</td>
<td>n=108/134</td>
<td>78%</td>
<td>n=90/121</td>
<td>70%</td>
<td>0.637</td>
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<tr>
<td>Oyster group</td>
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<tr>
<td>Non-oyster group</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>p</td>
<td></td>
<td>0.489</td>
<td></td>
<td>0.268</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Severe Anemia (Hb &lt; 8 g/dL)</td>
<td>n=26/134</td>
<td>11%</td>
<td>n=26/121</td>
<td>23%</td>
<td>0.008*</td>
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<tr>
<td>Oyster group</td>
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<td>Non-oyster group</td>
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<tr>
<td>p</td>
<td></td>
<td>0.018*</td>
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<td>0.668</td>
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<tr>
<td>Ferritin, ng/mL</td>
<td>n=133</td>
<td>14.6 (2.0)</td>
<td>n=120</td>
<td>8.9 (1.3)</td>
<td>0.0001*</td>
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<tr>
<td>Oyster group</td>
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<td>p</td>
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<td></td>
<td>0.958</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Iron deficiency (Ferritin &lt; 15 ng/mL)</td>
<td>n=65/133</td>
<td>54%</td>
<td>n=75/120</td>
<td>70%</td>
<td>0.008*</td>
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<td>Oyster group</td>
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<tr>
<td>Non-oyster group</td>
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</tr>
<tr>
<td>p</td>
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<td></td>
<td>0.131</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Deficiency Anemia (Hb &lt; 12 g/dL and Ferritin &lt; 15 ng/mL)</td>
<td>n=61/132</td>
<td>52%</td>
<td>n=58/120</td>
<td>50%</td>
<td>0.782</td>
<td></td>
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</tr>
<tr>
<td>Oyster group</td>
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<tr>
<td>p</td>
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<td>0.733</td>
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<td></td>
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</tbody>
</table>

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.
Table 4. Unadjusted and adjusted beta (\( \beta \)) coefficients of hemoglobin and serum ferritin predictors during the closed and open oyster harvesting seasons and dietary iron intake in all Ghanaian women of reproductive age enrolled in the study.

<table>
<thead>
<tr>
<th>Season</th>
<th>Biomarker</th>
<th>n</th>
<th>( \beta ) (SE)</th>
<th>p</th>
<th>( \beta ) (SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Closed Season</td>
<td>Open Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=119</td>
<td>n=123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed Season</td>
<td>Hemoglobin</td>
<td>134</td>
<td>0.026 (0.093)</td>
<td>0.782</td>
<td>0.026 (0.094)</td>
<td>0.783</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>Adjusted(^1)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferritin</td>
<td>133</td>
<td>0.019 (0.094)</td>
<td>0.843</td>
<td>0.020 (0.094)</td>
<td>0.822</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adjusted(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open Season</td>
<td>Hemoglobin</td>
<td>121</td>
<td>0.185 (0.090)</td>
<td>0.044</td>
<td>0.174 (0.090)</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
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</tr>
<tr>
<td></td>
<td>Adjusted(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferritin</td>
<td>120</td>
<td>0.015 (0.092)</td>
<td>0.872</td>
<td>-0.046 (0.096)</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adjusted(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Models adjusted for marital status, CRP, and AGP collected at enrollment
\(^2\)Models adjusted for food insecurity score, parity, and marital status collected at enrollment. Ferritin adjusted for CRP and AGP using the BRINDA method.
Table 5. Comparisons of hemoglobin, serum ferritin concentrations, and anemia by dietary intake groups during the closed and open oyster harvesting seasons among all Ghanaian women of reproductive age enrolled in the study.¹

<table>
<thead>
<tr>
<th>Dietary intake groups, n</th>
<th>Closed Season Mean (SE)</th>
<th>Open Season Mean (SE)</th>
<th>Closed Season Frequency</th>
<th>Open Season Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>High dietary iron intake</td>
<td>60/119</td>
<td>62/123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>59/119</td>
<td>61/123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>10.0 (0.3)</td>
<td>10.5 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>10.0 (0.3)</td>
<td>9.2 (0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.424</td>
<td>0.0007*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia (Hb &lt; 12 g/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>75%</td>
<td>73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>85%</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.118</td>
<td>0.794</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe Anemia (Hb &lt; 8 g/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>19%</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>20%</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.844</td>
<td>0.002*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>17.5 (2.6)</td>
<td>12.2 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>14.7 (1.9)</td>
<td>9.0 (1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.907</td>
<td>0.0004*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency (Ferritin &lt; 15 ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>40%</td>
<td>68%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>56%</td>
<td>57%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.062</td>
<td>0.187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency Anemia (Hb &lt; 12 g/dL and Ferritin &lt; 15 ng/mL.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>37%</td>
<td>48%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>53%</td>
<td>48%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.06</td>
<td>NS²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹High and low dietary iron intake groups determined by a median split.
²Not significant (p > 0.99)
### Table 6. Comparison of ferritin concentrations during the closed and open oyster harvesting seasons between women with high and low dietary iron intake stratified by AGP

<table>
<thead>
<tr>
<th></th>
<th>Closed Season Mean (SE)</th>
<th>Open Season Mean (SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>60</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>59</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td><strong>Normal AGP (&lt;1.0 g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>17.3 (3.2)</td>
<td>11.1 (1.3)</td>
<td>0.919</td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>14.9 (2.3)</td>
<td>8.6 (2.0)</td>
<td>0.0005*</td>
</tr>
<tr>
<td><strong>High AGP (≥1.0 g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dietary iron intake</td>
<td>17.7 (4.5)</td>
<td>15.1 (3.4)</td>
<td>0.924</td>
</tr>
<tr>
<td>Low dietary iron intake</td>
<td>14.3 (3.2)</td>
<td>9.3 (2.4)</td>
<td>0.207</td>
</tr>
</tbody>
</table>

### Table 7. Comparison of ferritin concentrations by oyster harvesting season and oyster group stratified by AGP

<table>
<thead>
<tr>
<th></th>
<th>Closed Season Mean (SE)</th>
<th>Open Season Mean (SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal AGP (&lt;1.0 g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oyster group</td>
<td>13.4 (2.2)</td>
<td>7.6 (1.2)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Non-oyster group</td>
<td>19.9 (3.3)</td>
<td>13.5 (2.6)</td>
<td>0.016*</td>
</tr>
<tr>
<td>p</td>
<td>0.512</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td><strong>High AGP (≥1.0 g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oyster group</td>
<td>18.8 (4.8)</td>
<td>14.2 (4.6)</td>
<td>0.200</td>
</tr>
<tr>
<td>Non-oyster group</td>
<td>14.4 (3.1)</td>
<td>10.2 (2.2)</td>
<td>0.014*</td>
</tr>
<tr>
<td>p</td>
<td>0.544</td>
<td>0.637</td>
<td></td>
</tr>
</tbody>
</table>

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.
Figure 1. Hypothesized effect of increased oyster consumption on iron and markers of iron status. We would expect increased oyster consumption to increase iron intake. Higher iron intake is expected to increase hemoglobin and ferritin concentrations and decrease the prevalence of anemia, iron deficiency, and iron deficiency anemia. Other factors may influence the effect of oyster consumption on iron status. Other dietary sources of iron would increase total dietary iron intake. Phytate intake from plant-based sources and heavy metals may decrease iron absorption. Inflammation, measured by alpha-1 glycoprotein (AGP) or C-reactive protein (CRP) may elevate ferritin concentrations but under-represent the prevalence of iron deficiency and iron deficiency anemia.
Figure 2. Study Design calendar showing the two data collection timepoints, open and closed oyster harvesting seasons, and major and minor rainy seasons in southern Ghana.
Figure 3: Flowchart of participant enrollment and anemia outcomes in a pilot study on the association between dietary iron intake, oyster consumption, and anemia in Ghanaian women.
Figure 4: Flowchart of participant enrollment and anemia outcomes in a pilot study on the association between dietary iron intake, oyster consumption, and anemia in Ghanaian women for the High and Low Dietary Iron Intake groups.
Figure 5. Effect modification of AGP by oyster group and oyster harvesting season on ferritin concentrations (mean (SE))

Note: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.
Figure 6. Effect modification of AGP, CRP, and age by oyster group and oyster harvesting season on the prevalence of iron deficiency (ID) (%)

a. Normal AGP and High AGP

b. Normal CRP and High CRP

c. 18-34 y and 35-49 y

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.
REFERENCES


12. WHO. WHO Guideline on Use of Ferritin Concentrations to Assess Iron Status in


49. Ahamed M, Kaleem M, Siddiqui J. Environmental lead toxicity and nutritional factors. doi:10.1016/j.clnu.2007.03.010

MANUSCRIPT #3

This manuscript was prepared in accordance with author guidelines for the Journal of Nutrition. It is currently in preparation for submission.
Abstract

Background: Oysters are an excellent source of iron and zinc but may be contaminated with cadmium, a toxic metal that may increase the risk of iron deficiency. Iron deficiency is high among women living near oyster harvesting communities in Ghana, and the relationship between oysters, cadmium, zinc, and iron is unknown.

Objectives: To determine the association between oyster consumption, zinc, and cadmium concentrations, and to determine whether the associations between iron intake and ferritin or hemoglobin concentrations are mediated by zinc or cadmium.

Methods: We enrolled women in the Bortianor/Tsokomey area in Ghana in a 6-month, longitudinal pilot study, with 66 women who consumed oysters only during the open season (oyster group) and 70 women did non consume oysters in either the open or closed oyster seasons (non-oyster group). We collected data at two time points: 1) March 2020 (end of closed season) and 2) August 2020 (5 months into the open season). We collected the amount and frequency of dietary iron intake and oyster consumption using a 30-day food frequency questionnaire. We analyzed plasma zinc and urinary cadmium (ug/g creatinine) concentrations and used paired t-tests to analyze the difference in zinc and cadmium concentrations and between seasons and used independent t-tests between groups. We also used a pathway analysis to understand the relationships between dietary iron intake or oyster consumption and zinc, cadmium, and serum ferritin or hemoglobin concentrations.

Results: At enrollment, mean±SD age was 34±8 y and BMI was 26±5 kg/m². For women in both the oyster and non-oyster group, zinc concentrations were lower during the closed season compared to the open season and cadmium concentrations were higher
during the open season compared to the closed season. No women presented with zinc
deficiency (< 66 ug/dl) or cadmium toxicity (≥5 ug/g crea) during the closed season and
only 2-5% had zinc deficiency and 0% had cadmium toxicity during the open season.
Oyster consumption was not associated with zinc or cadmium concentrations in
continuous models. Cadmium and zinc did not mediate the association between dietary
iron intake or oyster consumption and ferritin or hemoglobin concentrations.

**Conclusions:** We found that zinc was lower and cadmium concentrations were higher in
the open compared to the closed season, and within a healthy range during both time
points. However, there was no association between oyster consumption and zinc or
cadmium concentrations. Based on low cadmium concentrations among women who
consumed oysters, cadmium contamination of oysters may not be of great concern in this
community and oysters may continue to serve as a valuable nutrition source for women of
reproductive age in Ghana. However, future research should evaluate whether oyster
consumption increases the risk of exposure to other heavy metals such as mercury, and the
association with iron deficiency, which was high in this population.
ASSOCIATIONS BETWEEN OYSTER CONSUMPTION, ZINC, AND CADMIUM CONCENTRATIONS IN GHANAIAN WOMEN: A PILOT STUDY

INTRODUCTION

Iron deficiency in women of reproductive age is common in Sub-Saharan Africa and associated with adverse short- and long-term maternal and child health outcomes.¹ Oysters are an excellent source of iron, and oyster harvesting and consumption is common in the Bortianor/Tsokomey area in Ghana.² Oyster harvesters and local stakeholders in this community instituted an annual, 5-month closed season (November-March) in 2017, during which time oyster harvesting is discontinued, followed by an open season the rest of the year.² Oysters are less often consumed during the closed season of oyster harvesting, but may be consumed regularly during the open season. With increased oyster consumption we would expect to observe increase markers of iron status such as ferritin and hemoglobin concentrations. However, we previously found that anemia and iron deficiency were high in this population during both the closed and open seasons and ferritin concentrations were lower during the open season, when oysters are harvested and consumed. There was also no difference in iron status between women who consumed oysters and those who did not (manuscript in preparation, Chapter 2 of this dissertation). The high prevalence of anemia and iron deficiency in this population is of great concern and it is unclear why increased oyster consumption was not protective against iron deficiency and anemia.

In addition to iron, oysters are an excellent source of zinc, but can also be contaminated with cadmium. It is possible that heavy metal exposure and high zinc intake
decrease the absorption and utilization of iron, increasing the risk for iron deficiency anemia. Cadmium concentrations have previously been negatively associated with iron and zinc status,\textsuperscript{3,4} and zinc is also a competitive inhibitor of iron absorption.\textsuperscript{5} It is possible that cadmium and zinc mediate the association between oyster consumption or dietary iron intake and hemoglobin and ferritin concentrations. Therefore, our objectives are to 1) determine the association between oyster consumption, zinc, and cadmium concentrations in Ghanaian women, and 2) determine whether the associations between iron intake and ferritin or hemoglobin concentrations are mediated by zinc or cadmium concentrations.

**METHODS**

*Study Design, Setting, and Participants*

Details of the study methods have been described previously (manuscript in preparation, Chapter 2 of this dissertation). Briefly, we collected data during both the closed and open oyster harvesting seasons (Figure 1) on oyster consumption and dietary iron intake using a 30-day food frequency questionnaire and collected blood samples for iron status (hemoglobin and serum ferritin concentrations). We enrolled 136 women who were classified into two groups. The two groups were based on data collected during the open season: Group 1, an oyster group for women who reported consuming oysters in the last 30 days ($n = 66$), and Group 2, a non-oyster group of women who did not consume oysters in the last 30 days ($n = 70$), acting as a control group. Among the whole sample, only a few women ($n=12$) reported eating oysters during the closed season. The University of Rhode Island IRB (BI1920-005) and the Ghana Health Service Ethical IRB (GHS-ERC 012/12/19) approved this study. Additional methodology relevant to this analysis is described below.
Data Collection

Blood and Urine Collection and Lab Analysis

A trained phlebotomist collected blood samples from women during both timepoints of data collection. A plasma sample was collected to assess zinc and a serum sample to assess ferritin concentrations, and the inflammatory markers alpha-1 glycoprotein (AGP) and C-reactive protein (CRP). Serum samples were allowed to clot for 30 minutes and all blood samples were centrifuged and aliquoted into 2 mL vials prior to freezing. We assessed hemoglobin concentrations using a Hemocue Analyzer 201.

After the blood draw, we provided supplies for take-home urine collection. Women were instructed to collect urine for 24 hours and return the full sample to the study site. Participants were instructed to start urine collection the morning of the day following the blood draw and to skip the first urine of the day and collect until the same time the following morning. When returned to the study site, we aliquoted samples into a 5 ml vial.

Blood and urine samples were stored in a -20°C freezer at the project site. Aliquoted samples were transferred to the University of Ghana on ice at the end of each data collection day. Samples were stored in a -80°C freezer until shipment on dry ice to the University of Rhode Island for storage and analysis. Ferritin, α-1 acid glycoprotein (AGP), C-reactive protein (CRP), and creatinine were analyzed at the University of Rhode Island and analysis methods have been described previously (manuscript 2). Urinary creatinine was assessed using the Jaffe method. Samples were measured in duplicate. The intra-assay coefficients of variation (CVs) were: ferritin (5%), AGP (8%), CRP (5%), and creatinine
(3%). The inter-assay CVs were: ferritin (8%), AGP (13%), CRP (4%), and creatinine (6%).

In this study, inductively coupled plasma mass spectrometry (ICP-MS; Agilent GC-QQQ-MS) was used to analyze plasma zinc and urinary cadmium concentrations at the University of Massachusetts - Amherst. We prepared plasma and urine samples separately. We added 0.4 mL concentrated trace metal grade nitric acid and 0.2 mL 20% hydrogen peroxide to 0.2 mL plasma. Samples were capped tightly and heated at 60 °C for 90 min followed by cooling to room temperature. We added 4.2 mL 2% trace metal grade nitric acid, including internal standard (final volume 5 mL). Therefore, each plasma concentration has been multiplied by 25 from the measured value to obtain concentration corrected for dilution. We added 7.2 mL 2% trace metal grade nitric acid, including internal standard to 0.8 mL urine. Each concentration was multiplied by 10 from the measured value to obtain concentration corrected for dilution. We corrected urinary cadmium by dividing urinary cadmium by urinary creatinine to account for urine concentration.

Statistical Analysis

We used the BRINDA method to adjust serum ferritin for inflammation. We tested the difference in zinc and cadmium concentrations between the oyster and non-oyster groups at both timepoints using independent t-tests and tested the difference within-group between the open and closed oyster harvesting seasons using dependent t-tests. McNemar’s test was used to test the difference between groups for zinc deficiency (pZn <66 μg/dl) and cadmium toxicity (uCd ≥5 μg/g creatinine). P-values < 0.05 were considered statistically significant. Unadjusted and adjusted multiple linear regression
models were used to determine the beta coefficients for the associations between oyster consumption and zinc and cadmium concentrations.

We used a pathway analysis to understand the mediating role of zinc or cadmium between oyster consumption or dietary iron intake and serum ferritin or hemoglobin concentrations. We evaluated the direct effect of oyster consumption or dietary iron intake on ferritin or hemoglobin concentrations. We also determined the indirect effect, which considers mediation through zinc or cadmium on the pathway, as well as total effect, which is the sum of the direct and indirect effects. Bootstrapping was used to calculate the 95% confidence intervals (CI).

We checked continuous models for normally distributed residuals using the Shapiro-Wilk statistic. In cases where variables did not exhibit a normal distribution, a log transformation was applied. Outliers were identified using the median absolute deviation (MAD) method, and a rejection criterion of 3 was applied to exclude biologically implausible values. MAD is particularly recommended for smaller sample sizes as it is less influenced by outliers. To evaluate heteroscedasticity, residual versus fit plots were examined and scatterplots were used to assess the linearity between independent and dependent variables. Data were examined using SAS software 9.4.

Adjusted regression models included confounding variables that had a statistically significant association (p < 0.1) with the outcome in bivariate regressions. A total of 10 predetermined covariates, based on existing literature, were considered for adjusted analyses. These covariates, recorded at enrollment, included age, parity, education, BMI, height, household food insecurity assessment scores, CRP and AGP concentrations, malaria status, and marital status. We assessed collinearity for continuous predictors using
variance inflation factors (VIF) and running models with covariates. None of the covariates presented a VIF greater than 2.

We evaluated variables that may modify the association between oyster consumption and zinc or cadmium concentrations. We included interactions that were significant (p<0.1) between the oyster consumption group and baseline covariates. To avoid collinearity, effect modifiers were considered separately in the regression model. Potential effect modifiers selected a priori included age, parity, BMI, CRP and AGP.

RESULTS

Background characteristics of study participants

Table 1 presents participant characteristics. We enrolled 136 participants and lost 11 participants to follow-up due to: pregnancy (n=5), illness (n=2), or travel during the open season (n=4). The mean (SD) age of participants was 35.0 (8.2) years in the oyster and 33.4 (8.3) years in the non-oyster group. The mean BMI mean (SD) was for the oyster group and non-oyster group was 25.8 (4.5) and 27.1 (5.5) kg/m². There were two participants during the closed season and two participants during the open season whose blood could not be drawn or refused a blood draw. The prevalence of malaria was low in both groups during both seasons (< 2%). Of participants with zinc data, no women had zinc deficiency during the closed season and only 3 of 56 participants in the oyster group and 1 of 64 participants in the non-oyster group had zinc deficiency during the open season. Variables that were significantly associated with zinc and included in adjusted regression models included BMI and AGP at enrollment. Variables that were significantly associated with cadmium and included in adjusted models were BMI and age.

Associations between oyster consumption and zinc concentrations
During the open season, there was no association between oyster consumption and zinc concentrations in unadjusted analyses or adjusted models including BMI and AGP (Table 2). Plasma zinc was significantly lower during the open season compared to the closed season for the oyster group (p<0.0001) and the non-oyster group (p=0.007). Zinc was not significantly different between groups in either season (Table 3).

**Associations between oyster consumption and cadmium concentrations**

There was also no association between oyster consumption and cadmium concentrations during the open season in unadjusted or adjusted models including BMI and age (Table 2). Urinary cadmium was significantly higher during the open season compared to the closed season for the oyster group (p<0.0001) and non-oyster group (p<0.0001), and cadmium was not significantly different between groups in either season (Table 3). However, no women presented with cadmium toxicity during either season in either group. We observed that higher cadmium concentrations were associated with higher BMI and age.

**Effect modifiers**

We found that BMI modified the effect of oyster consumption and zinc and stratified analyses by BMI category (underweight as <18.5 kg/m²; healthy weight as 18.5-24.9 kg/m²; overweight/obese as ≥25 kg/m²). We also found that age modified the effect of oyster consumption and cadmium and stratified analyses by age. We created two groups for age: <35 and ≥35 years based on the median age of 35 years. However, there were no significant differential results after stratification for either variable.

**Mediation Models**
We assessed the mediating role of plasma zinc or urinary cadmium concentrations on the relationship between oyster consumption and serum ferritin concentrations (Figure 3). The results revealed that the direct effect of oyster consumption on ferritin was not significant (Table 4). Recently, there has been increased discussion on evaluating mediation pathways when there is no direct effect. It is possible that although there is no direct effect, there may still be a significant indirect and total non-significant effect in the pathway. This may happen when the indirect effect is not large and with increased sample variability. Previous research has found that the indirect effect has greater power than the test of the total effect, increasing the likelihood that the indirect effect is significant. We also hypothesized the mediation pathway with justification a priori, as it is possible that there is a true and mediated relationship between oyster consumption and iron status, thus supporting the use of the model in the absence of a significant direct effect. However, when we tested the indirect effect of oyster consumption on ferritin (mediated by plasma zinc or urinary cadmium), the findings were not significant. Therefore the impact of oyster consumption on ferritin was not mediated by plasma zinc or urinary cadmium concentrations in this population. Similar results were found with dietary iron intake as a predictor and hemoglobin as an outcome. (Figure 3, Table 4).

DISCUSSION

This study examined the relationships between oyster consumption and zinc and cadmium concentrations, and whether zinc and cadmium impact the relationship between oyster consumption and markers of iron status. Our findings show that oyster consumption was not associated with zinc or cadmium concentrations in linear models and there was no difference in zinc or cadmium concentrations between women who consumed oysters and
those who did not. We previously found that anemia and iron deficiency were high in this population, including among women who consumed oysters and those who had high iron intake. It is possible that high zinc intake and cadmium exposure from oysters influences the impact of oysters on markers of iron status (figure 2). However, neither zinc nor cadmium mediated the relationship between oyster consumption or dietary iron intake with markers of iron status (hemoglobin or ferritin concentrations).

The variability of oyster micronutrient content may influence the impact of oyster consumption on micronutrient concentrations in this population. Oysters accumulate micronutrients and metals in their tissues by acquiring it from the environment through the process of filter-feeding. As such, the micronutrient and metal content of oysters and the contribution to micronutrient intake and metal exposure in humans is highly dependent on the surrounding environmental conditions. The rainy seasons in Ghana may influence the iron, zinc, and heavy metal concentrations in oysters. A study in Ghana conducted in 1996 found that oysters (*Crassostrea Tulipa*) had the highest iron content in November and December, during the dry season, and lowest concentrations between March and September with a range of 9.81 mg/100g to 62.03 mg/100g. It is possible that oysters harvested and consumed in July and August had low iron concentrations, limiting the impact of oyster consumption on iron status. Therefore, it is understandable that zinc and cadmium would not impact the relationship between oyster consumption and iron status if the iron content of oysters was low and the iron provided had low bioavailability.

Metal concentrations in oysters may also fluctuate depending on season. In the neighboring country of Coté d’Ivoire, oysters were found to have significantly higher cadmium during the rainy season, and higher lead concentrations in the dry season.
Based on the low cadmium concentrations among women who consumed oysters in this population, cadmium contamination during the open season may not be of great concern in this community and oysters may continue to serve as a valuable food source for women of reproductive age in Ghana.

Oyster consumption was not associated with cadmium concentrations, however, other heavy metals such as lead and mercury may influence iron absorption and utilization. While oysters are listed under the best choices category based on the methylmercury content by the Food and Drug Administration, \textsuperscript{15} we previously found that smoked, dried oysters from Ghana were contaminated with cadmium, lead, arsenic, and most significantly with mercury (manuscript 1, in preparation). Further research is needed to determine if mercury acts as a mediator between dietary iron intake and ferritin concentrations and the impact of rainfall on oyster content in Ghana. Oysters in Ghana that are being grown for harvesting and consumption should be regularly monitored for heavy metals. It is possible that there is a more optimal time to implement the closed and open season of oyster harvesting that maximizes micronutrient intake and minimizes heavy metal exposure for people who harvest and consume oysters.

It is interesting to observe that in this study, zinc was adequate in the same sample while iron deficiency was high. This may be due to challenges in measuring zinc status.\textsuperscript{16} Currently, there are no biomarkers that are generally accepted for measuring the zinc status of an individual. The relationship between the biological functionality of zinc and plasma zinc concentrations remains unclear.\textsuperscript{17} Zinc is well regulated with efficient utilization in the body and no clear symptoms indicating deficiency or excess in non-pregnant women of reproductive age. We used plasma zinc because it may be used at a
population level, to predict functional responses to an intervention, declines when there is low dietary zinc intake (<2 mg/day), and has established reference limits that we can compare the sample population to. However, it is possible that while plasma zinc concentrations are adequate, dietary zinc intake is low and biological functionality is impaired. There is high heterogeneity in research determining the relationship between dietary zinc intake and plasma zinc concentrations. Previous studies have also estimated that Sub-Saharan Africa is a region with high zinc deficiency due to diets high in plant-based foods. Furthermore, plasma zinc can be influenced by a range of factors, such as oral contraceptive use, severe stress, and diurnal variations, which we could not adjust for. However, we did adjust for inflammation, age, and used standardized blood collection procedures that allow for more accurate estimations of zinc status.

Limitations to this study include the use of self-report for dietary intake and oyster consumption, which allows for recall bias to influence our results. We previously discussed that the food frequency questionnaire overestimated dietary iron intake, and so we are unable to present mean daily intake. However, we did include multiple biological markers of iron status. Another limitation is that it is unknown how the COVID-19 pandemic may have influenced oyster consumption, dietary zinc intake, or exposure to cadmium. Strengths of this study include the use of accurate markers of zinc and cadmium and gold-methods standards for evaluating minerals and metals in plasma and urine. We also were able to conduct within group comparisons, which reduce external variability error. The presence of a control group helped us evaluate variability due to external factors such as COVID-19.
In conclusion, we did not find a significant association between oyster consumption and plasma zinc or urinary cadmium concentrations and neither zinc nor cadmium mediated the relationship between oyster consumption or dietary iron intake with markers of iron status. The etiology of high iron deficiency remains unclear and supplementation of iron that is highly bioavailable should be recommended to increase the iron status in this population. Regular monitoring and regulation of oyster heavy metal content is also needed to ensure that heavy metals like mercury and lead do not contribute to the underlying causes of iron deficiency and anemia.
TABLES

Table 1. Characteristics of Ghanaian women of reproductive age by oyster consumption group enrolled during the closed season of oyster harvesting into a pilot study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Oyster Group</th>
<th>Oyster Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD) or %</td>
<td>Mean (SD) or %</td>
</tr>
<tr>
<td>n</td>
<td>70</td>
<td>66</td>
</tr>
<tr>
<td>Age, years</td>
<td>33.4 (8.3)</td>
<td>35.0 (8.2)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>58%</td>
<td>47%</td>
</tr>
<tr>
<td>Secondary School</td>
<td>42%</td>
<td>53%</td>
</tr>
<tr>
<td>Married or Cohabitating</td>
<td>64%</td>
<td>74%</td>
</tr>
<tr>
<td>Household Food Insecurity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Secure</td>
<td>36%</td>
<td>34%</td>
</tr>
<tr>
<td>Food Insecure</td>
<td>64%</td>
<td>66%</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>14%</td>
<td>5%</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 (5.5)</td>
<td>25.8 (4.5)</td>
</tr>
<tr>
<td>Positive Malaria Test</td>
<td>1.6%</td>
<td>1.8%</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.01 (.31)</td>
<td>1.38 (.21)</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>1.00 (0.06)</td>
<td>0.79 (0.05)</td>
</tr>
</tbody>
</table>

CRP and AGP show geometric means and SE

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season
Table 2. Unadjusted and adjusted beta ($\beta$) coefficients of plasma zinc and urinary cadmium predictors during the open oyster harvesting season and oyster consumption in Ghanaian women of reproductive age enrolled in a pilot study.

<table>
<thead>
<tr>
<th>Oyster Consumption</th>
<th>Plasma Zinc</th>
<th>Urinary Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ (SE)</td>
<td>$p$</td>
<td>$p$</td>
</tr>
<tr>
<td><strong>Plasma Zinc</strong></td>
<td>n=133</td>
<td>n=132</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.159 (0.134)</td>
<td>0.175 (0.133)</td>
</tr>
<tr>
<td>Adjusted$^1$</td>
<td>0.164 (0.137)</td>
<td>0.197 (0.133)</td>
</tr>
</tbody>
</table>

$^1$Models adjusted for BMI and AGP collected at enrollment
$^2$Models adjusted for BMI and age collected at enrollment.

Table 3. Comparisons of plasma zinc, zinc deficiency, urinary cadmium, and cadmium toxicity by oyster consumption groups and oyster harvesting seasons among Ghanaian women of reproductive age enrolled in a pilot study.

<table>
<thead>
<tr>
<th></th>
<th>Closed Season</th>
<th>Open Season</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Zinc, $\mu$g/dL</strong></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td></td>
</tr>
<tr>
<td>Number of plasma samples</td>
<td>133</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Oyster group</td>
<td>116.4 (2.0)</td>
<td>101.7 (2.8)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Non-oyster group</td>
<td>118.5 (1.9)</td>
<td>108.2 (3.2)</td>
<td>0.007*</td>
</tr>
<tr>
<td>$p$</td>
<td>0.794</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>Zinc deficiency (&lt; 66 $\mu$g/dL)</td>
<td>n=0/133</td>
<td>n=4/120</td>
<td></td>
</tr>
<tr>
<td>Oyster group</td>
<td>0%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Non-oyster group</td>
<td>0%</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

| **Urinary Cadmium, $\mu$g/g creatinine** | | |
| Number of urine samples | 132           | 121         |      |
| Oyster group           | 0.023 (0.001) | 0.037 (0.003) | <0.0001* |
| Non-oyster group       | 0.025 (0.003) | 0.039 (0.003) | <0.0001* |
| $p$                    | 0.279         | 0.881       |      |

Footnote: Oyster group includes women who reported consuming oysters in the last 30 days and the non-oyster group includes women who did not consume oysters in the last 30 days during the open season.
Table 4. Total effect, direct effect, and indirect effect of the mediation models showing the associations between dietary iron intake or oyster consumption and hemoglobin or serum ferritin mediated by plasma zinc or urinary cadmium.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Total Effect</th>
<th>Direct Effect</th>
<th>Indirect Effect</th>
<th>Confidence Interval</th>
<th>t-statistics</th>
<th>Mediation Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td><strong>Dietary Intake Mediation Models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Iron Intake → uCd → Hb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.731 (0.034)</td>
<td>0.741 (0.033)</td>
<td>-0.011</td>
<td>-0.123</td>
<td>0.106</td>
<td>-0.20</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.709 (0.046)</td>
<td>0.707 (0.048)</td>
<td>0.002</td>
<td>-0.089</td>
<td>0.129</td>
<td>0.04</td>
</tr>
<tr>
<td>Dietary Iron Intake → pZn → Hb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.710 (0.041)</td>
<td>0.694 (0.049)</td>
<td>0.016</td>
<td>-0.133</td>
<td>0.138</td>
<td>0.24</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.697 (0.052)</td>
<td>0.665 (0.069)</td>
<td>0.032</td>
<td>-0.149</td>
<td>0.194</td>
<td>0.38</td>
</tr>
<tr>
<td>Dietary Iron Intake → uCd → SF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.039 (0.796)</td>
<td>0.033 (0.829)</td>
<td>0.006</td>
<td>-0.039</td>
<td>0.062</td>
<td>0.25</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.042 (0.794)</td>
<td>-0.052 (0.747)</td>
<td>0.010</td>
<td>-0.055</td>
<td>0.073</td>
<td>0.33</td>
</tr>
<tr>
<td>Dietary Iron Intake → pZn → SF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.025 (0.872)</td>
<td>0.001 (0.994)</td>
<td>0.023</td>
<td>-0.030</td>
<td>0.086</td>
<td>0.83</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.058 (0.719)</td>
<td>-0.091 (0.573)</td>
<td>0.034</td>
<td>-0.028</td>
<td>0.112</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Oyster Consumption Mediation Models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oyster consumption → uCd → Hb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.327 (0.279)</td>
<td>0.283 (0.359)</td>
<td>0.044</td>
<td>-0.063</td>
<td>0.189</td>
<td>0.71</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.331 (0.276)</td>
<td>0.282 (0.360)</td>
<td>0.049</td>
<td>-0.055</td>
<td>0.212</td>
<td>0.75</td>
</tr>
<tr>
<td>Oyster consumption → pZn → Hb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.327 (0.279)</td>
<td>0.406 (0.178)</td>
<td>-0.080</td>
<td>-0.413</td>
<td>0.070</td>
<td>-0.65</td>
</tr>
</tbody>
</table>
Oyster consumption → uCd -> SF

<table>
<thead>
<tr>
<th></th>
<th>Adjusted</th>
<th>Unadjusted</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>uCd</td>
<td>0.331 (0.276)</td>
<td>0.421 (0.169)</td>
<td>-0.089</td>
<td>-0.482</td>
<td>0.059</td>
<td>-0.64</td>
<td>None</td>
</tr>
<tr>
<td>pZn</td>
<td>0.143 (0.230)</td>
<td>0.135 (0.270)</td>
<td>0.008</td>
<td>-0.049</td>
<td>0.065</td>
<td>0.31</td>
<td>None</td>
</tr>
</tbody>
</table>
| Hemoglobin models adjusted for marital status, CRP, and AGP, BMI, and age collected at enrollment. Ferritin models adjusted for food insecurity score, parity, and marital status, BMI and age collected at enrollment. Ferritin adjusted for CRP and AGP using the BRINDA method.

Abbreviations: uCd, urinary cadmium; pZn, plasma zinc; Hb, hemoglobin; SF, serum ferritin

Oyster consumption measured in grams using a 30-day food frequency questionnaire.
Figure 1. Study Design calendar showing the two data collection timepoints, open and closed oyster harvesting seasons, and major and minor rainy seasons in southern Ghana.
Figure 2: Flowchart of participant enrollment and anemia outcomes in a pilot study on the association between dietary iron intake, oyster consumption, and anemia in Ghanaian women.

139 Screened

- 2 not eligible
  - Did not meet health criteria

137 recruited

- 1 not enrolled
  - non-oyster group enrollment complete

66 categorized into oyster group

- 64 anemia outcomes during closed season
- 56 anemia outcomes during open season

70 categorized into non-oyster group

- 70 anemia outcomes during closed season
- 64 anemia outcomes during open season
Figure 3. Adjusted mediation analysis for the association between the independent variable (oyster consumption or dietary iron intake), mediator variables (urinary cadmium or plasma zinc), and dependent variable (hemoglobin or serum ferritin).  

a.  

b.  

c.
Figure 3 (cont.)

1Values present a’, b’, and c’ coefficients representing indirect and direct associations. The direct effect of dietary iron intake or oyster consumption on ferritin or hemoglobin concentrations is not mediated through other variables, whereas the indirect effect is mediated through zinc or cadmium on the pathway. The total effect is the direct and indirect effects added together. Hemoglobin models adjusted for marital status, CRP, and AGP collected at enrollment. Ferritin models adjusted for food insecurity score, parity, and marital status collected at enrollment. Ferritin adjusted for CRP and AGP using the BRINDA method.
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LITERATURE REVIEW

Oysters are globally valued due to their nutritional value, positive ecological impact, sustainability, and economic benefits. They are nutrient dense and contain protein, omega-3 fatty acids, and provide a good source of vitamins and minerals such as zinc, iron, and vitamin B12.\(^1\)-\(^3\) Oysters are also considered to be a sustainable food source and have a low impact on greenhouse gas emissions, creating a favorable nutrient density to environmental impact ratio.\(^3\) Oyster populations can be managed to ensure long-term sustainability, with practices such as regulated harvesting. Management of oyster harvesting can include the implementation of an open and closed season of oyster harvesting,\(^4\) which allows the oysters to spawn and grow bigger. Communities can also incorporate a shell recycling program to support the growth of new oyster reefs and fisheries.\(^5\)

As filter feeders, oysters can have a positive ecological and economic impact on their environment. Oysters filter and remove toxic metals, excess nutrients, sediments, and pollutants from the water.\(^6\)-\(^9\) By doing so, they can create a healthier aquatic ecosystem. Oyster reefs also contribute to the habitat and shelter for various marine species, promoting biodiversity and improving coastal ecosystems.\(^6\) Oysters can positively impact the economy at a local and national level. Oyster farming and harvesting provides a source of income in coastal communities, supporting the local economy.\(^4,10\) Oysters can also contribute to trade and export, increasing revenue domestically and internationally.\(^11\)

Oysters can be a good source of iron. Iron is an essential micronutrient involved in various physiological processes and critical for women of childbearing age. Iron is needed to transport oxygen throughout the body and attached to hemoglobin, a protein in
red blood cells that is used to determine anemia status. Iron plays a role in the electron transport chain and is therefore involved in energy production. It can also function as an antioxidant at healthy levels and protects cells from damage. Iron can also influence DNA synthesis and repair and contributes to the production of white blood cells which strengthen the immune system. Iron is critical during and after pregnancy, as it supports proper growth and development of the fetus, continuing into infancy and childhood.

Iron deficiency results in decreased and altered red blood cell production, lower hemoglobin, and lower ferritin concentrations, the storage protein of iron and most accurate marker of iron status. Iron deficiency anemia is the presence of both anemia (Hb < 12 g/dL) and iron deficiency (ferritin < 15 ng/ml). There are many adverse health outcomes associated with iron deficiency and iron deficiency anemia. Iron deficiency can negatively impact oxygen transport and energy production, leading to feelings of fatigue, weakness, and cognitive impairment. Consequentially, iron deficiency can negatively impact work performance and day-to-day activities. Due to its role in the immune system, a deficiency can make a person more vulnerable to infection and decreased ability to recover. During pregnancy, iron deficiency increases the risk for adverse birth outcomes, such as stunting and preterm birth. Iron deficiency is also associated with delayed cognitive development in children.

Oysters may also be a good source of zinc. Zinc is an essential micronutrient that is involved in various enzymatic reactions and is required for proper growth, development, and immune function. Zinc supports the production and activation of immune cells, which supports the immune system to fight infection, and is involved in DNA synthesis and repair, as well as cell division and growth. Zinc is also important as
an anti-oxidant. Zinc has a role in regulating cytokine expression, which reduces inflammation, and is an important co-factor used to activate enzymatic antioxidants. The antioxidant activity of zinc can decrease oxidative stress by reducing reactive oxygen species that may harm cellular function. Zinc is also involved in lipid metabolism and influences blood pressure and cholesterol levels.

Because of the important physiological roles of zinc, zinc deficiency can lead to impaired immune function, delayed wound healing, and decreased fetal and childhood growth and development. Maternal zinc status has also been associated with low birth weight and pre-eclampsia. Stunting is a low height-for-age, and an indicator of zinc deficiency at a population level. Zinc supplementation has been shown to reduce stunting in populations that are zinc deficient. When the prevalence of stunting is >20% among children < 5 years, it is labeled as a public health problem by the World Health Organization. A study by Wessells et al. estimated the global prevalence of zinc deficiency using the prevalence of stunting and national food supplies. They found that inadequate zinc intake is common, and of particular concern in Sub-Saharan Africa. In Ghana, stunting was found to be >20% and is therefore a public health problem in the country.

Oysters may be a source of cadmium, a toxic heavy metal. Exposure to cadmium, primarily through dietary intake, can lead to kidney damage, increased oxidation and cellular damage, and increased risk for cancers and fetal abnormalities. Cadmium can also contribute to increased risk for cardiovascular disease and hypertension. Cadmium has also been associated with adverse birth outcomes. A study by Cheng et al. evaluated cadmium exposure at different times during pregnancy and the associations with fetal
growth and birth weight. They found that higher maternal cadmium was associated with lower birth size and weight in girls. Another study by Zhang et al. found that higher maternal urinary cadmium was associated with shortened telomere length in newborns, which is a marker of increased risk for chronic disease such as diabetes, cardiovascular disease, and certain cancers. Cadmium also interacts with other micronutrients and has been associated with iron deficiency. A study by Gallagher et al. evaluated body iron stores and the association with cadmium concentrations in non-pregnant women of reproductive age. They found that lower urinary cadmium was associated with higher iron and that iron deficiency was associated with higher cadmium concentrations.

Micronutrients can be obtained through food sources or dietary supplements. Some of the benefits of receiving micronutrients from food sources include intake alongside other valuable nutrients from nutrient dense food sources and synergistic effects. For example, in addition to iron and zinc, oysters can provide protein and omega-3 fatty acids. The physical structure of the food, especially from plant sources, is also important to intestinal health. This is especially important for plant-based sources of iron. The form of iron in plants, ferric iron, has lower bioavailability compared to animal based sources. However, when consumed with vitamin C, ferric iron can be converted to ferrous iron, which is more easily absorbed in the intestines. Some of the challenges related to micronutrients from food sources include meeting daily requirements for normal physiological function, and availability and accessibility to nutrient dense foods. In these cases, supplementation may be a more feasible way to regularly meet daily requirements while also reducing the risk to heavy metal exposure from food sources.
Ghana is located in Sub-Saharan Africa, with agriculture, mining, and oil production as the major contributors to the economy. The Ghanaian diet is primarily comprised of traditional dishes made from locally available ingredients. The diet is high in grains, vegetables, fruits, meat, and fish. One staple foods in Ghana include fufu, which is made of boiled cassava, yam, or plantains and turned into a smooth, dough-like consistency. Fufu is usually served with soups or stews, such as light soup or groundnut soup, which are made with vegetables, herbs, spices, and sometimes fish or meat. Banku is another food commonly consumed, made of fermented corn and cassava dough that is cooked and usually eaten with a soup or stew. Fish is an important part of the Ghanaian diet and accounts for 60% of animal protein consumed. The national average for dietary intake of seafood is also >200% of the recommended intake.

Like many countries that experience economic development, Ghana has been undergoing a nutrition transition. Nutrition transition refers to a shift from primarily traditional, often plant-based diets to western diets that are higher in energy dense foods and lower in nutrient dense foods. A study by Rousham et al. conducted a meta-analysis that evaluated changes in Ghana in the context of the nutrition transition between 1971-2018. They found that consumption of sugar sweetened beverages was high and consumption of fruit and vegetables was low. There was also a wide range of dietary patterns, where some areas and groups consumed more traditional foods, while people in more urban areas were more likely to follow westernized food patterns.

In Ghana, malnutrition remains a significant challenge. Despite Ghana's progress in meeting nutrition goals, micronutrient deficiencies are still highly prevalent. The 2017 Ghana Micronutrient Survey determined that 22% of non-pregnant women have anemia
and 14% have iron deficiency, respectively. However, 45% of pregnant women were found to have anemia. The 2014 Demographic and Health Survey also found that 19% of children were stunted and 11% were underweight, and over 40% of women in Ghana were found to be overweight or obese. The presence of high rates of overweight and obesity occurring alongside micronutrient deficiencies emphasizes the double burden of malnutrition.

In conclusion, oysters offer numerous benefits as a valuable food source. They are highly nutritious, containing essential nutrients such as protein, omega-3 fatty acids, zinc, and iron. However, it is important to consider potential risks associated with oysters, such as the presence of cadmium, a toxic heavy metal. In Ghana, despite progress in addressing malnutrition, micronutrient deficiencies and the double burden of malnutrition persist. Efforts should focus on improving access to nutrient-rich foods like oysters, decreasing exposure to toxic metals, and addressing nutritional disparities to combat malnutrition effectively.
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Appendices

**Appendix 1.** Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the NON-OYSTER GROUP during the open and closed seasons.
Appendix 2. Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the OYSTER GROUP during the open and closed seasons.
Appendix 2 cont.
Appendix 3. Distribution of daily dietary iron intake during the open and closed season among all women enrolled in the study

Footnote:
This shows that daily dietary iron intake was likely overestimated by the 30-day FFQ. Previous studies have shown that average iron intake in women in Ghana is 14 mg/day, respectively. In contrast, the range presented above is ~1 mg-170 mg/day, which is highly unlikely.
Appendix 4. Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the LOW DIETARY IRON INTAKE GROUP during the OPEN season
Appendix 4 cont.
Appendix 5. Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the HIGH DIETARY IRON INTAKE GROUP during the OPEN season.
Appendix 5 cont.
Appendix 6. Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the LOW DIETARY IRON INTAKE GROUP during the CLOSED season.
Appendix 6 cont.

![Graph of CRP (Closed Season) vs Normal Quantiles](image1)

![Graph of Creatinine (Closed Season) vs Normal Quantiles](image2)
Appendix 7. Distribution of hemoglobin, ferritin, AGP, CRP, and creatinine concentrations in the HIGH DIETARY IRON INTAKE GROUP during the CLOSED season