Regular crabmeat consumers do not show increased urinary cadmium or beta-2-microglobulin levels compared to non-crabmeat consumers.

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Short title*:* Cadmium exposure in crabmeat consumers

Abbreviations*:* Cadmium (Cd); beta-2-microglobulin (B2M); Inductively-coupled plasma mass spectrometry (ICP-MS); European Food Safety Authority (EFSA), Selenium (Se).

Abstract

Cadmium (Cd) is a toxic metal that can be relatively high in brown meat from crab and there is concern that it may accumulate in long-term crabmeat consumers posing a health risk. Sixteen healthy habitual crabmeat consumers and twenty five healthy non-crabmeat consumers were recruited through completion of a seafood frequency questionnaire. Whole blood and urine samples were analysed for Cd levels and urinary beta-2-microglobulin, an established marker of Cd-induced kidney toxicity, to determine levels in crabmeat consumers. Whole blood Cd levels were significantly elevated in the crabmeat-consuming group, whereas urinary levels of Cd and beta-2-microglobulin were not. Whole blood Cd levels can be both a short and long-term marker for Cd intake and levels might be expected to be elevated in the crabmeat consumers as crabmeat can contain Cd. However, crabmeat consumers did not show increases in a more established long-term marker of Cd (urinary Cd) and consistent with this, no change in a Cd-induced kidney toxicity marker. Consequently, in conclusion, compared to consumers who reported very little crabmeat consumption, healthy middle-aged consumers who regularly consume brown crabmeat products (an average of 447g/week) for an average of 16 years showed no change in long-term Cd exposure or kidney toxicity.

Keywords: Cadmium; crab; brown crabmeat; beta-2-microglobulin; selenium

1. Introduction

Cadmium (Cd) is a heavy metal and a well-known environmental contaminant. In the non-smoking population, the principal exposure to Cd is from dietary sources. Although absorption of Cd from the diet in humans is comparatively low (3–5%), it is efficiently retained in the kidney and liver, with a long biological half-life (10 to 30 years) [1]. Therefore, accumulation of Cd is known to increase with age [2], and levels of urinary Cd (urinary Cd/g creatinine) reported to be maximal at 50 years old [3]. Cd is thought to be absorbed within the body in the intestine through the use of the same transport pathways as essential metals including zinc, calcium and iron. After absorption, Cd is transported in the blood to the liver where it forms a complex with metallothionein proteins. In the kidney, the Cd-metallothionein complex is filtered in the glomeruli and then reabsorbed in the renal tubules. It is in the kidney that Cd is known to be primarily toxic, especially to the proximal tubular cells where accumulation over time may cause a decrease in the glomerular filtration rate and eventually renal failure. Cd is also known to cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction and has been statistically associated with increased risk of cancer in the lung, endometrium, bladder and breast [4,5].

The major food groups contributing the most to Cd exposure are rice and grains, shellfish and seafood, and meat and vegetables. It is known that meat from crab can contain high levels of Cd both in the white meat but especially so in the brown meat (the hepatopancreas and liver) [6,7]. There is concern that Cd from brown crabmeat consumption can contribute greatly to the body burden of Cd, however, few studies have investigated whether the Cd present in crabmeat increases the levels of Cd in the body and similarly, it is not known whether higher Cd intakes in regular long-term brown crabmeat eaters is associated with increased incidence of kidney damage. There is some evidence to indicate that the bioavailability of Cd from crabmeat may be lower when compared to Cd in other foods [8]. Moreover, studies in rodents have suggested that selenium (Se) (and perhaps zinc), which are both present at high levels in crabmeat (and especially so in the brown meat), may counteract the toxicity of Cd [9] and other heavy metals [10,11]. This study aimed to investigate whether volunteers who reported regularly consuming crabmeat had higher body levels of Cd compared to volunteers who do not consume crabmeat. A specific food frequency questionnaire was designed to capture volunteers' total crabmeat intake and to estimate their Cd exposure based on their reported seafood consumption. This information was compared with the volunteers' body exposure of Cd (urinary Cd and blood Cd levels) to determine whether regular crabmeat consumers had an increased burden of Cd compared to non-crabmeat consumers. In addition, Se, zinc and the status of other heavy metals

was assessed in order investigate their relationship with crabmeat Cd and burden. Lastly, Cd toxicity was assessed through measurement of urinary levels of beta-2-microglobulin (a recognised marker of Cd-induced kidney toxicity) [12] to establish whether regular long-term crabmeat consumers showed greater signs of kidney toxicity compared to non-crab consumers.

2. Material and Methods

2.1 Study participants and study design

The study was a cross-sectional design with two groups of participants, non-crabmeat and habitual crabmeat consumers, who were assessed and compared for their Cd toxicity levels. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Rowett Ethics Review Panel, University of Aberdeen. Informed consent was obtained from all volunteers prior to the start of the study. Participants were recruited from mainland Scotland and Orkney via press releases, flyers, newspapers, websites, information boards, radio advertisements and during recruitment events. Interested male and female participants aged over 40 years old were screened through completion of a questionnaire (paper or online) to exclude those with current heart/circulatory, liver or kidney disease, anaemia, glomerular nephritis, cancer, pregnancy and addiction to any substances. Current or ex-smokers in the previous 5 years were also excluded since smoking would be an additional confounding factor contributing to Cd body burden [2]. Participants were also excluded if they reported environmental exposure to Cd through current or previous occupation (alloy maker, aluminium solder maker, ammunition maker, car mechanic, battery maker, bearing maker, brazier or solderer, cable, trolley wire maker, cadmium plater, cadmium vapour lamp maker, ceramics, pottery maker, copper-cadmium alloy maker, dental amalgam maker, electric instrument maker, electrical condenser maker, electroplater, engraver, glass maker, incandescent lamp maker, incinerator of municipal waste, jeweller, lithographer, lithopone maker, mining and refining worker, paint maker, paint sprayer, pesticide maker, pharmaceutical worker, photoelectric cell maker, pigment maker, plastic product maker, sculptor of metal, smelter, solder maker, textile printer, welder of Cd alloy or Cd-plate, working with phosphate fertilisers) or might have been exposed to Cd through living near a zinc, lead, or copper smelter or an iron or steel production facility. Additionally, vegetarians were excluded as they have been shown to have significantly higher blood Cd levels than non-smoking non-vegetarians [13]. Volunteers were screened and recruited into the two study groups based on their levels of crabmeat consumption and subsequent estimated Cd intakes. Sixty one volunteers were assessed for their entry into the study (Fig, 1). Twelve volunteers of

the potential crabmeat consuming group were excluded as they failed to meet the minimum required intake of Cd from brown crabmeat and a further 2 potential volunteers were excluded as they failed to meet the health criteria. Of the potential non-crabmeat consumers, 4 of these were calculated to have a Cd intake from crabmeat/seafood products above the permitted level for this group and a further 2 volunteers also failed to meet the health criteria to be included in the study. A total of forty-one participants (sixteen consuming crabmeat and twenty-five who did not) then attended either the human nutrition unit at the Rowett Institute in Aberdeen or a health practice on Orkney where anthropometric measurements and a 40ml non-fasted blood sample were taken. Prior to this visit, participants were sent a metal-free container to collect a midstream 20mL sample of first-morning urine on the morning of their visit. Blood and urine samples taken at the Rowett were processed and stored at -70^{o}C. Blood samples taken on Orkney were processed at Balfour hospital, Kirkwall (NHS Orkney) prior to shipment and storage at the Rowett. The study was registered at http://www.clinicaltrials.gov (NCT03104530).

2.2 Seafood and crabmeat questionnaire

A detailed questionnaire covering 55 questions was designed to determine the participants' current and historical intake of Cd from crabmeat and brown crabmeat as well as other foodstuffs known to contain relatively high levels of Cd (100% brown crabmeat (3.9µg/g wet weight), dressed crab (1.9µg/g), crab pastes and spreads (2.4µg/g), crab pâté, terrines and potted crab (4µg/g), tinned dressed crab (6.4µg/g), crab cakes (0.08µg/g), crab soup or bisque (1.35µg/g), ready-made mixed crab dishes (0.26µg/g), scallops, whelk, seaweed, and algae supplements (algae capsules/powders, astaxanthin, chlorella, silica, spirulina, sea plasma, vegetarian or vegan algal omega 3 fats)) (Supplementary File 1). For each food product, participants were asked how long they had been consuming the food, whether consumption had changed ('increased', 'decreased' or 'stayed the same') in that period, how often ('number of portions per day', 'per week', per month' or 'per year') and how much they consumed in standard portion size ('small', 'medium' or 'large portion' using photographs as visual aids). Together, this questionnaire was used to exclude all volunteers with potentially higher than average Cd body status, either through occupational exposure to Cd or through consumption of non-brown crabmeat products, such as seaweed, scallops, whelk and algal formulations which are known to contain elevated levels of Cd. Moreover, the questionnaire was used to calculate participants' average consumption of crabmeat/brown crabmeat products and subsequently, their estimated weekly intake of Cd from these products (Supplementary File 2).

This estimate was based on data from relatively up-to-date analysis of Cd levels in foods present in the UK diet [14,15] and recent UK survey data based on 399 individual samples of brown crabmeat products [16]. Participants with calculated intakes of ≥156 μg Cd/week from brown crabmeat products eaten consistently for 5 years or more were recruited into the regular brown crabmeat group whereas those who had never consumed or consumed very little crab meat (less than an average of two 60g portions/y and having Cd intakes from crabmeat of ≤10 μg Cd/week) over at least 5 years were recruited into the non-crabmeat group. The Cd intake limits for each group were calculated to provide a sufficient difference in intakes of Cd in order to detect differences in urinary Cd levels between those groups should they be present [17]. In addition, setting a Cd intake level of ≥156 μg Cd/week from crabmeat, when combined with the likely Cd intake from the average background diet (1.1μg Cd/kg body weight/week [14]), would result in a level above the tolerable weekly intake (TWI) for Cd of 2.5μg Cd/kg body weight/week.

2.3 ICP-MS analysis

Whole blood samples (0.95ml) were added to water (0.05ml) and were digested in nitric acid (65% (v/v)) using the MARS 6 digestion system (CEM, Matthews, USA) and then stored overnight at room temperature. Samples were ramped from room temperature to 210 °C and then held at this temperature for 10 min before being cooled. Digested whole bloods and undigested urine samples were diluted in decomposition matrix prior to ICP-MS analysis. The decomposition matrix was nitric acid (2% (v/v)) and hydrochloric acid (0.5% (v/v)) in distilled deionized water (Millipore, UK), which was used for preparation of all solutions. The measured isotopes analysed by ICP-MS were ^{66}Zn, ^{78}Se, ^{111}Cd, ^{202}Hg, and ^{208}Pb. All element standards were used in stock solutions of 1000 mg/L, which served for the preparation of calibration solutions and internal standard solution. The ICP-MS measurements were carried out using the Agilent 7700X spectrometer (Agilent Technologies) equipped with a MicroMist nebulizer and nickel sampler and skimmer cones. The flow of standards or samples was joined together with a flow of erbium internal standard solution (1 mg/L). The mixed flow (approximately 500 μL /min) was delivered by the peristaltic pump to the nebulizer of the ICP-MS setup. Duration of ICP-MS analysis was 3.0 min. Data acquisition was one point, five replicates and 100 sweeps per replicate. The accuracy of the method was assessed using two certified materials: digested whole blood (Seronorm Whole Blood L-2) and undigested urine (Seronorm Trace Elements Urine L-1) (SERO AS, Billingstad, Norway). The median recovery values of the relevant elements were within the certified ranges as indicated by the supplier.

2.4 Urine creatinine

Spot urine samples were collected from volunteers and aliquoted and stored at −70°C until analysis. After thawing, creatinine levels were measured using a creatinine assay kit in accordance with the manufacturer's instructions (Abcam, UK).

2.5 Urine beta-2-microglobulin levels

Urinary concentrations of beta-2-microglobulin were measured using the human beta-2-microglobulin ELISA Kit (Abcam, UK) in accordance with the manufacturer's instructions. Urine samples were initially stored at −70°C then thawed on ice prior to assay and quantified using the *u*-quant microplate spectrophotometer (Bio-Tek Instruments Inc., USA).

2.6 Statistical analysis and power calculation

A power analysis revealed adequate power for detecting a change in the primary outcome measure of urinary Cd (μg Cd/g Cr). The within group standard deviation in urinary Cd was calculated to be 0.32 μg Cd/g Cr, after adjustment for age and gender. This would give a percentage standard deviation of 59% and with group sizes of 16 and 25, there would have been power to detect differences in mean urinary Cd between the two groups of about 0.3 μg Cd/g Cr with 80% power. This would be sufficient to detect effects of around 50-60% (with mean urinary μg Cd/g Cr being 0.54). As the study anticipated that the intake level difference of 150 μg Cd/wk (between the crabmeat consumers and the non-consumers) would result in a predicted difference in urinary Cd of around 60% [18] this was within the detection limits of the study. Data describing volunteers' characteristics as well as crabmeat, seafood and estimated Cd intake levels (Tables 1-3) was analysed by Student's paired t-tests. All other data were analysed by linear models with terms for age, gender and crab-eating-group. Location was too unbalanced and so a term for this was not included. For some variables with a skewed distribution, analysis was repeated on a log scale, and where appropriate this is reported. A *p*-value of 0.05 or less was considered to indicate statistical significance. Correlation coefficients were calculated to indicate the association between variables, and their significance tested by linear regression. Statistical analysis was carried out using R 3.2.4 (R foundation for statistical computing, Vienna).

3. Results

3.1 Subject characteristics

Forty one volunteers completed the study (16 volunteers in the crabmeat-consuming group and 25 in the non-crabmeat group) and their characteristics are shown in Table 1. Both groups were well-matched for age with an overall average of 52. Although average volunteer heights were

similar in each group, as males made up a greater percentage of the crab-consuming group, this group had significantly higher average weight and therefore BMI.

3.2 Reported crabmeat intake levels and estimated cadmium intakes.

The reported volunteer intakes of crabmeat from different crabmeat products containing brown crabmeat that were assessed in the study are shown in Table 2. Some very limited crabmeat consumption was reported by some subjects in the non-crabmeat group but when averaged across the group these amounts were very low. The crabmeat group consumed an average of 447g /wk of brown crabmeat products compared to 0.3g /wk in the non-crabmeat group ($p<0.0001$, Table 2). Most of the crab intake was due to consumption of 100% brown crabmeat (28%), dressed crab (30%) and crab soups or bisques (23%) products (Table 2). The reported intakes of crabmeat and other seafood in the questionnaire were used to estimate the average amount of Cd intake/wk in each volunteer across all of these products (Table 3). Those volunteers assigned to the crabmeat-consuming group had much higher estimated total amount of Cd intake (μg /wk) compared to those in the non-crabmeat group (1022 ± 1045 versus 2.3 ± 4.0 respectively, $p<0.001$). In addition, the majority of the weekly Cd intake in the crabmeat consuming volunteers was estimated to be from consumption of crabmeat (1003 ± 1044 and 0.8 ± 2.7 respectively, $p<0.001$) (Table 3). In addition, these volunteers reported consuming these or similar levels over an average time period of 16 years (Table 3). When expressed relative to body weight, the average value of the total Cd intake from crabmeat calculated for each individual was 14.19 μg/kg body weight for the crabmeat consumers compared to 1.11 μg/kg body weight for the non-crabmeat consumers when including the Cd intake from the average background diet (Table 3). Both groups reported consuming seafood (fish, shellfish, seaweed) for a similar period (average of 20 years) but volunteers in the crabmeat consumption group reported consuming more four times as much seafood overall compared with the non-crab consumers (362 ± 284 and 92 ± 99 g/wk respectively, $p<0.001$) (Table 3).

3.3 Urinary and whole blood levels of cadmium and other elements in the crabmeat and non-crabmeat consuming groups.

Cumulative Cd retention within volunteer groups was assessed by measuring urinary levels of Cd per gram creatinine [18] which is considered a valid biomarker of lifetime kidney accumulation of Cd [19]. Levels of Cd in single spot, first morning urine have been shown to exhibit good-to-excellent temporal stability of Cd, indicating that such urine Cd is suitable for use as a biomarker of long-term Cd exposure [20]. There was no significant difference detected in urinary Cd levels (urinary Cd/ g creatinine) in crabmeat compared to non-crabmeat consumers after adjusting for potential confounding factors (age and sex) (p=0.420, Table 4).

Overall, across all volunteers, urinary Cd levels were found to be significantly higher in females compared to males (0.698 ± 0.376 versus 0.325 ± 0.213 μg Cd/g Cr respectively) (p=0.001) (not shown), but were not correlated with estimated levels of Cd intake in the crabmeat consumers (not shown). However, whole blood Cd levels from crabmeat consumers were found to be significantly higher compared to non-crab consumers (0.46 ± 0.27 and 0.35 ± 0.15 μg/l respectively, p=0.005, Table 4). Whole blood Cd levels were also positively correlated with urinary Cd levels (μg Cd/ g Cr) (r=0.43, p=0.005) and were strongly associated with crabmeat intake (r=0.51, p<0.0007) but not with volunteers' seafood intake (p=0.69) after accounting for age and gender.

While blood zinc levels showed no change between groups, whole blood Se and mercury levels were significantly increased by 20% (p<0.001) and 290% (p<0.001) respectively, in the crabmeat consuming volunteers compared to non-crab consumers. Additionally, higher whole blood lead levels in crabmeat consumers approached significance when compared to non-crab consumers (p=0.051) (Table 4).

Urinary levels of Se increased in the crabmeat consuming group by 74% (p=0.016) and whole blood Se levels were positively correlated with urinary Se levels (r=0.49, p<0.0013) but also with whole blood mercury (r=0.59, p<0.0001), lead (r=0.57, p<0.0002) and with urinary Cd levels (r=0.34, p<0.03). Whole blood Se also showed a positive correlation with both crabmeat intake (p=0.04) and seafood intake (p=0.03).

3.4 Urinary levels of beta-2-microglobulin.

Urinary levels of beta-2-microglobulin (B2M) have been recognized as an important marker for the toxic effects of Cd and it has been established as the standard biomarker in Cd toxicity meta-analyses [12,21] and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Agency for Toxic Substances and Disease Registry (ASTDR) and European Food Safety Authority (EFSA) [22-24]. The reference range of urinary B2M is 0 – 300 μg B2M/g Cr, whilst levels ≥300 μg B2M/g Cr have been associated with accelerated decline of age-related loss of renal function [25,26]. In the crabmeat consumers, both the urinary B2M levels, as well as the creatinine-corrected B2M levels were lower than levels in the non-crab consumers (p=0.006 and p=0.011 respectively) (Table 5). B2M levels in both groups of volunteers were within the reference range (<300 μg B2M/g Cr) for this biomarker.

4. Discussion

This study investigated whether healthy, regular crabmeat consumers aged ≥40 years who had been consuming crabmeat for more than five years had an elevated body burden of Cd

compared to a similar group of volunteers who did not consume crabmeat. Results showed that while whole blood Cd levels were significantly increased in the crabmeat consuming group, the levels of urinary Cd (urinary Cd/g creatinine) and those of urinary B2M were not. Whole blood Cd is thought to reflect a combination of both long term (11-16 y) and more recent exposure (3-4 months) based on studies regarding occupational exposure to Cd inhalation [23,27] whereas urinary levels of Cd are recognised as the most reliable biomarker of cumulative, long term exposure [19,20]. Therefore, the finding of a lack of difference in urinary Cd values indicates that volunteers who consume brown crabmeat on a regular basis (for an average of 16 years) do not show an increase in long term Cd exposure. Indeed, the actual levels of urinary Cd detected within both volunteer groups (0.50 ± 0.37 and 0.56 ± 0.37 μg Cd/g Cr) were similar to those reported in non-smoking populations (0.47 ± 0.50 μg Cd/g Cr) and below levels reported in either former or current smokers (0.69 ± 0.88 and 0.91 ± 0.81 μg Cd/g Cr respectively) [18]. Additionally, and consistent with this finding, measurement of a recognised biomarker for Cd-induced kidney toxicity (urinary B2M levels), indicated that crabmeat consumers also displayed no detectable increase in Cd-induced toxicity.

The tolerable weekly intake (TWI) level for Cd in the European Union is 2.5 μg/kg body weight [4], therefore the average estimated total weekly Cd intake for the crabmeat consuming group (14.2 μg/kg b.w.), which included the weekly Cd intake from the average background diet (1.1μg Cd/kg body weight) [14] was greater than five times the level regarded as safe. Indeed, all of the crabmeat-consuming volunteers had estimated intakes above the TWI and 70% of these had reported consuming these levels for at least 20y, with the rest reporting at least 5y consumption. In contrast, levels of estimated Cd intakes within the non-crabmeat consuming group, including the Cd intake from the average background diet, were all below the TWI for Cd. Whilst occasional exceedance of the TWI is recognised as being of limited concern to health, long term exceedance of the TWI is expected to lead to adverse effects on kidney function [4], effects which were not evident in the current study.

One limitation of the study however, was the inability to validate the reported crabmeat intake levels derived from the questionnaire used to assign volunteers the study groups. However, in mitigation, due to the potential toxic effects of the Cd being long-term in nature, the questionnaire attempted to estimate current, medium and long-term crabmeat intake over a ≥ 5y period and in such cases, a 24h dietary recall or measured food diary would not sufficiently capture an individuals' weekly, monthly or even yearly habitual diet and would also be unlikely to capture intake of a food such as crabmeat that is often consumed on a seasonal basis. Indeed, in one previous survey where two 24h dietary recalls were carried out, this was found not to

supply reliable information about consumption of foods not eaten on a daily basis when assessing the risk of Cd in crabmeat consumption [28]. Other studies have also indicated that using standard food frequency questionnaires to estimate dietary Cd intakes are of limited use particularly at low Cd intake levels [28]. Other limitations of the study was that only single samples of blood and urine were measured and also that Cd intake levels from the rest of the diet were not estimated and were assumed to be similar between the two groups, although we did exclude volunteers with significant intakes of Cd from other seafood sources known to have high Cd levels. In addition, levels of urinary Cd measured in the non-crabmeat consuming group were similar to levels reported in the average adult non-smoking population which provides some support in the assumption that the non-crabmeat group at least, consisted of volunteers with intake levels of Cd within the average range.

The finding that whole blood Cd, Se and mercury levels were found to be significantly increased in the crabmeat relative to the non-crabmeat consuming group however, supports the use of the self-reported crabmeat intake questionnaire in the assignment of volunteers to their respective groups. As mentioned above, whole blood Cd levels can be both a short and long-term marker for Cd intake and levels might be expected to be elevated in the crabmeat consumers as crabmeat can contain significant levels of Cd compared to other foods [16]. Indeed, whole blood Cd levels were positively correlated with reported crabmeat intake but not with seafood intake as might be expected. Blood Cd levels in the non-crab consuming group (mean 0.35 μg/l) were higher than those reported in a Swedish study (mean 0.27 μg/l) [17], but volunteers in this latter study were generally younger (mean age 37y) and blood Cd levels are known to increase with age. However, whole blood Cd levels in both groups were similar to those previously measured in non-smoking populations in other countries including Belgium, Korea and the US [30-32], indicating that these levels, including within those consuming crabmeat, are within established population ranges. Increased whole blood Se and mercury levels within crabmeat consumers also validates the self-reported crabmeat/seafood intake questionnaire since seafood and crabmeat are rich sources of both Se and mercury [33, 34] and higher intakes would also be expected to result in an elevated status of these elements. Volunteer blood mercury levels were however, observed to be below the biological limit value [34] and no increase in urinary mercury levels indicative of long term exposure were detected in the current study.

A further limitation of the study was that more females were recruited into the non-crabmeat consuming group compared to the crabmeat group. As Cd has a high affinity for the main intestinal iron transporter [35], the absorption of Cd is influenced by body iron status and

therefore females, with their lower body iron/plasma ferritin levels [36] tend to show a higher accumulation of Cd [17]. However, the effect of gender would be expected to be minimised in this study due to the recruitment of older women (mean age 52 y) as the effect of gender on Cd accumulation becomes much reduced/lost after the menopause when iron status improves [37]. A strength of the study was that it excluded volunteers having significant exposure to Cd from sources other than from crabmeat including occupational and dietary and non-dietary sources. Consequently, differences in the estimated Cd intakes between the two groups were most likely due to their reported crabmeat intakes, albeit it assumed that Cd intakes from the background diet was similar between both groups. Crabmeat consuming volunteers required high average crabmeat intakes over at least 5y, and this condition combined with the strict exclusion criteria outlined above, together with the requirement to be healthy, meant that the study was limited by a modest sample size. Indeed, previous studies including the UK-wide National Diet and Nutrition Survey identified that that crabmeat consumers are relatively rare within the UK [38]. Very few studies on the effect of crabmeat consumption on Cd burden in humans exist. One Korean study on intake levels of marinated brown crabmeat also found that consumers who ate crabmeat more than five times/week had significantly increased blood Cd levels compared to those consuming lower amounts [39]. However, it did not investigate whether urinary Cd or markers of kidney toxicity were also affected.

The reason why consumers with habitually high intakes of brown crabmeat known to contain significant levels of Cd do not show increases in urinary Cd levels is not known. One possibility may relate to the actual levels of Cd within the crabmeat. Volunteer Cd intakes were estimated using data from a recent UK-wide survey of crab/crabmeat products but within this survey Cd levels in individual crab/crabmeat products varied widely and, in addition, these levels are also known to vary widely depending on season [6]. In the current study, 3.9 mg/kg was taken as the mean level of Cd in 100% brown crabmeat, but individual samples ranged from 0.11 to 26 mg/kg [16]. Therefore, volunteers may have reported their crabmeat intakes accurately, but corresponding Cd intakes may have been overestimated meaning these lower Cd intakes were still detectable in the short term (in whole blood) but were not high enough to accumulate (at least not within the reported consumption time frame) and to affect established long term exposure markers (urinary Cd) or kidney toxicity (urinary B2M). However, previous studies have shown that crabmeat sourced from the Orkney/Shetland area contained 3-5 times higher Cd levels than crabmeat from other UK areas [40,41] and, since the majority of the crabmeat-consuming group were recruited from the Orkney area and likely consumed locally-caught crab products, this may provide some evidence against this likelihood. A second possibility may be

that some of the Cd present within the crabmeat is in a form that may not be detectable long term or is less able to affect urinary B2M levels within the consumer. Some support for this comes from studies where the bioavailability of Cd from crab was shown to be lower compared with Cd in mushrooms or inorganic Cd, at least in a rodent model [8]. Additionally, other studies in rodents suggest that Se, which is also present at high levels in crabmeat, may counteract the toxicity of Cd [9] and other heavy metals [11]. In the present study, blood Se levels were found to be significantly correlated with blood Cd, mercury and lead as well as with urinary Se levels. Increased urinary Se levels in the crabmeat consumer group observed within the current study have also been demonstrated to occur in workers exposed to heavy metals (including Cd) due to the formation of metal-Se complexes [42]. Indeed, studies in mammals have also shown that Se in tissues can antagonise mercury and Cd toxicity by storing and reallocating these toxic metals thus lessening their potential adverse effects [43-45]. Therefore, potentially, Se and/or other nutrients may play a role in sequestering at least some of the Cd within brown crabmeat and modifying its apparent toxicity within humans.

In conclusion, the current study found that, compared to consumers who reported very little crabmeat consumption, regular consumption of products containing brown crabmeat (an average of 447g per week) for an average of 16 years within a group of healthy middle-aged consumers did not result in increased long-term Cd exposure or kidney toxicity.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Authorship

A.A.S. designed the study, K.D. recruited all volunteers and together with S.B. generated the data, G.W.H. analysed the data and A.A.S. wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Characteristics of the non-crab and crabmeat consumer groups. Values are means ± sd. Statistical analysis was carried out using *t-tests*.

	Crabmeat group	Non-crabmeat group	p-value
No participants	16	25	
Male	10	7	
Age	54.0 ± 9.9	51.5 ± 7.2	0.350
Height (m)	1.69 ± 0.11	1.66 ± 0.08	0.433
Weight (kg)	81.7 ± 12.4	70.3 ± 14.5	0.013
BMI (kg/m^2)	28.4 ± 2.9	25.2 ± 3.8	0.007

Table 2: Reported intake levels of all crabmeat products in the non-crab and crabmeat consumer groups. Values are means ± sd. Statistical analysis was carried out using *t-tests.*

Product	Crabmeat group (g/wk)	Non-crabmeat group (g/wk)	p-value
100% brown crabmeat	127 ± 120	0.0 ± 0.0	<0.00001
Dressed crab	132 ± 171	0.2 ± 1.0	0.0005
Crab pastes/spreads	0.2 ± 0.3	0.0 ± 0.0	0.016
Crab pate/terrines/potted crab	13.4 ± 38.5	0.0 ± 0.0	0.09
Tinned dressed crab	8.1 ± 32.3	0.1 ± 0.3	0.23
Crab cakes	4.5 ± 10.6	0.0 ± 0.0	0.04
Crab soup/bisque	102 ± 224	0.0 ± 0.0	0.03
Ready-made mixed crab dishes	61 ± 225	0.0 ± 0.0	0.19
Total crabmeat intake (g/wk)	***447 ± 518***	***0.3 ± 1.1***	*<0.001*

Table 3. Weekly cadmium intake levels estimated from crabmeat and seafood questionnaire, estimated total weekly cadmium intake including background diet and seafood intakes of participants. (Values for total weekly cadmium intake including background diet are estimated from reported crabmeat, whelk, scallop and seaweed intakes and the average Cd intake from the rest of diet). Values are means ± sd. Statistical analysis was carried out using *t-tests.*

	Crabmeat group	Non-crabmeat group	p-value
Est Cd intake (µg/wk) from crabmeat and seafood questionnaire			
Total	1022 ± 1046	2.3 ± 4.0	<0.0001
From crabmeat	1003 ± 1044	0.8 ± 2.7	<0.0001
(range)	*(156 - 3571)*	*(0.0 - 10.3)*	
Est total Cd Intake including background diet			
(µg Cd/kg bw)	14.19 ± 15.83	1.11 ± 0.04	<0.001
(range)	*(2.74 – 60.73)*	*(1.10 – 1.25)*	
Duration consumed crab (y)	16.3 ± 6.0	0.0 ± 0.0	<0.0001
Duration consumed seafood (y)	20 ± 0.0	19.2 ± 4.0	0.431
Portions seafood/wk	3.3 ± 2.2	1.2 ± 1.1	<0.001
Ave seafood intake (g/wk)	362 ± 284	92 ± 99	<0.0001

Table 4. Urinary and whole blood cadmium and element levels in crabmeat and non-crabmeat consumers. Values are means ± sd. Data were analysed by a linear model with terms for age, gender and crab-eating-group. (Cr (creatinine); Zn (zinc), Se (selenium), Hg (mercury), Pb (lead), ns (non-significant)).

	Crabmeat	**Non-crabmeat**	p-value
Urine			
Cd (µg/l)	0.40 ± 0.43	0.29 ± 0.17	0.181
Cr (mg/l)	904 ± 504	668 ± 429	0.763
µg Cd/g Cr	*0.503 ± 0.366*	*0.563 ± 0.370*	0.420
Zn (µg/l)	379 ± 245	248 ± 359	0.501
Se (µg/l)	33.6 ± 16.2	19.3 ± 12.3	0.016
Hg (µg/l)	1.40 ± 1.45	0.57 ± 0.45	0.095
Pb (µg/l)	1.57 ± 1.02	1.19 ± 0.61	0.540
Whole Blood			
Cd (µg/l)	0.46 ± 0.27	0.35 ± 0.15	0.005
Zn (µg/l)	6567 ± 1184	5890 ± 754	0.223
Se (µg/l)	129 ± 23	107 ± 14	0.0001
Hg (µg/l)	4.95 ± 3.39	1.71 ± 1.30	0.0001
Pb (µg/l)	20.5 ± 9.2	14.0 ± 5.9	0.051

Table 5: Levels of urinary beta-2-microglobulin (B2M) in non-crab and crabmeat consumers. Values are means ± sd. Data were analysed by a linear model with terms for age, gender and crab-eating-group. Cr (creatinine).

Analyte	Crabmeat group	Non-crabmeat group	p-value
B2M (µg/l)	13.32 ± 11.52	44.79 ± 35.65	0.006
Cr (mg/l)	904 ± 504	668 ± 429	0.763
µg B2M/g Cr	24.61 ± 31.14	108.06 ± 106.57	0.011

Figure Captions

Figure 1. Study trial profile