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Postprandial zinc stable isotope response in human blood serum†

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In recent years, considerable advances have been made in the field of medical isotope metallomics, but numerous fundamental physiological processes remain to be investigated. Past studies report that blood serum Zn concentrations decrease by between about 10 and 25%, depending on the size of meal, approximately three hours postprandially (i.e. after eating), before returning to baseline values if no meals are consumed over the following four to five hours. Nine participants were recruited for this study to investigate whether this postprandial Zn concentration decrease is accompanied by a stable isotope response. A baseline serum sample was collected from participants in the morning after overnight fasting. A 576 kcal meal was then provided and additional serum samples were taken 90 and 180 minutes post-meal to coincide with the postprandial response. Serum Zn concentrations decreased postprandially by an average of 21 ± 9% (1SD), but this was not accompanied by a change in stable Zn isotope composition (mean $\Delta^{66}Zn_{180\text{-minute} - \text{Baseline}}$ = 0.01 ± 0.09‰, 2SD). We propose that hemodilution and the rapid, efficient postprandial transfer of albumin-bound Zn from serum to the liver and pancreas is responsible for the lack of postprandial serum Zn isotopic response. These results indicate that studies examining solely the distribution of Zn isotopes in serum may obtain samples without considering timing of the most recent meal. However, future studies seeking to compare serum Zn concentrations with $\delta^{66}Zn$ values should draw blood samples in the morning after overnight fasting.

Significance to metallomics

Zinc concentrations in blood serum decrease by between about 10 and 30% three hours after eating, putatively due to postprandial sequestration by the liver and pancreas to participate in phosphorylation reactions and the synthesis of digestive enzymes, respectively. Given the magnitude of this decline, it is notable that no accompanying zinc stable isotope response is observed ($\Delta^{66}Zn$ = 0.01 ± 0.09‰ 2SD). The data allow us to model the differences in Zn isotope composition between serum Zn pools, albumin and alpha-2-macroglobulin, and therefore better constrain the nature of this post-meal redistribution.

Introduction

Zinc (Zn) has five stable isotopes (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, and ^{70}Zn), typically occurs in divalent form (Zn^{2+}), and is the second most abundant transition metal in organisms after iron.[1] Zinc is a component of approximately 3000 proteins,[2] a cofactor or component of more than 300 metalloenzymes,[1,3] and has many roles in the human body including in normal growth and development, immunity, cellular homeostasis, cell survival, and biochemical functions.[1,4,5] The Zn content of the human body ranges from 1.5 to 3 g with the daily requirement of 2 to 3 mg Zn determined by loss via stool, urine, and sweat.[6] The recommended daily adult intake of approximately 10 mg Zn significantly exceeds the daily requirement of 2 to 3 mg due to the limited bioavailability of Zn from foods[6,7] and results in a turnover time in the body of 150 to 300 days.[8] However, Zn is more labile in some compartments than others, with blood serum Zn having a turnover rate of over 150 times per day.[9] Serum is the main transporter of nutrients to body tissues and it is the compartment through which all absorbed Zn passes. Approximately 65 to 85% of Zn in serum is loosely bound to albumin,[10–14] accounting for as much as 98% of the exchangeable fraction of Zn in serum.[13] The majority of the remaining serum Zn is held tightly by α2-macroglobulin.[15] Only about 2% of circulating albumin molecules are bound to Zn[16,17] and albumin effectively acts as an extracellular "Zn buffer" that controls the concentrations of "free" Zn^{2+} ions that are available to other serum proteins or for cellular uptake through membrane-bound Zn transporters.[18] Blood plasma, a similar but distinct fluid is sometimes referred to and used interchangeably with serum,[19] but it is important to note their differences. Both serum and plasma are devoid of blood cells, but plasma contains fibrinogens and coagulation proteins that serum lacks.[19] Despite this, there is no significant difference in the Zn concentration of serum and plasma,

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and for the sake of simplicity, this paper will generally refer to "serum Zn" for both types of specimens.[20]

With the development of multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) and ion exchange chromatography procedures that can efficiently purify metals and metalloids with significant interferents from even complex sample matrices prior to isotopic analysis, isotope geochemists have been able to resolve subtle mass dependent changes in the isotope compositions of numerous elements in the environment.[21] Subsequently, this has paved the way for novel research that investigates the distribution of metal stable isotopes in the human body, and the processes that govern their allocation. Given its numerous biological roles, Zn has been a key element of interest in this context. For example, investigators have sought to establish reference ranges for Zn isotopic compositions in the blood compartments of healthy subjects[22–26] so as to understand changes observed in those suffering from conditions where metal dyshomeostasis is fundamental to disease pathogenesis.[27–34] Sex, age, and time of blood draw must be considered when determining serum Zn concentrations to assess the Zn status of populations, whereas it appears dietary and supplemental Zn do not affect serum Zn concentrations.[35] So far, age,[36] diet,[26] menopausal status,[36] and cold stress-induced differences in basal metabolic rate[36] have been shown to potentially influence the Zn isotopic compositions of blood compartments and must be taken into account. In contrast, sex does not appear to have an impact on the Zn isotopic status of blood compartments.[22,37]

Despite advances in the field of medical isotope metallomics,[8,38] fundamental questions remain unanswered that have implications for researchers concerned with blood sampling protocols for such research. Depending on the element of interest and material being investigated, it may be necessary to consider or control for potential diurnal or postprandial effects, as is the case for assessing serum Zn concentrations.[35] Serum Zn concentrations do not appear to deviate significantly from baseline values during fasting,[39] whereas they fall by between about 10 and 25% postprandially (i.e. after eating), depending on the size of meal, with a subsequent gradual return to baseline values if no meals are consumed over the following four to five hours.[39–44] The mechanism and physiological relevance of the postprandial serum Zn response are poorly understood, but there is evidence that serum Zn is redistributed postprandially to the liver to supply Zn for hepatic fuel metabolism.[40] Specifically, serum phosphorous concentrations are 10% below baseline values 90 minutes after a meal, indicating that serum Zn may be used by the liver postprandially to participate in phosphorylation reactions.[41] Serum Zn may also be redistributed to the pancreas to replenish as much as 4.8 mg of Zn that is secreted into the duodenum during digestion to aid with the synthesis of pancreatic enzymes.[45]

In contrast to the postprandial behaviour of serum Zn concentrations, no systematic diurnal or postprandial variations in serum copper (Cu) concentrations have been observed.[46,47] Additionally, as expected, there appear to be no diurnal variations in serum Cu isotope compositions.[48] However, a Zn stable isotope response may accompany this postprandial serum Zn concentration decrease because metal stable isotope fractionation reflects the energies of bonding for specific biological pathways.[49] Possible postprandial changes in the Zn stable isotope composition of serum are thus far unstudied and have, therefore, not been considered in sampling protocols.[26,36,50,51] The current investigation addresses this lack of knowledge through the measurement of overnight-fasting baseline, and 90- and 180-minute post-meal serum Zn concentrations and isotope compositions. These results will help further refine the optimal sampling protocols for Zn isotopic analysis of serum. A fasting period prior to drawing blood in future studies investigating Zn isotope compositions of human serum may help resolve the subtle differences in isotopic composition that exist in this isotope system. The data will also help further our understanding of the nature of this postprandial Zn redistribution.

Experimental

Sample collection

This study received approval from the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (TRAQ#: 6021543; Department Code: GEOSC-002-17). Nine participants (four males, five females) were recruited to participate in this study. Participant E was unable to provide a 180-minute postprandial blood sample and was subsequently excluded from the study. Upon the receipt of informed consent, participants completed a survey regarding their sex, age, diet, and health status (Table S1, ESI†). All participants were omnivores. Male participant ages ranged from 27 to 30 years with a mean of 28 ± 1.4 years (1SD), whereas female ages ranged from 22 to 65 years with a mean of 35 ± 20 years (1SD). One male participant (H) has Crohn's disease that was diagnosed in 2002, and at the time of sampling had been in remission for four years with no inflammatory episodes. The participants arrived at Providence Care Hospital (Kingston, Ontario) at 7:30 a.m. in the fasting state (from 10 p.m. the night before). After height and weight were measured, a fasting-state blood sample was drawn into 6ml BD Vacutainer Plastic Blood Collection Tubes for Trace Element Testing with Serum Clot Activator to serve as a baseline. Participants then consumed a meal consisting of whole-grain bread, raspberry jam, butter, yogurt, and apple juice (Table 1). This 575.6 kcal meal provided 80% of energy as carbohydrate, 14% as protein, and 6% as fat, and is similar to 'BRKFT x 2' provided in Lowe *et al*.[39] (640.1 kcal).

Table 1 Composition of breakfast meal provided to participants

Food item	Amount (g)	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)
12-grain bread	76	200	34	8	3
Butter	4.5	125.6	0	0	3.5
Raspberry jam	10	70	18	0	0
Apple juice	200	90	23	0	0
Cherry yogurt	100	90	14	8	0

The participants were instructed not to eat anything further until the test was concluded; drinking water was permitted *ad libitum*. Additional blood samples were drawn 90 and 180 minutes after eating to coincide with the expected peak postprandial response (~180 minutes).[39] After collection, each sample was gently inverted 180° and back ten times to mix the blood and clot activator before being transported to the Queen's School of Kinesiology and Health Studies to undergo ten minutes of centrifugation at 1,300g. Serum was separated from the clot under a Biological Safety Cabinet in a Level 2 Biosafety Laboratory by pipetting and transferring to acid-cleaned 1.5 ml microfuge tubes.

Sample preparation

Between 0.22 and 1.1 g of serum was mixed with 2.8 ml of 15 M HNO_3 and 0.25 ml of 30% H_2O_2 (per 0.25 g of sample) under metal-free laminar flow hoods in the Clean Room Laboratory at the Queen's Facility for Isotope Research and digested at 110°C overnight on a hotplate. Separation of Zn from the serum matrix was achieved by anion exchange column chromatography using Bio-Rad AG® MP-1M (100-200 mesh) resin in hydrochloric acid media under ISO Class 4 metal-free laminar flow hoods in the MAGIC Clean Room Laboratory at Imperial College London.[52] Quartz-distilled acids diluted with ≥18.2 MΩ cm H_2O (Millipore) were used throughout sample preparation.

Concentration measurements and isotopic analysis

An initial determination of Zn concentrations by isotope dilution was carried out for each sample to ensure that an appropriate sample aliquot was digested for isotopic analysis. The sample solutions were mixed in optimal proportion with a ^{64}Zn-^{67}Zn double spike to enable the correction of any isotope fractionation incurred during chromatographic separation and measurement. The subsequent coupled Zn isotope and concentration measurements with the double spike technique followed previously described techniques.[52,53] They utilized a Nu Instruments Nu Plasma HR MC-ICP-MS at low resolution with either an Aridus II (CETAC) or DSN-100 desolvation system (Nu Instruments Ltd.) for sample introduction fitted with a glass nebuliser that had a typical uptake rate of approximately 100 μl min^{-1}. Samples were run interspersed between and relative to analyses of isotopic reference material IRMM-3702 Zn, which was used to monitor and correct for within- and between-session changes in instrumental mass bias.[52,53] Following the collection of raw data, the double spike data reduction was performed offline using an iterative procedure that corrects for instrumental (and any other laboratory-induced) mass fractionation, and polyatomic (Ni) and doubly charged ion (Ba) interferences.

As variations in the ratio (R) $^{66}Zn/^{64}Zn$ are small, isotopic data are reported in $\delta^{66}Zn$ notation, which denotes the parts per thousand (‰) change in the $^{66}Zn/^{64}Zn$ value of the sample relative to a standard (Std; Equation 1).

$$\boldsymbol{\delta^{66}Zn_{Std}}\ (‰) = \left(\frac{\mathbf{R^{66/64}_{Sample}}}{\mathbf{R^{66/64}_{Standard}}} - \mathbf{1}\right) \cdot \mathbf{1{,}000} \quad (1)$$

Furthermore, differences in the $\delta^{66}Zn$ values of two samples (A and B) are denoted using $\Delta^{66}Zn_{A\text{-}B}$ as defined in Equation 2.

$$\Delta^{66}Zn_{A\text{-}B} = \delta^{66}Zn_A - \delta^{66}Zn_B \quad (2)$$

The $\delta^{66}Zn$ values reported here were originally determined relative to IRMM-3702 Zn ($\delta^{66}Zn_{IRMM}$) but were then recalculated so that all results are given relative to the JMC-Lyon Zn isotope reference material ($\delta^{66}Zn_{JMC}$) using Equation 3.[54]

$$\boldsymbol{\delta^{66}Zn_{JMC}} = \left[\left(\frac{\boldsymbol{\delta^{66}Zn_{IRMM}}}{\mathbf{1{,}000}} + \mathbf{1}\right) \cdot \left(\frac{\boldsymbol{\Delta^{66}Zn_{IRMM-JMC}}}{\mathbf{1{,}000}} + \mathbf{1}\right) - \mathbf{1}\right] \cdot \mathbf{1{,}000} \quad (3)$$

A value of 0.30‰, was used for $\Delta^{66}Zn_{IRMM–JMC}$, based on results obtained in the interlaboratory calibration of the new Zn isotope reference material AA-ETH Zn ($\Delta^{66}Zn_{AA–JMC}$ = –0.28‰ and $\Delta^{66}Zn_{AA–IRMM}$ = 0.02‰).[54]

Results

Quality control

Zinc blank contributions were monitored and remained below 0.55 ng or less than 1.1% of sample Zn. The reference material, BCR®-639 Human Serum, was processed through the same procedure as serum samples to ensure the inclusion of a matrix-matched reference material, allowing for the monitoring of the efficacy of the entire procedure in producing accurate and reproducible results. Accuracy and reproducibility were also monitored by measuring both column-processed and unprocessed aliquots of an in-house Zn standard solution, London Zn, throughout measurement sessions, and by measuring sample duplicates. These measurements yielded mean $\delta^{66}Zn$ values of 0.11 ± 0.08‰ (2SD, *n* = 30) for London Zn and –2.97 ± 0.23‰ (2SD, *n* = 15) for BCR®-639, in good agreement with previously reported results.[50,52,54] A mean between-run sample $\delta^{66}Zn$ reproducibility of ±0.07‰ (2SD) was achieved in this study and is hereafter referred to as 'measurement reproducibility'.

Zinc concentrations

Zinc concentrations were determined in three serum samples (baseline fasting-state, 90 minutes post-meal, 180 minutes post-meal) taken from each of eight participants (Table 2). Baseline serum Zn concentrations measured in the overnight fasting state range from 655 to 1006 ng g^{-1} (mean = 877 ± 116 ng g^{-1}, 1SD, *n* = 8). Zinc concentrations in samples taken 90 minutes after consumption of the breakfast meal range from 562 to 831 ng g^{-1} (mean = 741 ± 79.8 ng g^{-1}, 1SD, *n* = 8). Zinc concentrations in samples taken 180 minutes after the consumption of the breakfast meal ranged from 575 to 819 ng g^{-1} (mean = 691 ± 106 ng g^{-1}, 1SD, *n* = 8). The 90-minute postprandial Zn concentrations are between 79.2 and 93.9% relative to baseline values (mean = 84.8 ± 4.8%, 1SD, *n* = 8), whereas the 180-minute postprandial Zn concentrations are between 66.3 and 90.7% relative to the baseline (mean = 79.1 ± 8.9%, 1SD, *n* = 8) (Fig. 1).

Table 2 Results for serum Zn concentrations and isotopic compositions (mean, 2SD) for samples from participants and reference materials

Identifier	Sex	Age	Description	*n (m)*	Zn (ng g^{-1})	$\delta^{66}Zn_{JMC}$ (‰)	$\Delta^{66}Zn_{Postprandial - BL}$ (‰)
A	M	27	Fasting-state baseline	3 (1)	950	0.60 ± 0.04	
			90 minutes post-meal	3 (1)	801	0.61 ± 0.01	0.01
			180 minutes post-meal	3 (1)	862	0.60 ± 0.12	0.00
			Mean	9 (3)		0.60 ± 0.07	
B	F	65	Fasting-state baseline	6 (2)	838	0.46 ± 0.08	
			90 minutes post-meal	3 (1)	742	0.49 ± 0.05	0.03
			180 minutes post-meal	3 (1)	584	0.46 ± 0.06	-0.01
			Mean	12 (4)		0.47 ± 0.07	
C	F	23	Fasting-state baseline	3 (1)	655	0.53 ± 0.04	
			90 minutes post-meal	3 (1)	562	0.58 ± 0.04	0.05
			180 minutes post-meal	3 (1)	575	0.54 ± 0.12	0.02
			Mean	9 (3)		0.55 ± 0.08	
D	F	22	Fasting-state baseline	3 (1)	864	0.59 ± 0.07	
			90 minutes post-meal	1 (1)	734	0.60 ± 0.05*	0.00
			180 minutes post-meal	5 (2)	669	0.71 ± 0.01	0.11
			Mean	9 (4)		0.66 ± 0.12	
F	F	29	Fasting-state baseline	3 (1)	786	0.67 ± 0.06	
			90 minutes post-meal	3 (1)	738	0.65 ± 0.05	-0.02
			180 minutes post-meal	3 (1)	637	0.66 ± 0.06	-0.01
			Mean	9 (3)		0.66 ± 0.05	
G	M	28	Fasting-state baseline	3 (1)	1006	0.38 ± 0.08	
			90 minutes post-meal	3 (1)	831	0.40 ± 0.06	0.01
			180 minutes post-meal	3 (1)	738	0.39 ± 0.05	0.00
			Mean	9 (3)		0.39 ± 0.06	
H	M	30	Fasting-state baseline	3 (1)	968	0.62 ± 0.11	
			90 minutes post-meal	3 (1)	768	0.66 ± 0.08	0.04
			180 minutes post-meal	4 (1)	642	0.60 ± 0.01	-0.02
			Mean	10 (3)		0.62 ± 0.08	
I	M	27	Fasting-state baseline	3 (1)	946	0.48 ± 0.01	
			90 minutes post-meal	3 (1)	749	0.52 ± 0.03	0.04
			180 minutes post-meal	3 (1)	819	0.44 ± 0.06	-0.04
			Mean	9 (3)		0.48 ± 0.08	
London Zn			Column-unprocessed	16 (4)	-	0.09 ± 0.05	
			Column-processed	14 (5)	-	0.12 ± 0.09	
			Mean	30 (9)		0.11 ± 0.08	
BCR-639			Reference material	15 (3)	2296	-2.97 ± 0.23	

m = number of separate aliquots prepared, *n* = total number of Zn isotope ratio measurements performed on each material by MC-ICP-MS, * 2SD of bracketing standards run alongside sample (in all other cases, 2SD is calculated from repeated measurement of individual samples). Mean $\delta^{66}Zn$ values and 2SD calculated from all measurements performed on samples from each individual participant, and column processed and unprocessed London Zn. Sex is denoted as M for males and F for females. $\Delta^{66}Zn_{Postprandial - BL}$ represents the difference between $\delta^{66}Zn$ values determined in serum samples taken 90 and 180 minutes post-meal relative to baseline.

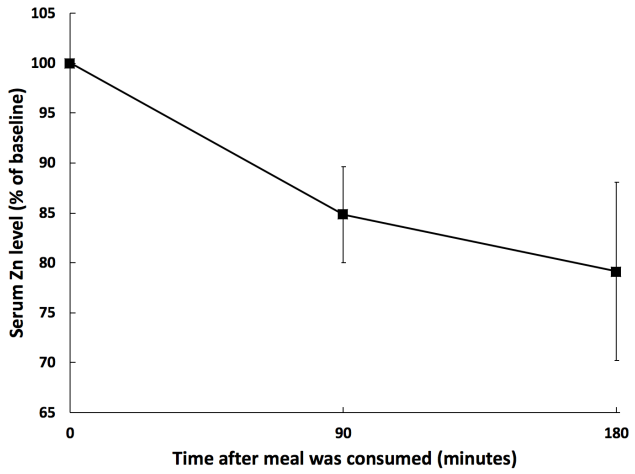

Fig. 1 Zinc concentrations over time expressed as a percentage variation of the fasting-state baseline Zn concentration measured before breakfast meal consumption. Values are means for eight subjects, with their 1SDs represented by vertical bars. Samples at time '0' were taken from participants in an overnight fasting state and the remaining samples were taken at 90 and 180 minutes after breakfast meal consumption.

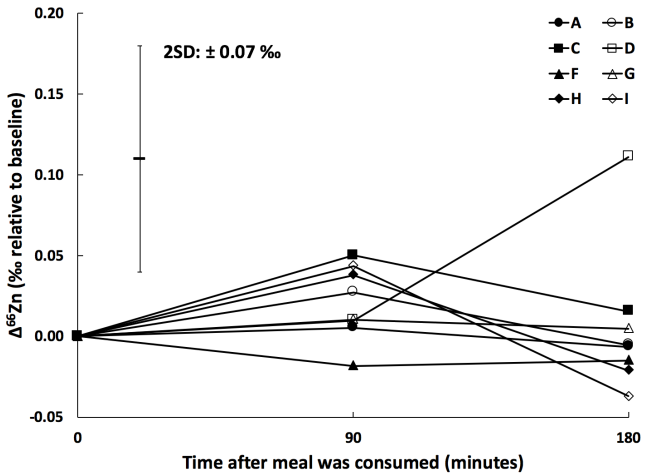

Fig. 2 $\Delta^{66}Zn$ value over time expressed as the permille variation of the fasting-state baseline $\delta^{66}Zn$ value measured at time = 0 before breakfast meal consumption. Samples at time '0' were taken in the morning from participants in an overnight fasting state and the remaining samples were taken 90 and 180 minutes after breakfast meal consumption. The error bar represents the mean between-run sample $\delta^{66}Zn$ reproducibility achieved in this study.

The change in the mass of Zn present in serum after consumption of the breakfast meal (Table S2, ESI†) was estimated by calculation of (1) blood volume from height and weight using Nadler's Formula;[55] (2) serum volume from (1) based on male and female packed cell volumes of 0.46 and 0.42, respectively;[56] (3) serum mass using a density of 1.02385 g ml^{-1};[57] (4) the postprandial change in serum Zn concentrations after breakfast meal consumption; and (5) postprandial change in serum Zn mass after breakfast meal consumption by combining the results of (3) and (4). These calculations reveal that the total serum Zn mass was reduced postprandially by as little as 171 μg and as much as 682 μg relative to individual baseline values over the study period, excluding participant H. For participant H with Crohn's disease, the total serum Zn mass was 622 μg lower than baseline 90 minutes after eating and 935 μg lower than baseline 180 minutes post-meal.

Zinc isotopes

Zinc isotope compositions were determined for three serum samples (baseline fasting-state, 90 minutes post-meal, 180 minutes post-meal) taken from each of the eight participants (Table 2). Baseline serum $\delta^{66}Zn$ values measured for the overnight fasting state range from 0.38 to 0.67‰ (mean $\delta^{66}Zn_{BL}$ = 0.54 ± 0.10‰, 1SD, *n* = 8). Serum $\delta^{66}Zn$ values in samples taken 90 minutes after consumption of the breakfast meal range from 0.40 to 0.66‰ (mean $\delta^{66}Zn_{90\text{-}Min}$ = 0.56 ± 0.09‰, 1SD, *n* = 8), whereas serum $\delta^{66}Zn$ values in samples taken 180 minutes after eating range from 0.39 to 0.71‰ (mean $\delta^{66}Zn_{180\text{-}Min}$ = 0.55 ± 0.11‰, 1SD, *n* = 8). The 90-minute postprandial serum $\Delta^{66}Zn$ value is between –0.02 and 0.05‰ relative to baseline (mean $\Delta^{66}Zn_{90\text{-}Min\text{–}BL}$ = 0.02 ± 0.05‰ 2SD, *n* = 8), whereas the 180-minute postprandial serum $\Delta^{66}Zn$ value is between -0.04 and 0.12‰ relative to baseline (mean $\Delta^{66}Zn_{180\text{-}Min\text{–}BL}$ = 0.01 ± 0.09‰, 2SD, *n* = 8). This suggests that if a change in postprandial serum Zn isotopic composition is present, it is too small to be detectable given the measurement reproducibility of ±0.07‰ (Fig. 2). Only for one participant (D) was a change in the 180-minute postprandial serum $\Delta^{66}Zn$ value recorded that exceeds the measurement reproducibility with $\Delta^{66}Zn_{180\text{-}Min\text{–}BL}$ = 0.11‰ (Table 2). With the exclusion of participant D, the 180-minute postprandial serum $\Delta^{66}Zn$ values range between -0.04 and 0.02‰ relative to baseline with a mean $\Delta^{66}Zn_{180\text{-}Min\text{–}BL}$ value of –0.01 ± 0.03‰ 2SD (*n* = 7), significantly reducing the postprandial variability of $\Delta^{66}Zn_{180\text{-}Min\text{–}BL}$.

Discussion

Postprandial serum Zn decrease

The quantification of the absolute rate and magnitude of the serum Zn decline was beyond the scope of this work. However, the observed serum Zn concentration decrease of between about 10 and 30% three hours after eating is in agreement with data from past studies of postprandial serum Zn homeostasis, which reported results from a similar number of subjects consuming comparably-sized meals.[39–44] As the same meal was provided to each participant in the current study, the observed differences in the magnitude of the postprandial response may be due to differences in individual basal metabolic rates.[58] Together, the data confirm that, in addition to sex and age,[35] time of blood draw must be controlled for assessments of the Zn status of populations.

Zinc correlation with body mass index

The co-variation between the maximum measured decrease in the postprandial serum Zn concentration of each participant and their body mass index (BMI) was assessed using the Pearson's Correlation Coefficient (ρ). The results for participant H with Crohn's disease were excluded from the statistical analysis as an outlier. A strong negative correlation was found, with ρ(5) = -0.93, $p < 0.01$ (Fig. 3). The finding that a greater relative decrease in postprandial serum Zn concentrations occurs in participants with lower BMIs was not unexpected as all participants consumed the same breakfast meal. Participants with a higher BMI have increased caloric intake requirements to maintain weight, so the same sized meal will require

a higher proportion of serum Zn be reallocated to aid with processes such as digestive enzyme synthesis than for a participant with a lower BMI.

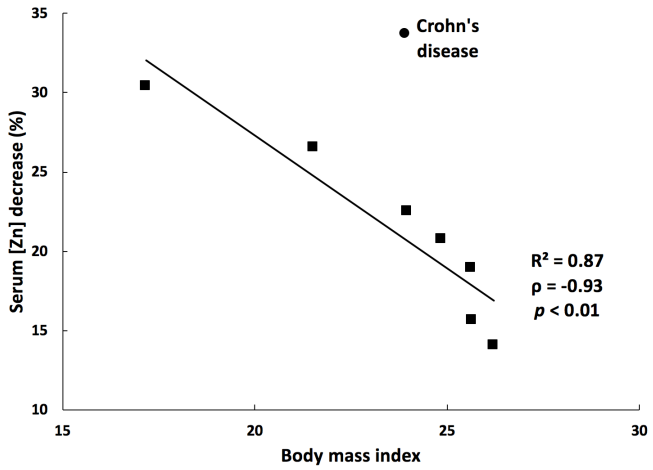

Fig. 3 Maximum measured postprandial serum Zn concentration decrease observed in participants as it relates to body mass index. Participant H with Crohn's disease is an outlier and was excluded from Pearson's Correlation Coefficient calculation. R^2 = coefficient of determination; ρ = Pearson's Correlation Coefficient where a value of 1 indicates a perfect positive linear correlation, 0 indicates no linear correlation, and -1 indicates a perfect negative linear correlation; p = p-value, where a value of ≤ 0.01 indicates significance at the 99% confidence interval.

Participant H was diagnosed with Crohn's disease in 2002 and at the time of sampling had been in remission for four years, with no inflammatory episodes. Participant H had a baseline Zn level that was similar to that of the other three healthy male subjects, but experienced the largest postprandial decrease in serum Zn concentration observed in this study, with a reduction from 968 to 642 ng g^{-1}. This represents a 33.7% decrease, which is well above the study average of 20.9%. The participant cites dietary and activity adjustments as playing a role in the four-year disease remission. A possible explanation for this large postprandial serum Zn decrease could be painless hyperamylasemia or hyperlipasemia.[59] One previous study found elevated levels of amylase and lipase, two digestive enzymes synthesized in the pancreas to aid with the breakdown of carbohydrates and fats, respectively, in the serum of 15.8% of Crohn's disease patients.[59] The activity of amylase, lipase, and other pancreatic enzymes are correlated with increased serum Zn levels in animal studies.[60,61] Given this, it is conceivable that digestive enzyme overproduction from hyperamylasemia and hyperlipasemia may lead to a greater-than-normal postprandial serum Zn decrease.

Postprandial metabolism

Given the decrease in postprandial serum Zn concentration, it is interesting that no change in serum δ^{66}Zn value is associated with this decline. Isotope tracer studies have been used to develop mathematical models of postprandial serum Zn kinetics[62–66] and showed that a minimum of two exponential terms were required to fit the postprandial serum ^{70}Zn decay, with the first pool composed of primarily serum Zn (pool a) and the second pool suggested to be predominantly located in the liver (pool b).[39,65] The pattern of disappearance of the isotope from serum indicate the number, size, and turnover rates of the metabolic Zn pools. The Zn turnover rate of pool (a) increased by nearly 10% after meals in comparison with fasting. Of the total amount of Zn flowing out of pool (a), 93% went into pool (b), with the remaining 7% transported to other pools. Whilst erythrocyte Zn exchanges rapidly with serum Zn,[62] there is no evidence that erythrocyte uptake of Zn accounts for the postprandial decline in serum Zn concentrations.[67]

As the liver is the primary site of postprandial, non-lipid nutrient metabolism, it has been proposed that serum Zn is redistributed postprandially to the liver to supply Zn for hepatic fuel metabolism.[65] In a previous study, serum phosphorous concentrations were observed to be 10% below baseline values 90 min after a meal.[68] Based on this, the authors concluded that serum Zn may be sequestered by the liver postprandially to participate in phosphorylation reactions. In addition, the postprandial decline in serum Zn may be due to Zn uptake by the pancreas to facilitate the synthesis of pancreatic digestive enzymes that are released into the duodenum.[39,42,45] The most important stimulation of pancreatic enzyme secretion occurs during the intestinal phase of digestion and continues throughout the course of gastric emptying.[39] The stimulation of the pancreas to secrete enzymes is controlled by the hormones secretin and cholecystokinin, which are released from duodenal cells when duodenal pH declines below 5.0. Gastric contents reach a pH of 3.5 approximately 60 minutes after the ingestion of a meal and this coincides with the postprandial decrease in serum Zn. Over the pH range of 0 to 3, the release of secretin is directly proportional to the amount of acid entering the duodenum. Therefore, the larger the meal, the greater the amount of enzyme synthesis required, and in turn more secretin is released.[39] This is further supported by the previously reported absence of a postprandial serum Zn decrease for an individual with pancreatitis.[42] Thus, the postprandial fall in serum Zn concentration may be due to uptake and utilization of Zn by both the liver and pancreas,[39] but this requires further investigation in additional pancreatitis patients.

The hematocrit, or the ratio of the volume of red blood cells to the total volume of blood, has been reported to decrease postprandially by an average of 6%.[69] It was previously hypothesized that the decreased hematocrit level may be associated with hemodilution that occurred after ingestion of foods and fluids.[70] As dilution has no impact on isotope compositions, it is conceivable that the lack of a postprandial Zn isotope response is in part, due to hemodilution. However, an additional mechanism is required to explain why the bulk of the postprandial serum Zn concentration decrease occurs without a change in δ^{66}Zn.

Modelling of postprandial δ^{66}Zn values for serum

Most likely, the postprandial decrease in serum Zn concentrations primarily reflects reallocation of Zn weakly bound to albumin from the serum to the liver and pancreas. It is conceivable that this reallocation of Zn occurs without isotope selectivity, possibly because the Zn transfer is a rapid and/or a nearly quantitative process. The isotopic consequences of such a process can be evaluated with a mixing equation, based on the assumption that the Zn present in serum is bound primarily to albumin and α2-

macroglobulin, whereby these two Zn pools likely record different Zn isotope composition due to the distinct bonding environments.

In such a simplified two component system (Equation 4), the serum $\delta^{66}Zn_{Ser}$ is defined by the isotope composition and mass balance of Zn bound to albumin (Alb) and α2-macroglobulin (Mgl):

$$\delta^{66}Zn_{Ser} = \delta^{66}Zn_{Alb} \times F_{Alb} + \delta^{66}Zn_{Mgl}\,(1 - F_{Alb}) \qquad (4)$$

where F_{Alb} is the molar fraction of total serum Zn that is loosely bound to albumin.

For the modelling, it was determined that when F_{Alb} is varied from 65 to 85%,[10–14] a 20% decrease of the serum Zn concentration is achieved when between 23.5 and 30.8% of albumin-bound Zn is removed from serum. It was then explored how large the isotope fractionation between Zn bound to albumin and α2-macroglobulin ($\Delta^{66}Zn_{Alb–Mgl}$) must be until the postprandial decrease of serum Zn content from loss of albumin-bound Zn generates a serum $\delta^{66}Zn_{Ser}$ response that exceeds the measurement reproducibility of ±0.07‰. With these boundary conditions, the model indicates that the isotope fractionation between serum Zn bound to albumin and α2-macroglobulin, $\Delta^{66}Zn_{Alb–Mgl}$, must exceed 0.80 to 1.87‰ for serum Zn album fractions of 65 to 85%, to generate an analytically significant postprandial $\delta^{66}Zn_{Ser}$ response (Fig. 4).

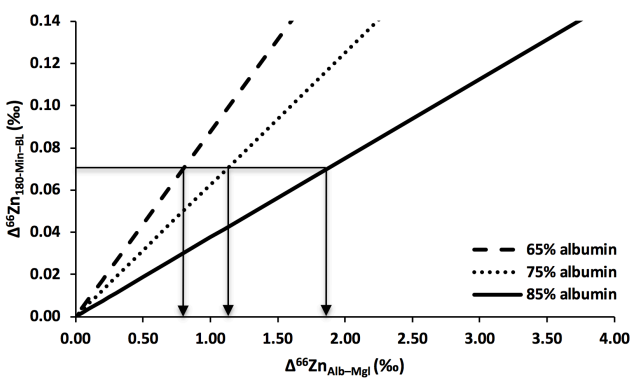

Fig. 4 The difference between the $\delta^{66}Zn$ values of serum albumin and α2-macroglobulin ($\Delta^{66}Zn_{Alb–Mgl}$) vs. the change in $\delta^{66}Zn$ values of serum postprandially relative to fasting-state ($\Delta^{66}Zn_{180\text{-}Min–BL}$). This figure illustrates that $\Delta^{66}Zn_{Alb–Mgl}$ must be larger than between ~0.80‰ at a serum albumin Zn fraction of 65% and ~1.85‰ at a serum albumin Zn fraction of 85% to shift the Zn isotopic composition of serum postprandially by greater than measurement reproducibility (± 0.07‰ 2SD).

In the context of the available literature on Zn isotope variations in biological and abiotic systems, an inferred fractionation of $\Delta^{66}Zn_{Alb–Mgl}$, ≥0.80‰ is notably large. This conclusion is supported by a wealth of literature data, which suggests that natural Zn isotope variations are typically rather moderate, especially compared to similar elements, such as Fe and Cd. For example, the total natural $\delta^{66}Zn$ variability observed for terrestrial surface and marine reservoirs as well as the human body does not exceed 2‰. In the latter case, the most extreme Zn isotope compositions are found in bones (0.77‰),[71] and the liver (–1.05‰).[72] However, within blood, the variability is more limited. The mean $\delta^{66}Zn$ values of serum,[50,51] erythrocytes,[22,25,26,72–75] and whole blood[22,26,36] are approximately 0.45‰. Previously,[37] it was proposed that Zn bound to albumin, where it is presumably complexed by the amine groups of histidine,[76] would be isotopically heavier with higher $^{66}Zn/^{64}Zn$ than Zn bound to α2-macroglobulin, where it is most likely complexed through the thiol groups of cysteine.[77] This suggestion is supported by *ab initio* calculations, which predict that Zn-histidine complexes have $\delta^{66}Zn$ values which exceed those of Zn-cysteine compounds by about 0.6‰ (at 37°C).[78] A different fractionation is expected, however, when these amino acids act as part of complex proteins, which commonly feature several structurally different binding sites for the same metal. For example, *ab initio* modelling also suggests that Zn-histidine complexes feature a $\delta^{66}Zn$ that exceeds the value of Zn bound to cysteine-rich metallothionein, but only by about 0.2‰ (at 37°C).[78] Considering this evidence, it is unlikely that $\Delta^{66}Zn_{Alb–Mgl}$ exceeds 0.80‰. If correct, this conclusion indicates that the observed lack of a postprandial serum Zn isotope response is a natural and expected consequence of the limited within serum Zn isotope fractionation.

Outlook for studying Zn homeostasis in serum

Zinc stable isotopes in blood and serum have shown potential utility in monitoring longitudinal changes during the recovery of patients who have undergone bariatric surgery to achieve weight loss.[51] The changes in Zn isotopic composition after bariatric surgery are not accompanied by changes in Zn concentration, making isotopic compositions potentially more sensitive to physiological changes than concentrations, thereby providing added value and supporting the case for further research in this context.[51] However, the lack of serum Zn concentration change following bariatric surgery is not uniform across other surgeries as there are well-documented decreases in serum Zn concentrations after severe surgical interventions.[79–82] Serum Zn is mostly redistributed to the liver in the hours following surgery,[83] but Zn is also integrally involved in all stages of wound healing, including matrix remodelling and scar formation, which can persist for months to years.[84] The variation of both serum Zn concentrations and isotope ratios due to homeostatic alterations may provide greater insights into the role of Zn in wound healing and enhance the monitoring of post-surgery recovery. Such studies comparing serum Zn levels with $\delta^{66}Zn$ values should obtain blood samples in the morning after overnight fasting so that serum Zn levels are comparable between participants. However, postprandial changes in serum $\delta^{66}Zn$ values are generally absent or small, so future investigations examining solely the distribution of Zn isotopes in serum may draw blood samples without considering timing of the most recent meal.

Conclusions

The 90-minute postprandial Zn concentration was 84.8 ± 4.8% 1SD (*n* = 8) relative to baseline, whereas the 180-minute postprandial Zn concentration was 79.1 ± 8.9% 1SD (*n* = 8) relative to baseline, representing a serum Zn concentration decrease of just over 20% three hours after the consumption of the breakfast meal. This is in agreement with previously reported results.[39–44] All 90-minute postprandial serum $\delta^{66}Zn$ values were identical, within measurement reproducibility, to the baseline results (mean $\Delta^{66}Zn_{90\text{-}Min\,–\,BL}$ = 0.02 ± 0.05‰ 2SD, *n* = 8), and with one exception this statement is also

correct for the 180-minute postprandial serum δ^{66}Zn values (mean $\Delta^{66}Zn_{180\text{-}Min - BL}$ = 0.01 ± 0.09‰ 2SD, *n* = 8). Given the magnitude of the postprandial serum Zn concentration decrease, it is notable that no change in serum δ^{66}Zn value is associated with this decline. We propose hemodilution and the rapid, efficient postprandial transfer of Zn bound to serum albumin to the liver and pancreas to participate in phosphorylation reactions and the synthesis of digestive enzymes, respectively, to explain the lack of postprandial serum Zn isotopic response. The proposed mechanism is supported with modelling that suggests that the difference between the δ^{66}Zn values of serum albumin and α2-macroglobulin ($\Delta^{66}Zn_{Alb - Mgl}$) must be larger than between 0.80‰ at a serum albumin Zn fraction of 65% and 1.87‰ at a serum albumin Zn fraction of 85% to shift the Zn isotopic composition of serum postprandially by greater than measurement reproducibility (±0.07‰ 2SD). A difference this great is unlikely due to the limited natural variability of Zn isotopic compositions.

Going forward, studies comparing serum Zn levels with δ^{66}Zn values should obtain blood samples in the morning after overnight fasting so that serum Zn levels are comparable between participants. However, postprandial changes in serum δ^{66}Zn values are generally absent or small, so future investigations examining solely the distribution of Zn isotopes in serum may draw blood samples without considering timing of the most recent meal.

Conflicts of interest

There are no conflicts to declare.

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