








Zinc absorption from maize-based meals enriched with edible house crickets: a randomized crossover stable-isotope study in Kenyan pre-school children

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Edible insects have been proposed as a novel and sustainable source of protein and other essential nutrients for human consumption but nutrient absorption efficiency is still uncertain. We investigated zinc absorption from house crickets (*Acheta domesticus*) in a single-center and single-blinded cross-over study with children aged 24–36 months old in Kenya from September–November 2021. For this, children were randomized to consume two different experimental meals labeled with stable isotopes of zinc (Zn) at two different days, separated by a wash-out period of one month. Primary endpoints were the differences in amount of absorbed zinc (AZ) from maize-based meals enriched with intrinsically ⁶⁷Zn-labeled house crickets (2.61 mg Zn, *n* = 28) in comparison with meals enriched with ⁶⁸Zn (low-enriched: 0.90 mg Zn, *n* = 29); high-enriched: 3.24 mg Zn, *n* = 28) or with intrinsically ⁶⁷Zn-labeled low-chitin cricket flour (2.51 mg Zn, *n* = 25), whereas the secondary endpoints were the differences in fractional zinc absorption. We found that AZ from meals with whole crickets (geometric mean: 0.36 mg; 95%CI: 0.30, 0.43) was 2.6 times higher than from low-enriched maize meals (0.14 mg; 0.11, 0.16; *P* < 0.001), while it was not different from low-chitin cricket flour meals. Absorbed zinc from both cricket meals was higher than that from high-enriched meals. No severe adverse side events were reported. We conclude that edible house crickets are a good source of well-absorbable zinc, and their increased consumption could contribute to the alleviation of zinc deficiency. This trial was registered at the Pan African Clinical Trials Registry as PACTR202104533831364.

Zinc deficiency is prevalent in human populations worldwide and is a significant contributor to the global burden of malnutrition. Impaired linear growth, which is associated with zinc deficiency, affects about a quarter of children under the age of five, putting them at a high risk of

mortality and other adverse consequences throughout their lives^{1–3}. Zinc plays a major role in several basic biological functions related to cell growth, metabolism, and neurological and immune functioning^{4,5}. Mild to moderate deficiency can be related to the impairment of

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physical growth and development⁶, and to increased susceptibility to communicable diseases, such as diarrhea, pneumonia and possibly also malaria, resulting in an increase in mortality among children^{6,7}. Severe zinc deficiency has major health effects on various organ systems, i.e., skin, gastrointestinal system, central nervous system, immune system, skeleton and reproductive organs⁸.

Zinc absorption by the body is strongly influenced by dietary composition, with the quantity of zinc and phytate being major determinants of lower fractional absorption of zinc. Nevertheless, a higher amount of zinc in a meal will increase the total amount of zinc absorbed^{9,10}. Zinc is found in high concentrations in whole-grain cereals and red meat, and in lower concentrations in fish, nuts, tubers, roots, and green leafy vegetables. Animal-source foods, which are often unavailable or unaffordable in low-income settings, exhibit higher zinc bioavailability than plant-based foods. This is because plant-based foods contain absorption inhibitors such as phytates, polyphenols, and fibers. In contrast, dietary protein and certain amino acid ligands enhance zinc absorption from both plant and meat sources¹¹.

Insect consumption offers a promising solution to food security challenges in low-resource settings, providing high nutritional value and sustainability benefits. With their minimal environmental footprint and efficient production, edible insects are poised to become a crucial intervention food, addressing both accessibility and sustainability concerns^{12,13}. Insects contain substantial amounts of proteins and zinc while phytate content is negligible^{14,15}. The presence of chitin, which is an abundant metal-binding polysaccharide composed of β -1,4-linked N-acetyl-d-glucosamine monomers¹⁶, might interfere with zinc uptake in the human gut; however, fractionation of the insect biomass to remove chitin could potentially enhance the bioavailability of zinc.

Orthopteran insects, including crickets, are widely consumed in African communities¹⁷, and house crickets have recently been judged safe for human consumption by the European Food Safety Authority (EFSA)¹⁸. Thus, to evaluate the potential of edible insects as a dietary source of absorbable zinc, our primary objective was to assess the amount of absorbed zinc (AZ) from maize-based meals enriched with whole house crickets (*Acheta domesticus*) in comparison with meals enriched with two levels of ZnSO₄ in young Kenyan children. Our secondary objective was to compare AZ from meals enriched with whole house crickets to meals enriched with low-chitin cricket flour. We hypothesized that the amount of absorbed zinc from maize-based meals complemented with crickets would be greater than from maize meals enriched with ZnSO₄; and that the meal with the low-chitin cricket flour would show the highest AZ.

Results

Composition of flours and test meals

The zinc concentration of the intrinsically labeled whole cricket (WC) flour and extracted cricket (EC) flour from which chitin had been removed was 16.3 ± 0.3 and 12.7 ± 0.3 mg/100 g of dry matter (DM) (Table 1), with a ⁶⁷Zn abundance of 16.8 ± 0.6 and 17.8 ± 0.1 atom % (Table 2), respectively. The iron and calcium concentration of WC and EC flours were comparable. Phytic acid concentration in the WC and EC flours were lower than the limit of detection (< 3.5 mg/100 g), whereas in maize flour this was high, as expected. Chitin concentration in the WC flour was 4.0 g/100 g DM while in the EC flour it was below the limit of detection (< 0.67 g/100 g DM). Fractionation of the insect biomass resulted in a ~ 1.5 times higher polyphenol concentration of EC (464 ± 20 mg/100 g flour DM) compared to WC flour (296 ± 11 mg/100 g flour DM). Participants consumed $\sim 81\%$ and $\sim 78\%$ of the 20 g of WC and EC meals administered to them. The amount of nutrients and anti-nutrients present in the test meals was calculated based on the average amount of the meals being consumed (Table 3). The total amount of zinc in the meals complemented with crickets was 2.61 ± 0.44 (WC) and 2.51 ± 0.59 (EC) mg, while for the other meals this

was 0.9 ± 0.00 (low-zinc, LZ) and 3.24 ± 0.00 (high-zinc, HZ) mg. Phytic acid:zinc molar ratio (PA:Zn) of both insect-based meals and of the HZ maize meal was 1.9, while for the LZ maize meal it was higher, namely 6.9.

In vitro study

Approximately 32% (95%-CI 29, 35%) of zinc was bio-accessible when only cricket flour was added to the digest, whereas it was higher in the presence of chitin (51–53%) and lower in the presence of chitosan (5%) (Table 4). A high mass ratio of calcium to zinc (10.9:1) led to similar bio-accessibility of zinc (32%, 95% CI 31, 33%), while a lower mass ratio (4.3:1) led to lower bio-accessibility (16%, 95%CI 13, 20%). When both chitin and calcium were added to the digests in various mass ratios, average bio-accessibility ranged between 22 and 23%.

Participant characteristics

Eligibility for participation was assessed in 154 children (Fig. 1). A total of 89 children were excluded due to malaria ($n=23$), hemoglobin < 7 g/dL ($n=3$), weight for height z-score < -2 ($n=7$), height for age z-score < -3 ($n=6$), inflammation ($n=19$), general health conditions ($n=4$), breastfeeding > 1 time/day ($n=14$), difficulties with anthropometric measurements ($n=9$), and difficulties with blood draws ($n=4$). Out of the 65 eligible participants, 5 withdrew consent. Out of the remaining 60 eligible subjects, 32 were randomly selected and enrolled in the study. A total of 17 participants dropped out during the first block of the study, either because they had difficulty consuming the meals ($n=10$) or because venipuncture was unsuccessful ($n=7$) (Fig. 1). Therefore, 16 participants were replaced in the first block of the study. In total, 25 participants completed both study blocks, and 4 additional subjects completed at least one study block hence contributing partially completed data. The baseline characteristics of the children are shown in Table 5. Of the children, 65% were zinc deficient, 55% were anemic, 23% were iron deficient, and 32% had iron deficiency anemia.

Table 1 | Dry matter content of minerals, protein, fat, total polyphenols and phytic acid in maize flour, whole cricket flour and low-chitin cricket flour^a

	Maize flour	Whole cricket flour (WC)	Low-chitin cricket flour (EC)
Micronutrients			
Calcium, mg/100 g	4.69 ± 0.04^c	143.01 ± 8.44^b	157.17 ± 4.68^a
Iron, mg/100 g	1.90 ± 0.07^b	5.82 ± 1.00^a	5.84 ± 0.26^a
Zinc, mg/100 g	2.37 ± 0.19^c	16.29 ± 0.34^a	12.66 ± 0.28^b
Copper, mg/100 g	0.16 ± 0.00^c	2.32 ± 0.15^a	2.23 ± 0.08^b
Phytic acid, mg/100 g	997.8 ± 69.6	nd	nd
Polyphenols ¹ , mg/100 g	na	296.4 ± 11.1^b	464.5 ± 20.4^a
Chitin, g/100 g	na	4.02 ± 0.13	nd
Macronutrients			
Protein, g/100 g	na	46.02 ± 1.23^a	37.86 ± 1.00^b
Soluble protein, g/100 g	na	2.07 ± 0.10^b	9.24 ± 0.21^a
Fat, g/100 g	na	19.53 ± 0.03^b	25.17 ± 0.35^a
Dry matter (%)	89.9 ± 0.0	95.8 ± 0.1	94.7 ± 0.3

^aValues are presented as means (mg/100 g dry matter) \pm SDs ($n=3$) for calcium, iron, zinc, copper, phytic acid and polyphenols, or as means (g/100 g dry matter) \pm SDs ($n=3$) for chitin, protein, soluble protein and fat. Test meal composition values were compared by two-sided pairwise t-tests or One-way ANOVA. Labeled means in a row without a common letter differ, with $P < 0.05$ considered to be statistically significant; ¹Expressed in GAEs, gallic acid equivalents, na not analyzed, nd not detectable.

Table 2 | Zinc isotopic abundances in intrinsically labeled whole cricket flour and low-chitin cricket flour^a

	Abundances, % (CV%)				
	⁶⁴ Zn	⁶⁶ Zn	⁶⁷ Zn	⁶⁸ Zn	⁷⁰ Zn
Whole cricket flour (WC)	42.10 (0.13)	24.09 (0.09)	16.83 (0.64)	16.45 (0.19)	0.53 (0.01)
Low-chitin cricket flour (EC)	41.58 (0.14)	23.84 (0.10)	17.76 (0.09)	16.29 (0.11)	0.52 (0.19)

^aValues are presented as mean % (CV%).

Table 3 | Average amounts of minerals, protein, fat, total polyphenols and phytic acid ingested by the four test meals^a

	With crickets		Without crickets	
	Whole	Low-chitin	High-zinc	Low-zinc
	WC	EC	HZ	LZ
Amount of flour (g) consumed per meal				
Maize flour	5.59 ± 0.94	5.31 ± 1.31	6.93 ± 0.01	6.93 ± 0.01
Insect flour	16.14 ± 2.70 ^a	15.54 ± 3.63 ^a	-	-
Amount of zinc (mg) consumed per meal				
Insect flour:	2.49 ± 0.42	1.89 ± 0.44	-	-
of which intrinsically labeled (⁶⁷ Zn) ¹	0.41 ± 0.07	0.33 ± 0.08	-	-
Maize flour:	0.12 ± 0.02	0.11 ± 0.03	0.15 ± 0.00	0.15 ± 0.00
Extrinsically added isotope-labeled (⁶⁸ Zn) ²	-	-	0.75 ± 0.00	0.75 ± 0.00
Extrinsically added natural isotope composition	-	0.51 ± 0.12	2.34 ± 0.00	-
Total zinc	2.61 ± 0.44^b	2.51 ± 0.59^b	3.24 ± 0.00^a	0.9 ± 0.00^c
PA:Zinc ³	1.9	1.9	1.9	6.9
Amount of other micronutrients (mg) consumed per meal				
Calcium	9.73 ± 0.04 ^a	23.36 ± 5.45 ^c	0.29 ± 0.00 ^b	0.29 ± 0.00 ^b
Iron	1.00 ± 0.17 ^a	0.95 ± 0.22 ^a	0.12 ± 0.00 ^b	0.12 ± 0.00 ^b
Copper	0.36 ± 0.06 ^a	0.34 ± 0.08 ^b	0.01 ± 0.00 ^c	0.01 ± 0.00 ^c
Amount of anti-nutrients consumed per meal				
Phytic acid, mg	50.18 ± 8.41 ^b	47.59 ± 11.71 ^b	62.19 ± 0.06 ^a	62.18 ± 0.07 ^a
Polyphenols, mg ⁴	45.85 ± 7.67 ^b	68.36 ± 15.95 ^a	-	-
Chitin, g	0.62 ± 0.10	nd	-	-
Amount of macronutrients (g) consumed from cricket flour				
Protein	7.12 ± 1.19 ^a	5.57 ± 1.30 ^b	-	-
Soluble protein	0.32 ± 0.05 ^b	1.36 ± 0.32 ^a	-	-
Fat	3.02 ± 0.51 ^b	3.70 ± 0.86 ^a	-	-
Amount of calories (kcal) per meal consumed				
	97.6 ± 12.2	94.9 ± 16.3	25.0 ± 0.0	25.0 ± 0.0
Amount of porridge (g) per meal consumed				
	113.12 ± 18.96	107.28 ± 26.39	120.18 ± 0.13	120.16 ± 0.13

^aValues are presented as means (mg/ meal) ± SDs for calcium, iron, zinc, copper, phytic acid and polyphenols, or as means (g/ meal) ± SDs for chitin, protein, soluble protein and fat calculated based on the average consumed amounts. The basis for all meals was refined maize flour. Comparison of test-meal composition values was assessed by two-sided pairwise t-tests or One-way Anova. Means in a row without a common letter differed, with $P < 0.05$ considered to be significant. ¹Whole cricket (WC) and low-chitin cricket (EC) flour containing meals were intrinsically labeled (⁶⁷Zn), hence intrinsically labeled zinc forms part of total zinc in insect flour. ²Meals without crickets (LZ and HZ) were extrinsically labeled (⁶⁸Zn). ³Phytic acid: zinc molar ratio (mol/mol). ⁴Expressed in GAEs, gallic acid equivalents. *nd* not detectable.

Fractional and total zinc absorption

Meal type significantly affected FAZ ($P < 0.001$) and AZ ($P < 0.001$), while the best model fit was achieved by including serum Zn (SZn) (FAZ, $P = 0.014$; AZ, $P = 0.015$) as fixed covariate in the linear model. Although inclusion of administration block and meal sequence within block as fixed effects in the models did not improve their fit, both were included in the final model due to the differences in meal composition and restricted randomization in study design. As expected, FAZ was lowest for the meal with the highest amount of zinc (EZ, geometric mean: 7.3%; 95% CI: 6.1, 8.8%), and highest for the meal with the lowest amount of zinc (LZ: 15.2%; 95% CI: 12.9, 18.1%; Table 6, Fig. 2A).

The amount of zinc absorbed (AZ) from the maize meal with whole crickets (WC, geometric mean: 0.36 mg; 95% CI: 0.30, 0.43 mg) was 2.6-fold greater than from the low-enriched maize meal (LZ, 0.14 mg; 95% CI: 0.11, 0.16 mg; ratio of geometric means: 2.6; 95% CI: 1.9, 3.6; $P < 0.001$; Table 6, Fig. 2B). The amount of zinc absorbed from

the meal with whole crickets did not differ from the amount absorbed from the meal with low-chitin cricket flour (EC, 0.34 mg; 95% CI: 0.28, 0.40 mg; $P = 1.000$). The amount of zinc absorbed from the cricket-containing meals was 1.5-fold (WC) and 1.4-fold (EC) higher than that from the high-enriched meal (HZ, 0.24 mg; 95% CI: 0.20, 0.29 mg; ratio of geometric means, WC vs. HZ: 1.5; 95% CI: 1.1, 2.1; $P = 0.004$; and EC vs. HZ: 1.4; 95% CI: 1.0, 2.0; $P = 0.019$, respectively). No correlations between FAZ or AZ and other variables, such as iron status indicators, serum zinc, and anthropometric indicators, were found.

Discussion

Our study shows that, compared to a maize meal low-enriched with zinc resembling a usual breakfast, addition of -16 g of dried edible house crickets resulted in a 2.6 times higher amount of zinc being absorbed by Kenyan children aged 24–36 months. In addition, maize meals with edible crickets yielded -1.5 times higher amounts of

Table 4 | Composition of digestive samples and mean (95%-CI) in vitro bio-accessibility of zinc (n = 3)

Sample	Cricket flour	Chitin	Chitosan	CaSO ₄ ¹	Ca ²	ZnSO ₄ ³	Zn	Zinc bio-accessibility ⁴
	mg	mg	mg	mg	mg	mg	mg	% (95%-CI)
Blank	-	-	-	-	-	-	-	-
1.	1250	-	-	-	1.44	-	0.27	32.2 (29.0, 35.4)
2.	-	1250	-	-	-	32.5	7.40	51.1 (28.1, 74.0)
3.	-	1250	-	-	-	8.34	1.90	52.8 (50.4, 55.1)
4.	-	-	1250	-	-	8.34	1.90	4.66 (2.95, 6.35)
5.	-	-	-	955	222	295	67.2	16.3 (12.7, 19.9)
6.	-	-	-	1133	263	117	26.6	32.2 (31.3, 33.0)
7.	-	1087	-	125	50	38.2	8.70	22.7 (22.0, 23.5)
8.	-	1190	-	45.6	18.3	13.9	3.17	21.6 (21.0, 22.2)
9.	-	1213	-	23.2	9.3	14.2	3.23	23.2 (22.1, 24.3)

¹As calcium sulfate dihydrate (23% calcium); ²In addition, 0.421 mg of CaCl₂(H₂O)₂ (= 0.11 mg of Ca) was added per INFOGEST protocol; ³As zinc sulfate heptahydrate (22.7% zinc); ⁴Adjusted for blank.

absorbed zinc than a maize meal highly enriched with zinc. Fractionation of cricket biomass to obtain a low-chitin protein-rich fraction neither enhanced nor inhibited zinc absorption compared to whole crickets. The in vitro experiment showed relatively high bio-accessibility of zinc from house crickets and no inhibition of zinc bio-accessibility by chitin alone, whereas there was some inhibition by chitosan and by combinations of chitin and calcium.

We have shown previously that absorption of iron from intrinsically labeled house crickets¹⁹ and yellow mealworms²⁰ in adults is likely affected by common inhibitors of mineral absorption, such as polyphenols and potentially also by chitin, both of which are present in the insect biomass. Our current findings suggest that zinc absorption is not affected by these inhibitors in a similar way, since we found FAZ and AZ from maize-based meals with house crickets to be comparable or superior compared to plain maize meals. Nevertheless, phytic acid from a cereal product such as maize flour is a known inhibitor of FAZ due to its high binding affinity to zinc^{11,21}. According to the World Health Organization (WHO)²² and International Zinc Nutrition Consultative Group (IZiNCG)²³, inhibition of FAZ occurs progressively with phytic acid molar ratios higher than 15:1 and 18:1, respectively. All meals provided in the present study were below this threshold with PA:Zn molar ratios ranging from 1.9 to 6.9. Also, it has been questioned if phytic acid has the same inhibitory effect in children as in adults²⁴. Taken together, inhibition of absorption by phytic acid likely played a minor role in our experiment. Also, the -1.4 times higher amount of polyphenols in the meal with low-chitin cricket flour did not affect FAZ, as also previously reported by Brnic et al. who found no inhibitory effect of polyphenols on zinc absorption from zinc-fortified maize meals in humans in the absence of phytic acid²⁵. Regarding enhancers, proteins have been reported to increase FAZ, especially when these proteins are derived from animal sources^{9,26}. Proteins, which are present in high concentrations in cricket flour (Table 1) might therefore counteract the negative effects of phytic acid or other inhibitors on FAZ^{27,28}.

The fractional absorption of zinc (FAZ) tends to decrease when the amount of zinc present in meals increases, while the total amount of absorbed zinc (AZ) increases^{9,11}. Therefore, AZ values, the resultant of FAZ and the amount of zinc present in a meal, are more relevant when evaluating edible insects as a dietary strategy to improve zinc status. The concentration of zinc in cricket biomass is higher compared to common livestock species such as beef, pork and chicken¹⁵, cereal grains such as maize, and legumes such as soy and pea²⁹. In Kenya, only 22% of children aged 6–23 months currently meet the minimum standards for meal frequency and minimum dietary diversity³⁰, while the prevalence of zinc deficiency among children below 5 years of age is 83%³¹ (65% in our study population). Based on

our findings, the daily addition of -16 g of dried house crickets to these children's basic staple meal can provide for 43–68% of their estimated physiological requirements for absorbed zinc as defined by WHO (0.83 mg/day)⁷, Institute of Medicine (IOM) (0.74 mg/day)³² and IZiNCG (0.53 mg/day)²³. We previously showed in a dietary optimization study that daily addition of 46.5 g of fresh crickets (which would translate to -12 g of dried crickets) to diets of Kenyan children aged 24–36 months can indeed increase average zinc intake above their requirements, while also ensuring intake adequacy of other nutrients³³.

Other studies measuring zinc absorption from cereal-based meals in children have reported results that are largely consistent with our results for the non-insect meals, although differences in amount of zinc and PA:Zn molar ratio of experimental meals should be considered when comparing findings. In the present study, we found that 0.14 mg of zinc was absorbed from a maize meal containing 0.9 mg of zinc and a PA:Zn molar ratio of 6.9, and 0.24 mg from a meal containing 3.24 mg of zinc and a PA:Zn molar ratio of 1.9. Supporting the importance of the PA:Zn molar ratio, a study in Burkinabe children aged 12–24 months found that 0.22 mg of zinc was absorbed from millet-based porridge meals containing 1.4 mg of zinc with added phytase (non-detectable PA), while only 0.13 mg was absorbed without added phytase (PA:Zn molar ratio of 7.7)³⁴. Similarly, a study in Sri Lankan children aged 4–7 years showed that 0.13 mg and 0.16 mg of zinc were absorbed from maize-based meals containing 1.5 mg of zinc with a PA:Zn molar ratio of approximately 8, under low and high iron concentrations, respectively³⁵. These findings indicate the critical role of the PA:Zn molar ratio in determining zinc absorption from meals, highlighting that lower PA:Zn is associated with higher zinc bioavailability.

In our study, the much higher FAZ of insect meals compared to the high Zn-enriched meal is explainable by their lower zinc content, but the higher AZ from insect meals was unexpected. The higher protein content of the cricket-based meals may have played a role. During protein digestion, soluble complexes of zinc with low molecular weight chelating peptides and amino acids can be formed and released, which enhances its absorption from the intestinal lumen^{36–38}. Although the meal with low-chitin cricket flour contained a higher amount of soluble proteins, nonetheless it did not show any additional enhancing effect on zinc bioavailability compared to the meal with whole cricket flour. In view of differences in meal composition and non-linearity of the inhibitory effect of amount of zinc as well as the enhancing effect of protein on absorption^{9,10}, a definite conclusion on superiority of zinc obtained from cricket biomass cannot yet be drawn.

A potential limitation to the use of extrinsic labelling of meals to measure zinc absorption is the assumption that the native zinc incorporated in the food matrix and extrinsically added (labeled) zinc enter

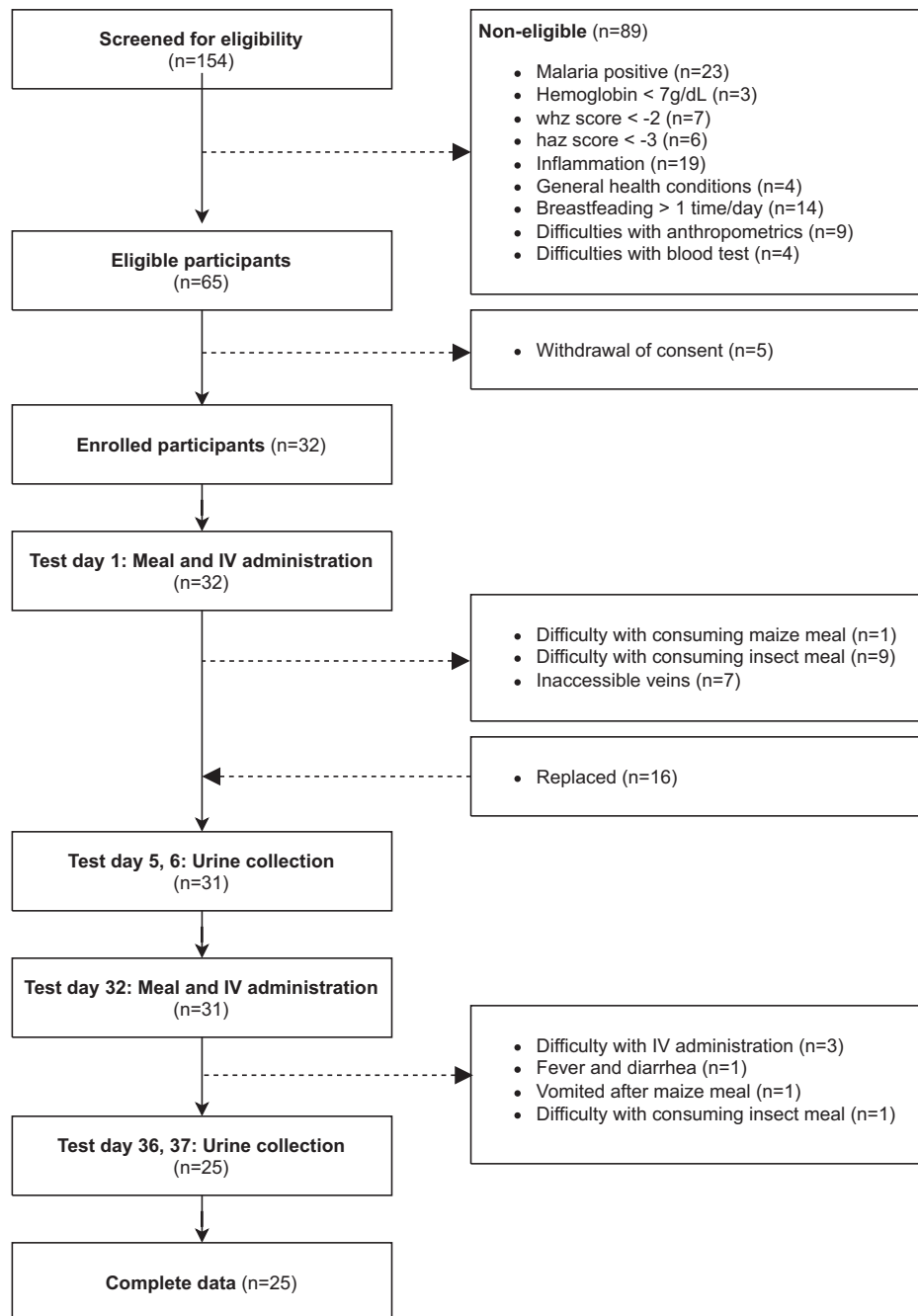


Fig. 1 | Flowchart of participant screening, enrolment and follow-up. Eligibility for participation was assessed in 154 children. A total of 89 children were excluded due to malaria ($n = 23$), hemoglobin $< 7\text{g/dL}$ ($n = 3$), weight for height z-score < -2 ($n = 7$), height for age z-score < -3 ($n = 6$), inflammation ($n = 19$), general health conditions ($n = 4$), breastfeeding > 1 time/day ($n = 14$), difficulties with anthropometric measurements ($n = 9$), and difficulties with blood draws ($n = 4$). Out of the 65 eligible participants, 5 withdrew consent. Out of the remaining 60 eligible subjects,

32 were randomly selected and enrolled in the study. A total of 17 participants dropped out during the first block of the study, either because they had difficulty consuming the meals ($n = 10$) or because venipuncture was unsuccessful ($n = 7$). Therefore, 16 participants were replaced in the first block of the study. In total, 25 participants completed both study blocks, and 4 additional subjects completed at least one study block hence contributing partially completed data. whz weight-for-height z-score, haz height-for-age z-score.

a common pool in the gastrointestinal tract³⁹. Donangelo et al. (2003) suggested that extrinsic zinc label and native zinc are absorbed to a similar extent, and that the method can be used to evaluate the effect of dietary and other factors on zinc absorption in humans⁴⁰. However, a human study measuring zinc absorption from beans reported a 10% overestimation of native zinc absorption when using extrinsic labeling²⁵. Thus, we may have slightly overestimated zinc absorption from the extrinsically labeled maize meals compared to the intrinsically labeled insect meals. Another issue is that the house crickets used

in this study were reared on optimized feed, and therefore zinc content may deviate from crickets reared elsewhere or caught in the wild. However, compared to reported values for zinc content¹⁵, the content of house crickets used for the current study was at the lower end of the range (16.3 mg Zn/100 g DM).

The strengths of our study include: a) we used a rigorous cross-over design, with three different stable zinc isotopes, thereby controlling for between-subject variation in the study outcomes; b) we reared and used intrinsically labeled house crickets by adding isotopic

zinc to the insect feed during growth, which accounts for the effect of the food matrix on zinc absorption; c) we used a dual-isotope technique, which is the reference method for determining zinc bioavailability in humans; d) we conducted the study in young Kenyan children, representing a population with a high prevalence of zinc deficiency. Our study also had some limitations, most importantly the fact that the stable isotope tracer technique employed in our study was burdensome to some subjects, which led to drop out of subjects due to: a) difficulty to access veins and to insert the canula intravenously; and b) the relatively large meal portion required to be consumed to allow tracer detection during analysis based on the low isotopic abundance of the intrinsic zinc of the labeled crickets.

Availability of insects for human consumption is particularly advantageous in low-resource settings where traditional protein sources are scarce or costly. Edible insects are not only rich in essential nutrients but also require significantly less land, water, and feed compared to conventional livestock, making them a sustainable option for addressing food insecurity^{41,42}. Furthermore, the minimal environ-

mental impact of insect farming, such as lower greenhouse gas emissions and reduced agricultural waste, aligns with global sustainability goals⁴³. Emphasizing these benefits can drive policy changes and promote the integration of edible insects into food systems to enhance nutrition and sustainability¹².

Approximately 15% of farmed insects in Africa are produced for human consumption. An enabling environment for scaling up cricket production and encouraging cricket consumption with *Acheta domesticus* as one of the most promising species exists in Kenya: cricket farming has expanded from the Western region of the country where smallholder farming was initiated in 2013, to now include the Central, Eastern and Rift Valley regions^{44–46}, one large-scale enterprise now farms and produces cricket snacks and powder for human consumption⁴⁷; the Kenya government has developed standards regulating production, handling and processing of edible insects⁴⁸; and there is ongoing investigation into mass production of edible insects⁴⁹. Our finding that crickets are a bioavailable source of zinc is a boost to these efforts. Considering the large differences in insect body composition, further research is needed to allow extrapolation of our findings to other insect species.

In conclusion, edible house crickets are high in zinc¹⁵, which is well absorbed and appears not to be strongly affected by chitin and other antinutritional factors. Therefore, house crickets can be a meaningful dietary source of zinc for humans, and, when locally available, this insect species may be incorporated in nutritious and sustainable diets to alleviate zinc deficiency.

Methods

Cricket rearing

Crickets (*Acheta domesticus* L.; Orthoptera: Gryllidae) were reared (Laboratory of Entomology, Wageningen University, Wageningen, the Netherlands) in a climate chamber at 30 °C, with a dark/light regime of 12 h/12 h and relative air humidity of 50%. Cricket eggs, laid in small containers with moistened vermiculite, were collected daily and transferred into a large plastic box until neonate hatching (55 × 38 × 28, BAUHAUS Twente, Hengelo, the Netherlands). Half carton egg trays were placed vertically in the box to increase surface area. A water dispenser (Gebroeders de Boon, Gorinchem, the Netherlands) provided the crickets with drinking water, and the dispenser opening was

Table 5 | Anthropometric measurements, zinc, iron and inflammation indices in Kenyan children (n = 31) at baseline^a

Participant's characteristics	Values
Age, mo	30.8 ± 3.0
Boys/girls, n	19/12
Body height, cm	88.2 ± 3.1
Body weight, kg	12.3 ± 1.3
Weight-for-height z-score, SD	-0.1 ± 0.1
Height-for-age z-score, SD	-1.1 ± 0.8
Hemoglobin, g/L	106 ± 11.8
Plasma ferritin, µg/L	15.3 (7.0, 32.7)
Serum zinc, µg/dL	58.0 ± 13.3
Plasma soluble transferrin receptor, mg/L	8.1 (7.1, 9.2)
Plasma C-reactive protein, mg/L	0.4 (0.2, 1.2)
Plasma α-1-acid-glycoprotein, g/L	0.9 (0.7, 1.1)

^aValues are Mean ± SD, or Median (25th, 75th percentiles).

Table 6 | Estimated marginal means of fractional zinc absorption (%) and amount of zinc (mg) absorbed from study meals, and mean differences (95% confidence intervals) between meals in Kenyan children aged 24–36 months^a

Meal type	Fractional zinc absorption (%) ¹			Amount of zinc absorbed (mg) ²		
	Geometric mean (95% CI)	Ratio of geometric means ³ (95% CI)	P-value	Geometric mean (95% CI)	Ratio of geometric means ³ (95% CI)	P-value
Primary outcome:						
LZ: no crickets – low Zn enriched	15.2 (12.9, 18.1)	Reference		0.14 (0.11, 0.16)	Reference	
WC: whole cricket flour	13.8 (11.6, 16.4)	0.9 (0.7, 1.2)	1.000	0.36 (0.30, 0.43)	2.6 (1.9, 3.6)	< 0.001
Secondary outcomes:						
WC: whole cricket flour	13.8 (11.6, 16.4)	Reference		0.36 (0.30, 0.43)	Reference	
EC: low-chitin cricket flour	12.6 (10.6, 15.0)	0.9 (0.7, 1.2)	1.000	0.34 (0.28, 0.40)	0.9 (0.7, 1.3)	1.000
HZ: no crickets – high Zn enriched	7.3 (6.1, 8.8)	Reference		0.24 (0.20, 0.29)	Reference	
WC: whole cricket flour	13.8 (11.6, 16.4)	1.9 (1.4, 2.6)	< 0.001	0.36 (0.30, 0.43)	1.5 (1.1, 2.1)	0.004
EC: low-chitin cricket flour	12.6 (10.6, 15.0)	1.7 (1.3, 2.4)	< 0.001	0.34 (0.28, 0.40)	1.4 (1.0, 2.0)	0.019
LZ: no crickets – low Zn enriched	15.2 (12.9, 18.1)	2.1 (1.5, 2.9)	< 0.001	0.14 (0.11, 0.16)	0.6 (0.4, 0.8)	< 0.001

^aValues are geometric means (95% CIs). Linear mixed model with subject as random factor; meals, block and meal sequence within block as fixed factors; and serum zinc (SZn) as fixed covariate. P-values reflect two-sided testing with Bonferroni correction for multiple comparisons. ¹Fractional zinc absorption (%) from test maize flour meal with WC) 16.14 g intrinsically labeled (0.41 mg ⁶⁷Zn) whole cricket flour (n = 28); EC) 15.54 g intrinsically labeled (0.33 mg ⁶⁷Zn) low-chitin cricket flour + unlabeled 0.51 mg ZnSO₄ (n = 28); HZ) 0.75 mg ⁶⁸ZnSO₄ + unlabeled 2.34 mg ZnSO₄ (n = 25); and LZ) 0.75 mg ⁶⁸ZnSO₄ (n = 29), respectively, in Kenyan children. ZnSO₄, zinc sulfate. ²Calculated based on the fractional absorption and zinc content of the test meals (Table 1). ³A ratio of geometric means < 1 should be interpreted as a negative effect compared to the reference, and a ratio > 1 as a positive effect compared to the reference. For instance, a ratio of 0.9 indicates that the treatment effect is 10% lower compared to the reference. A 95% CI that includes 1 does not rule out a null effect.

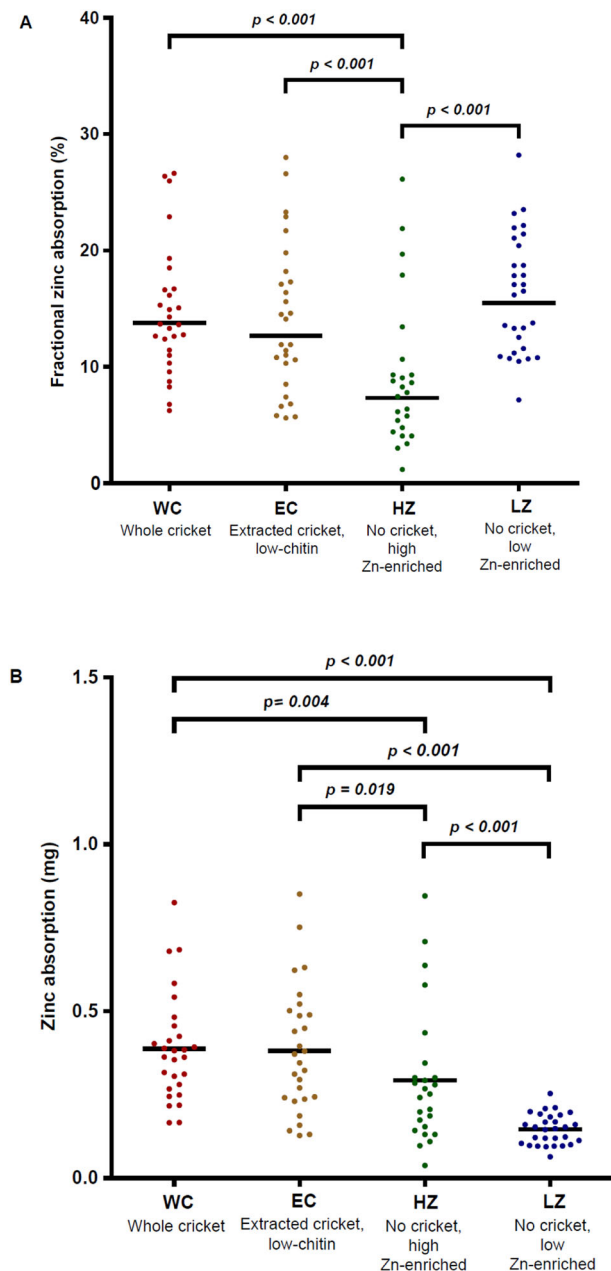


Fig. 2 | Zinc absorption from maize-based test meals by Kenyan children aged 24–36 months. **A** Fractional zinc absorption (FAZ) (%); **B**: amount of zinc absorbed (AZ) (mg). Meals were composed of maize porridge with: (WC, red dots) 16.14 g intrinsically labeled (0.41 mg ^{67}Zn) whole cricket flour ($n=28$); (EC, brown dots) 15.54 g intrinsically labeled (0.33 mg ^{67}Zn) low-chitin cricket flour and 0.51 mg unlabeled Zn (as ZnSO_4) ($n=28$); (HZ, green dots) 0.75 mg ^{68}Zn and 2.34 mg unlabeled Zn (as ZnSO_4) ($n=25$); and (LZ, blue dots) 0.75 mg ^{68}Zn (as ZnSO_4) ($n=29$), respectively. Horizontal lines represent the mean. We compared test meal effects (two-sided) by linear mixed model using ‘subjects’ as a random factor, ‘meals’, ‘block’ and ‘meal sequence within block’ as fixed factors, and ‘serum zinc (SZn)’ as a covariate, followed by *post hoc* tests with Bonferroni correction for multiple comparisons. ZnSO_4 , zinc sulfate.

filled with a paper tissue to prevent neonate crickets from drowning. Crickets received slices of carrot daily and a basal chicken feed diet (Kuikenopfokmeel 1, Kasper Faunafood, Woerden, the Netherlands) *ad libitum* during the first two weeks. Subsequently, they received an experimental diet (Research Diet Services, Wijk bij Duurstede, the Netherlands) with $^{67}\text{ZnSO}_4$ (see next section) until they reached the adult stage. Water and feed were checked daily and replenished when

necessary to provide an *ad libitum* supply. One day after the first adult cricket was seen in a box (after 31–34 days), crickets were relocated into a new box. They then received only carrots for 24 h, to remove the experimental diet from their gut. After 24 h, crickets were harvested and stored frozen at -21°C .

Preparation of experimental cricket diet

A total of 0.315 g of ^{67}Zn (as ^{67}ZnO with 90.6% isotopic enrichment; Chemgas, Boulogne, France) was dissolved in 0.1 mol/L H_2SO_4 to form $^{67}\text{ZnSO}_4$. This solution was sprayed at 1 bar with an HI/4VV-650067 nozzle over 2 kg of experimental cricket feed (Research Diet Services, Wijk bij Duurstede, the Netherlands) in a vacuum coater (Type 305, Dinnissen Process Technology, Sevenum, the Netherlands) at the feed processing facility of Wageningen University, Wageningen, the Netherlands. This mixture was dried overnight at 50°C . The 2 kg mixture containing the $^{67}\text{ZnSO}_4$ was then added to 14 kg of feed (Research Diet Services, Wijk bij Duurstede, the Netherlands) and mechanically mixed for five minutes using a feed mixer (Type F60, Halvor-Forberg, Telemark, Norway). The experimental diet was then packed in paper bags, sealed with plastic tape, and stored at -18°C until use.

Cricket processing

Frozen crickets were blanched for two minutes, chilled, and then quickly frozen to -40°C in a shock freezer. To obtain a whole cricket flour (WC), crickets were freeze dried (Lyofast S08 and Super Modulyo, Edwards, West Sussex, United Kingdom) and ground into a fine flour with an ordinary kitchen blender. To obtain a low-chitin cricket flour (EC), frozen crickets were first blended in a stainless-steel food blender with ultrapure water (1:2 w/w) at 60°C and pH 7.5. The soluble fraction was obtained by filtration through a cheese cloth; the obtained solution was heat-treated for 2 min at 90°C on a heating plate (Framo-Geratetechnik M20/1, Germany), left to cool at room temperature, transferred in plastic containers and stored at -18°C until freeze drying (Lyofast S08 and Super Modulyo, Edwards, West Sussex, United Kingdom) and fine grinding. Cricket flours were stored in the freezer (-18°C) until meal preparation and administration. Microbiological analysis was performed including enumeration of aerobic mesophilic bacteria, *Bacillus cereus*, *Clostridium perfringens*, *Enterobacteriaceae*, yeasts and molds, coagulase positive *Staphylococci* and *Escherichia coli*; and detection of *Listeria monocytogenes* and *Salmonella* spp. shortly before preparing the test meals.

In vitro study

We conducted in vitro experiments to evaluate potential inhibitors of zinc absorption present in crickets, i.e., chitin, its digestive breakdown product chitosan, and calcium. Chitin may inhibit mineral absorption in addition to phytate, calcium, and polyphenols found in insects^{30,31}. In vitro digestion was performed to assess the bio-accessibility of zinc according to the INFOGEST protocol⁵², with some modifications, as we described before for iron¹⁹. All samples were prepared in triplicate.

A range of samples were prepared to investigate the inhibitory effect of chitin, chitosan, and calcium on zinc absorption. Chemicals, i.e., ZnSO_4 (CAS: 7446-20-0), CaSO_4 (CAS: 10101-41-4), both with $\geq 99\%$ purity; and chitin (CAS: 1398-61-4) and chitosan (CAS: 9012-76-4) (both from shrimp shells), were all purchased from Sigma-Aldrich. Chitin from shrimp is fairly similar to the structure of chitin present in crickets⁵³. Since the sample weight was always 1.25 g, the amount of zinc (as ZnSO_4) added to a sample was limited by the weight of other components in the sample. We took the mass ratio of compounds as present in 100 g dry matter of crickets (zinc, 16 mg; chitin, 4 g; calcium, 140 mg) as guidance for deciding on the amounts of anti-nutrients and zinc to be added to each sample (Table 1). Crickets used for the in vitro study were taken from the same batch as for the human study. Zinc

content of input material and supernatants were analyzed at the Laboratory for Human Nutrition, ETH Zürich, Switzerland, by ICP-MS (iCap RQ, Thermo Fisher Scientific) after appropriate dilution with 1% nitric acid.

Mineral bio-accessibility, defined as the soluble mineral fraction in the supernatant, was calculated using the following formula:

$$\%Zn\ bio - accessibility = \frac{Zn\ in\ digested\ sample(mg) - Zn\ in\ blank\ sample(mg)}{Zn\ in\ the\ undigested\ sample(mg)} * 100 \quad (1)$$

The average zinc content of the blank was subtracted from all sample results included in the same run of 6 samples. Replicates of each condition ($n=3$) were used to calculate mean bio-accessibility with 95% confidence intervals.

Study site and participants

The human study was conducted between September and November 2021, with participants being enrolled between the 1st and 22nd of October. Caregiver-child pairs were recruited through public community information meetings from the catchment area of the Ober Kamoth Health Center in Kisumu, located at the shores of Lake Victoria in Kenya. Informed consent was provided by the children's legal caregiver or guardian with either a written signature or a fingerprint. The latter is accepted practice for biomedical research in Kenya in case of illiteracy. In such case, the informed consent form was also signed by a literate witness accompanying the child's caregiver. Before consenting, the purpose and procedures of the study were explained verbally to the child's caregiver, and time was taken for deliberations and to answer any remaining questions. Inclusion criteria for the children were: 1) age between 24 and 36 months; 2) willingness to consume edible house crickets; 3) weight-for-height z-score >-2 ; 4) height-for-age z-score >-3 . Exclusion criteria were: 1) intake of vitamin and mineral supplements <2 weeks before baseline (including milk formula, MNPs or other nutrient-fortified products); 2) current metabolic, gastrointestinal or chronic diseases; 3) long-term medication; 4) *P. falciparum* malaria infection; 5) C-reactive protein (CRP) concentration >5 mg/L; 6) Severe anemia, hemoglobin (Hb) <7 g/dL; 7) Allergic to crustaceans; 8) Breastfeeding ≥ 1 time per day. Data were collected in REDCap (version 11.2.2, RRVanderbilt University, USA).

Ethics and inclusion statement

The ethical review committee of the Kenyatta National Hospital/University of Nairobi (KNH-UON ERC, P571/10/2020) and the Pharmacy and Poisons Board (PPB) of the Kenyan Ministry of Health approved the study. This trial was registered at the Pan African Clinical Trials Registry as PACTR202104533831364. Roles and responsibilities of local researchers were agreed upon prior to study.

Study design

This study had a single blind partially randomized controlled cross-over design (Fig. 1). Children were assessed for eligibility. During screening, capillary blood from a finger prick was collected for determination of hemoglobin (Hb), C-reactive protein (CRP) and malaria infection. Weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm. The total duration of follow-up of the subjects was 36 days (Fig. 3). Eligible subjects were selected to complete two days of zinc absorption studies, with a wash-out period of 30 days in between. They received in total four different meals (two meals per experimental day). Two oral isotopic tracers, ^{67}Zn for WC and EC, and ^{68}Zn for HZ and LZ meals, were used; consequently, meals with the same isotopic tracer could not be administered at the same study day. Therefore, test meal administration was restricted to 16 possible test-meal sequences (Supplementary Table 1). Randomization

was accomplished by using a computer-generated list (Excel, Microsoft Office 2016) of 16 letter codes, each representing one of the test-meal sequences, and subjects were masked to the test meal order (Supplementary Table 2). Subjects were instructed to fast from 20:00 h onwards on the day before both test days. Before test-meal administration at test days 1 and 31, a baseline spot urine sample was collected for determination of urinary Zn isotopes, and a venipuncture blood sample was collected for determination of serum zinc, Hb, plasma ferritin (PF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α -1-acid glycoprotein (AGP). Two meals were administered per test day at 07:00 h (breakfast) and 11:00 h (lunch). In between the two meals (at 09:00 h), an intravenous zinc dose (^{70}Zn) was administered. Intake of food and drinks was not allowed between the two meals, except for drinking water (Planet Aqua, Planet), and children remained at the feeding site for 3 h after the second test meal, during which also no food and drinks were allowed, except for water. Four days as well as five days (as a confirmatory sample) after test-meal administration, on days 5-6 and 35-36, enriched spot urine samples were collected in the morning during home visits.

Test meals

For each test meal day, maize porridge was prepared in bulk according to a standardized protocol. A test meal portion consisted of 120 g of maize-based porridge (-8.7% dry weight), which contained -6.9 g refined maize flour, -3.5 g sugar (Mill White Sugar, Kenya's Sweetest), and -109.6 g water (Planet Aqua, Planet). The respective cricket flour and zinc isotopes were added to the maize porridge portion just before feeding. Four types of test meals were administered as: a) 20 g intrinsically labeled (0.52 mg ^{67}Zn) whole cricket flour (WC); b) 20 g intrinsically labeled (0.43 mg ^{67}Zn) low-chitin cricket flour (EC) + 0.66 mg unlabeled Zn (as $ZnSO_4$); c) 0.75 mg ^{68}Zn + 2.34 mg unlabeled Zn (as $ZnSO_4$, high zinc, HZ); and d) 0.75 mg ^{68}Zn (low zinc, LZ; Table 3). The LZ meal served as a reference meal that resembled the amount of zinc present in children's usual breakfast. The ^{67}Zn was given as an intrinsic label, while ^{68}Zn was added extrinsically (as $ZnSO_4$ in solution) on the meals prior to serving. Unlabeled $ZnSO_4$ was added to the low-chitin cricket flour meal (EC) to ensure its total Zn content was similar to the whole cricket flour meal (WC). After cricket flour addition, mixtures were heated in a microwave for 1 min at 600 W. All individual ingredients were pre-weighed, and the consumption of test meals was quantified by weighing the meals before and after consumption.

Stable-isotope tracers

Zinc oxide highly enriched in ^{67}Zn , ^{68}Zn and ^{70}Zn (90.6%, 99.0% and 98.8% isotopic enrichment respectively) was purchased from Chemgas, France. $^{67}ZnSO_4$ for intrinsic labelling of crickets and $^{68}ZnSO_4$ for extrinsic labelling of meals were prepared from ZnO enriched in ^{67}Zn and ^{68}Zn by dissolution in stoichiometric amounts of 0.1 mol/L H_2SO_4 . Sterile, non-pyrogenic solution of zinc chloride (^{70}Zn) in physiological saline solution was prepared, intended for use as an intravenously injectable marker, at the Cantonal Pharmacy of the University Hospital Zürich⁵⁴. For this, isotopically enriched zinc oxide (^{70}Zn) was converted to zinc chloride by dissolving it in hydrochloric acid (4 M), adjusted to pH 6 by adding $NaHCO_3$ (1 M), and diluted with NaCl 0.9% to the target zinc concentration of 22 μ g of ^{70}Zn (stable isotope of zinc) as zinc chloride ($ZnCl_2$) in 0.9% sodium chloride solution. The intravenous dose of ^{70}Zn (5.5 mL) was administered through a sterile injection system consisting of a two-way catheter and a septum injection port. The syringe containing the isotope solution was fixed at one port and a second syringe containing 5 mL physiological saline was fixed at the second port. To ensure quantitative isotope administration, 5 mL of saline was used to flush the injection system, and the weight of the syringe used was recorded before and after intravenous administration.

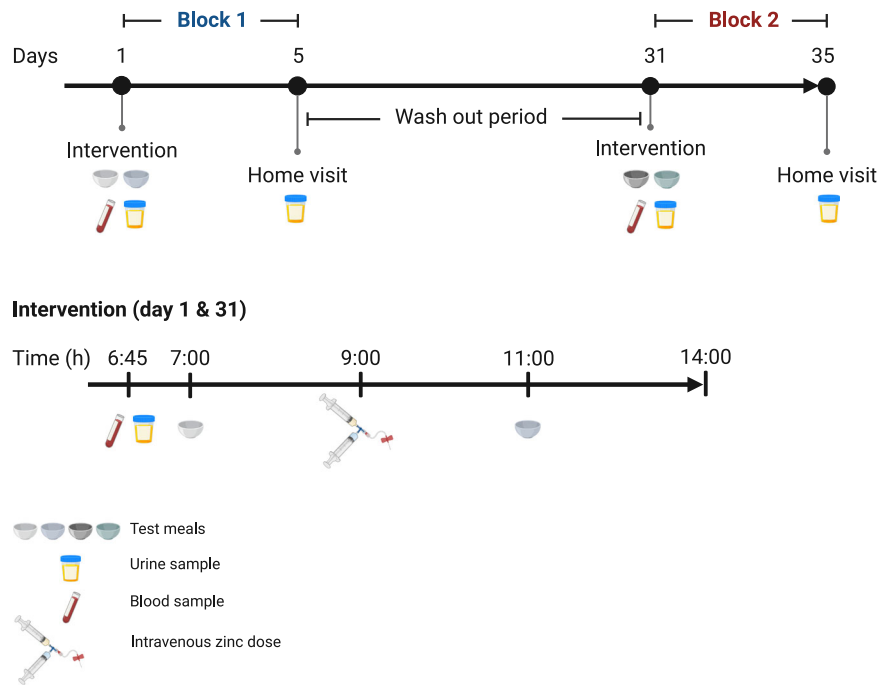


Fig. 3 | Study design and outline of the test days. The study was conducted in two blocks; days 1 and 31 were experimental days, during which blood and urine samples were collected, and two test meals were administered to participants at 7:00 h and 11:00 h, as well as an intravenous zinc dose at 9:00 h. Participants were allowed

to drink water until they left the study facility at 14:00 h. Urine samples for measurement of zinc isotope ratios were collected at days 5 and 35. Created in BioRender. Hilaj, N. (2024) <https://BioRender.com/t47d619>.

Composition of test meals

Dry matter of maize and insect flours was determined after drying the samples in a conventional oven (Binder BD-56, BINDER GmbH, Germany) at 100 °C overnight in triplicate. Mineral composition of meal ingredients was analyzed by Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS iCap RQ, Thermo Scientific) after microwave digestion (MLS-ETHOS plus; MLS) with the use of a mixture of HNO₃ and H₂O₂. Zinc isotopic ratios in the insect flours and solutions were analyzed by reversed isotope dilution mass spectrometry (Q-ICP-MS iCap RQ, Thermo Scientific) after mineralization by microwave digestion (TurboWave; MLS) in HNO₃. The phytic acid (PA) concentration was measured by adapting a method from Makower (1970)⁵⁵, in which iron was replaced by cerium in the precipitation step. After the mineralization of the precipitates, inorganic phosphate was determined according to Van Veldhoven and Mannaerts, (1987)⁵⁶ and converted into PA concentrations. The total polyphenol (PP) concentration was determined by using a modification of the Folin-Ciocalteu method⁵⁷ and was expressed as gallic acid equivalents. Chitin content was determined as described by Psarianos et al. (2022)⁵⁸. The fat content was determined according to ISO standard 1735:2004⁵⁹. The protein content of whole cricket flour (WC) and low-chitin cricket flour (EC) was determined by DUMAS (Flash EA 1112 Protein analyzer with Eager 200 software, ThermoFisher Scientific Inc., Waltham, USA) using nitrogen-to-protein conversion factor (Kp) of 4.75 and 5.60, respectively as described by Janssen et al. (2017)⁶⁰. The caloric content of meals containing maize flour with or without cricket flour was calculated by multiplying the weight of each ingredient in grams by its respective caloric value per gram. Maize flour contributes approximately 3.6 calories per gram, while cricket flour contributes about 4.5 calories per gram. The total caloric content of the meal was determined by summing the calories from each ingredient^{13,29}.

Blood analysis

Capillary blood from a finger prick was collected and hemoglobin (Hb) concentration was measured by Hemocue (Hb 301 System, Ängelholm,

Sweden). Venous blood samples were drawn into trace-element-free serum separator tubes (6 mL). Concentrations of ferritin (PF), soluble transferrin receptor (sTfR), α-1-acid glycoprotein (AGP), and C-reactive protein (CRP) in plasma were measured by using a multiplex immunoassay⁶¹. Serum zinc (SZn) concentration was measured by Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS iCap RQ, Neptune, Thermo Scientific) as described by Brnic et al. (2014)²⁵. Anemia was defined as Hb < 110 g/L⁶². Iron deficiency was defined as PF < 12 µg/L and/or sTfR > 8.3 mg/L⁶¹; and iron-deficiency anemia was defined as the combination of anemia and iron deficiency. Expected CRP and AGP concentrations for healthy children are < 5 mg/L and < 1 g/L, respectively⁶¹. Zinc deficiency was defined as SZn < 60 µg/dL for children < 10 years²³.

Calculation of absorbed zinc

Urine samples were prepared in duplicate as described by Brnic et al. (2014)²⁵. The isotopic ratios ⁶⁷Zn:⁶⁶Zn, ⁶⁸Zn:⁶⁶Zn and ⁷⁰Zn:⁶⁶Zn were measured in the prepared samples using a high-resolution double-focusing magnetic sector field multi-collector inductively coupled plasma mass spectrometer (Neptune, Thermo Scientific). The ⁶⁷Zn/⁶⁶Zn, ⁶⁸Zn/⁶⁶Zn and ⁷⁰Zn/⁶⁶Zn isotopic ratios measured in the urine samples following label administrations all showed detectable isotopic enrichments compared to baseline samples. The isotopic enrichments ranged from 0.9‰ to 26‰ for ⁶⁷Zn/⁶⁶Zn, 0.4‰ to 28‰ for ⁶⁸Zn/⁶⁶Zn and 30‰ to 260‰ for ⁷⁰Zn/⁶⁶Zn, whereas the limits of detection (based on 3 standard deviations of measured natural ratios) for isotopic enrichments were 0.14‰, 0.17‰ and 0.51‰ for respectively ⁶⁷Zn/⁶⁶Zn, ⁶⁸Zn/⁶⁶Zn and ⁷⁰Zn/⁶⁶Zn.⁶³ The fractional absorption of the ⁶⁷Zn and ⁶⁸Zn doses from the respective test meal was calculated from the spot urine sample collected 4 days after the tracer administration by using the oral-to-intravenous tracer ratio method, according to principles described by Friel et al. (1992):

$$FAZ = (u_{oral} : u_{iv}) * (d_{iv} : d_{oral}) * 100 \quad (2)$$

where u_{oral} and u_{iv} are the amounts of orally (^{67}Zn and ^{68}Zn) and intravenously (^{70}Zn) administered isotopic tracers in the urine sample, and d_{iv} and d_{oral} are the doses of intravenously and orally administered tracers, respectively. The oral-to-intravenous tracer ratio $u_{\text{oral}}/u_{\text{iv}}$ was calculated from the measured isotopic ratios using the principles of isotopic dilution, taking into account that neither tracer was mono-isotopic. The amount in mg of zinc absorbed (AZ) from the respective test meal was calculated by using the following equation:

$$AZ = FAZ * (\text{mg Zn consumed per meal}) / 100 \quad (3)$$

Sample size calculation

Assuming that the fractional absorption of zinc from maize porridge would be 26%, we expected a difference of 0.60 mg in absorbed zinc between the whole cricket (WC) and low zinc (LZ) meals. A smaller difference of 0.25 mg in absorbed zinc was expected between the whole cricket (WC) and extracted cricket (EC) meals. Based on a Student's t-test, our sample size calculation indicated that twenty-five subjects were required to detect a meaningful difference of 0.25 mg in AZ between meals, with a standard deviation of 0.35 mg, and a two-sided $\alpha = 0.05$ and $\beta = 0.10$.

Statistical analysis

We analyzed biochemical, anthropometric and zinc absorption data using MS Excel 2016 (Microsoft, Redmond, USA) and SPSS software (version 28; IBM). The graphical presentation of data was produced with GraphPad Prism (version 9, GraphPad Software Inc., San Diego, USA). Normality was assessed using the Shapiro-Wilk W-test. Values in the text and tables are provided as means \pm SDs if normally distributed and as medians (25th and 75th percentiles) if non-normally distributed. Due to heteroskedasticity of residuals, values for fractional zinc absorption (FAZ) and absorbed zinc (AZ) were converted to their logarithms in regression models, and reconverted to geometric means (95% confidence interval (CI)) for reporting. For CRP values below the limit of detection (0.2 mg/L), we imputed random values between 0 and the limit of detection ($n = 10$). A linear mixed model with unstructured covariance matrix was used to assess differences in FAZ or AZ between the meals using meal type as fixed factor and the subject ID as random factor (intercept). Differences are presented as ratios of geometric means, where a ratio < 1 should be interpreted as a negative effect compared to the reference, and a ratio > 1 as a positive effect compared to the reference. The effect of covariates on the model fit was explored by adding these as fixed effects (block and meal sequence within block, iron status indicators, serum zinc and anthropometrics) to the model. The final model was adjusted for serum zinc at baseline, administration block and meal sequence within block. A *post hoc* Bonferroni correction was applied to correct for multiple comparisons. In case of drop out, data were included in the analysis up to the point of exclusion. All cases who completed at least one full experimental block were retained in the analyses. Cases who did not provide a urine sample for the corresponding experimental block could not be retained because zinc absorption could not be determined. To explore associations between FAZ and/or AZ and other variables of interest (iron status indicators, serum zinc and anthropometrics, zinc content of the meals), we used Pearson correlations. Test meal composition values were compared by pairwise t-tests or one-way ANOVA.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. The individual de-identified trial participant data that support the findings of this study are available in 4TU. ResearchData with identifier <https://doi.org/10.4121/06596213-fdd5-4dcd-9f39-c52f3be933b6> under an infinite CC BY 4.0 license. No study protocol or statistical analysis plan is publicly available. Source data underlying Fig. 2 are provided with this paper.

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Author contributions

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Competing interests

The authors declare no competing interests.

Additional information

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