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Soil-type influences human selenium status and underlies widespread selenium deficiency risks in Malawi

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Selenium (Se) is an essential human micronutrient with critical roles in immune functioning and antioxidant defence. Estimates of dietary Se intakes and status are scarce for Africa although crop surveys indicate deficiency is probably widespread in Malawi. Here we show that Se deficiency is likely endemic in Malawi based on the Se status of adults consuming food from contrasting soil types. These data are consistent with food balance sheets and composition tables revealing that >80% of the Malawi population is at risk of dietary Se inadequacy. Risk of dietary Se inadequacy is >60% in seven other countries in Southern Africa, and 22% across Africa as a whole. Given that most Malawi soils cannot supply sufficient Se to crops for adequate human nutrition, the cost and benefits of interventions to alleviate Se deficiency should be determined; for example, Se-enriched nitrogen fertilisers could be adopted as in Finland.

S elenium is an essential micronutrient for humans[1], with 25 genes expressing selenoproteins in the human genome[2]. These include iodothyronine deiodinases, thioredoxin reductases and glutathione peroxidases (GPx) which have critical roles in thyroid function, redox homeostasis and antioxidant defence and can be compromised by Se deficiency[1–3]. Selenium deficiency also affects immune responses and is linked to lower CD4^{+} T cell counts, disease progression and mortality among individuals infected with HIV-1 (ref. 4). Several biomarkers are used to define human Se status, including its concentration in blood fractions and urine[1]. Whole-plasma GPx3 activity saturates at ~100 μg Se L^{-1}, corresponding to habitual Se intakes of ~1 μg Se kg^{-1} body mass d^{-1}, and this relationship is used by many expert bodies to set dietary recommendations of 25–75 μg Se person^{-1} d^{-1} at an individual level[1]. Few studies have been conducted on Se nutrition in Southern Africa, although Se deficiency is probably widespread based on intake data and extrapolation. For example, intakes of 17 μg Se d^{-1} were reported for adults in rural Burundi[5] and 15–21 μg Se d^{-1} for children in rural areas of Zomba District, Malawi[6]. The latter study corresponds to low plasma Se status (typically <60 μg L^{-1}) among adults in this area[7–9]. In a recent spatial survey of soil and maize grain in Malawi, >90% of the population were estimated to consume <7.5 μg Se person^{-1} d^{-1} from maize grain[10]. Maize provides >50% of dietary energy supply in Malawi based on retail-level food balance sheets[11] and household[12] surveys, but contributes more in some groups. On calcareous soils classified as Eutric Vertisols, grain Se concentrations were >10-fold higher than on other soil types[10] due to increased soil-to-crop Se transfer (Figure 1). This is likely to be due mainly to greater stability of soluble Se(VI) species at high pH, i.e. the most plant-available form of Se[13] and decreased strength of Se(IV) adsorption on soil colloids and differences in soil clay mineralogy[10]. However, soils of the Eutric Vertisols type comprise only 0.5% of the land area of Malawi and Se deficiency risk is likely to be high at a population level.

The aims of this study were (1) to test whether spatial variation in soil-to-crop transfer of Se due to soil pH corresponds with Se intake and Se status in individuals, and (2) to determine the risk of dietary Se inadequacy
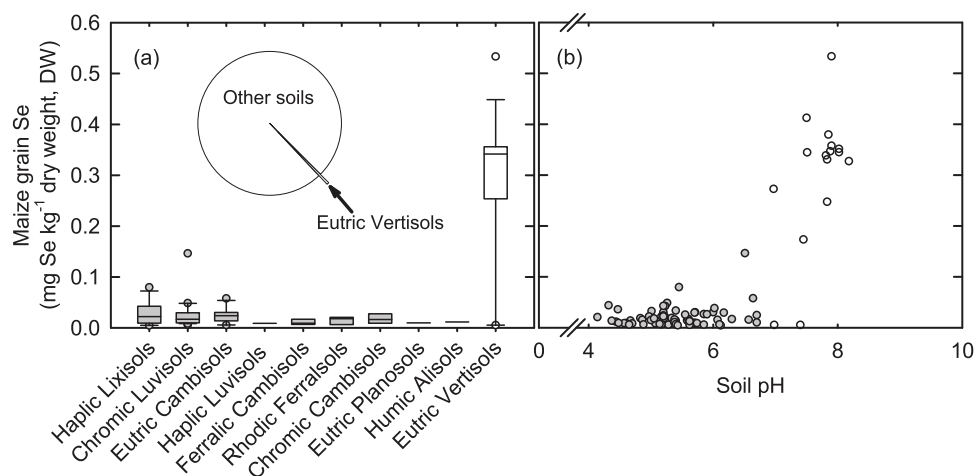
Figure 1 | Soil-to-maize grain Se transfer. (a, b) Most Malawi soils (grey symbols) have low soil-to-maize Se transfer linked to soil type including a low soil pH, in contrast to Eutric Vertisols (open symbols). In (a) boxes represent 25 and 75%-iles with median lines; whiskers are 5 and 95%-iles; circles are outliers. Inset in (a), pie chart shows that Eutric Vertisols comprise <0.5% of all soil types in Malawi. Data redrawn from Chilimba et al. (2011; ref. 10).

from net food availability and food composition tables. Where Se deficiency risks are high, it may be feasible to adopt agricultural-based programmes to enhance the mineral composition of food-stuffs, for example, by enriching fertilisers with Se[14–16].

Results

Soil pH markedly affected dietary Se intake and biomarkers of Se status in Malawi, as predicted from spatial surveys of maize[10]. Women from villages with acid soils in Zombwe Extension Planning Area (EPA) had median dietary Se intake of 6.5 µg Se d^{-1} (standard deviation (SD) 9.4, range 1.1–62.3 µg Se d^{-1}, n = 56) from all dietary sources including water (Figure 2a; Supplementary Tables 1, 2). Selenium intake was eight-fold higher in villages with proximal calcareous soils in Mikalango EPA (median 55.3 µg Se d^{-1}, SD 44.9, range 5.8–192 µg Se d^{-1}, n = 58). Plasma Se concentration in Zombwe EPA (median 53.7 µg L^{-1}, SD = 9.7, range 32.3–78.4, n = 60) was less than half of those in Mikalango EPA (median 117 µg L^{-1}, SD = 22.5, range 82.6–204, n = 60; Figure 2b). Urine Se concentration in Zombwe EPA (median 7.3 µg L^{-1}, SD = 2.0, range 4.1–13.3, n = 59) was one third that of Mikalango EPA (median
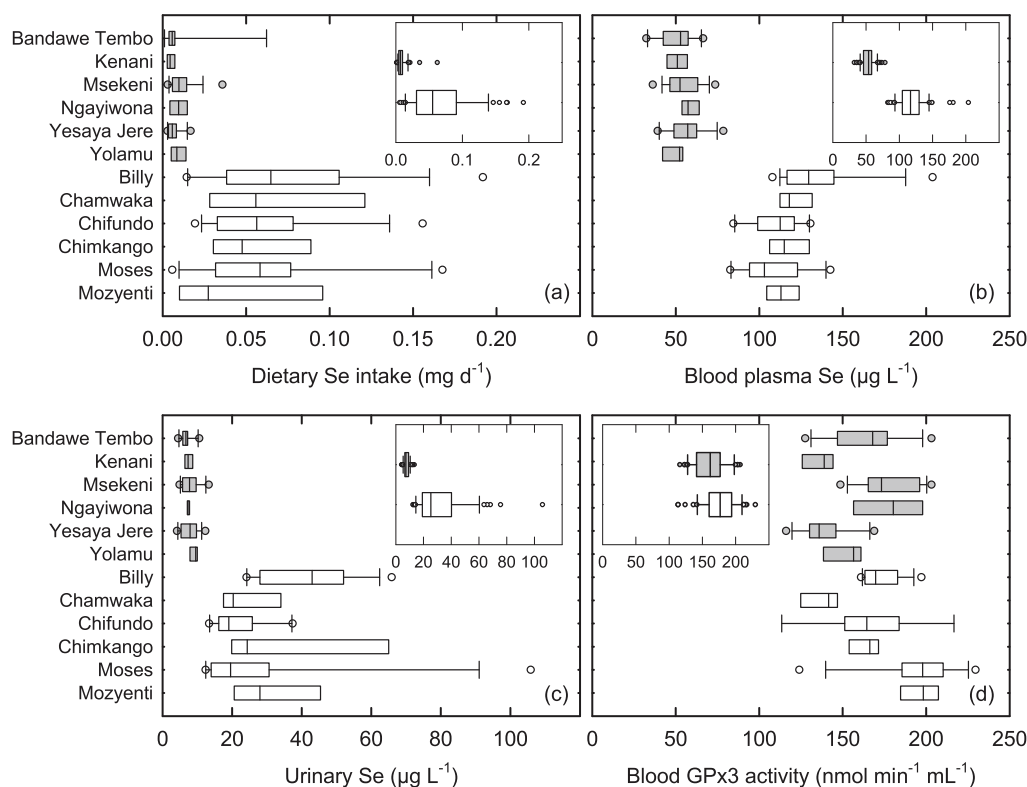

Figure 2 | Dietary Se intake and biomarkers of Se status in adult females in Malawi. (a) Dietary Se intake, (b) blood plasma Se concentration, (c) urine Se concentration and (d) blood plasma glutathione peroxidase 3 (GPx3) activity. Grey bars represent six villages from Zombwe Extension Planning Area (EPA) with low pH soils; open bars represent six villages from Mikalango EPA with high pH soils. Insets show these villages grouped by EPA. Boxes are 25 and 75%-iles with median lines; whiskers are 5 and 95%-iles; circles are outliers.

25.3 µg L^{-1}, SD = 18.9, range 12.4–106, n = 56; Figure 2c). Variation between EPAs was much greater than that between villages within an EPA for dietary Se intake and plasma and urine Se concentrations. EPA + village terms explained 43 + 2, 79 + 4 and 46 + 9% of the total variation in dietary Se intake, plasma and urine Se concentrations, respectively. Differences between EPAs were highly significant for dietary Se intake and plasma and urine Se concentrations ($P \ll 0.001$). Variation between villages within specific EPAs also occurred for plasma ($P = 0.004$) and urine ($P = 0.021$) Se concentration, but not for dietary Se intake ($P = 0.943$). GPx3 activity also differed between EPAs ($P = 0.002$). However, only 8% of the total variation in GPx3 activity was explained by EPA, whereas 46% of the variation occurred between villages within an EPA (Figure 2d). Thus, plasma GPx3 activity in Zombwe EPA (median 162 nmol min^{-1} mL^{-1}, SD 24.1, range 116–207, n = 60) was lower than in Mikalango EPA (median 177 nmol min^{-1} mL^{-1}, SD 25.6, range 113–230, n = 53). Residual variation was due to individual-level variation within villages. Given that approximately half of the variation in dietary Se intake, urine Se concentration and plasma GPx3 activity occurred among individual volunteers, it seems that plasma Se concentration is the most robust biomarker of Se status in these settings. Urine Se concentration will be more sensitive to short-term variation in Se intake than blood plasma Se concentration, whereas GPx3 may also vary due to oxidative stress conditions[1]. However, urine Se concentration measurements (Figure 3) could provide an effective non-invasive method for identifying Se deficiency risk in populations, once cut-off points to assess the severity of Se deficiency have been established, as is used routinely for iodine[17,18].

We estimated *per capita* supply of dietary Se available for human consumption in 46 African countries using an approach described previously for magnesium[19], by integrating Food and Agriculture Organization (FAO) Food Balance Sheets (FBSs) for 2007, and a food Se composition table (Supplementary Table 3). Risk of inadequate Se intake was estimated using an EAR cut-point method[19,20]. Mean Se supply in 2007 for Africa was 71 µg person^{-1} d^{-1}, ranging from 27 µg d^{-1} in Djibouti to 264 µg d^{-1} in Ghana (Figure 4; Supplementary Table 4). Selenium supply tended to be lower in Southern and Mid Africa. The risk of inadequate Se intake in Africa, is therefore 22% overall representing 230 M people (Figure 4). It must be stressed that the use of FBSs and food Se composition tables to estimate Se supply (i.e. and thereby infer intake and deficiency risks) must be conducted with great caution. For example, Se concentrations of fresh coconut in this analysis is from a single West Africa food composition table[21] which reports a very high value of 810 µg 100 g^{-1} FW. This value is much higher than that reported for coconut by the US Department of Agriculture[22] of 10.1 µg 100 g^{-1} FW. So in Ghana, for example, which has a FBS value for 2007 of 7.3 kg coconut person^{-1} y^{-1}, the estimated supply of Se from coconut could be either 162 or 2 µg Se person^{-1} d^{-1} depending on which source is used. Here, we elected to use the higher value, as it was more geographically relevant, but we note that our decision may underestimate the risk of Se deficiency. In a recent study of Se intakes of children (ages 12–15) in 3 residential care orphanages in Ghana from duplicate diets[23], the mean dietary intakes of Se were 58, 82, 92 µg Se person^{-1} d^{-1}. These are less than the supply figure of 264 µg Se person^{-1} d^{-1} derived from FBSs for Ghana, but interestingly, are greater than reported Se intakes in Malawi in our study. So whilst Se supply is likely be lower than suggested in Figure 4 for countries where coconut consumption is high, an approach based on FBSs and food composition tables can still identify populations at higher risks of deficiency and also guide efforts to improve food composition tables.

Discussion

These new biomarker measurements and analyses of *per capita* Se supply indicate that policies to alleviate Se deficiency should be considered alongside other micronutrient intervention programmes in Southern Africa. However, despite the known roles of Se in many communicable and non-communicable diseases[1], it is not yet possible to assess the health/economic impacts of Se deficiency at population scale due to a lack of suitable framework and input data. In contrast, frameworks have been established for other micronutrient deficiencies (e.g. iron, iodine, zinc and vitamin A) which co-exist in developing countries. These affect growth, immune function and cognitive development, cause disease and premature death, and constrain economic growth. For Malawi, we estimate that zinc deficiency (ZnD) leads to an annual loss of 6,500 "disability-adjusted life years" (DALYs), i.e. person-years lost to disability and shortened life, per million population (>99,900 DALYs in total), and >3,800 instances of child mortality per year using established methodologies[24,25]. This is higher than the 2,750, 2,020 and 1,660 DALYs estimated to be lost due to ZnD per million population in India, Honduras and Nicaragua, respectively[24]. These data suggest that ZnD alone imposes an economic burden on Malawi of ~$100 m yr^{-1}.

It is of course feasible to diversify diets in order to increase Se intake, for example, by increased consumption of Se-rich foodstuffs (e.g. including animal-based foodstuffs). However, such approaches are challenging when baseline Se intakes are low and where there is a lack of purchasing power for foods rich in Se, as in many low-income countries. Short- and medium-term policies to alleviate iron, iodine, zinc, and vitamin A deficiencies have been adopted in Malawi and elsewhere, including dietary iron supplements and salt iodisation[26,27], although such programmes are not used for Se. Longer-term crop-based approaches (biofortification) are also underway to increase iron, zinc and vitamin A intakes in the region[28,29], although the breeding potential to biofortify crops with Se *via* conventional breeding is low[14,29]. A public health precedent to alleviate dietary Se inadequacy was set in Finland in 1984 following the introduction of Se-enriched fertilisers (agronomic biofortification), which has continued to date[14,16]. Selenium fertilisation has successfully increased the Se concentrations of Finnish foods and dietary Se intakes and
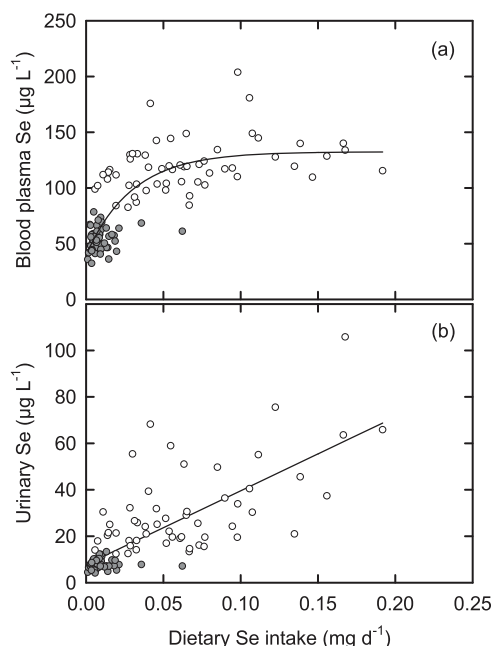

Figure 3 | Selenium biomarkers as functions of dietary Se intake among adult females in Malawi. (a) Blood plasma Se concentration and (b) urine Se concentration. Grey circles are adults from villages with low pH soils; open circles are adults from villages with high pH soils. Regression lines are (a) $y = 39.9 + (92.6 \ast (1 - (3.2 \ast 10^{-15})^x))$, $r^2 = 0.648$, $P < 0.0001$; (b) $y = 7.8 + (318 \ast x)$, $r^2 = 0.5707$, $P < 0.0001$.
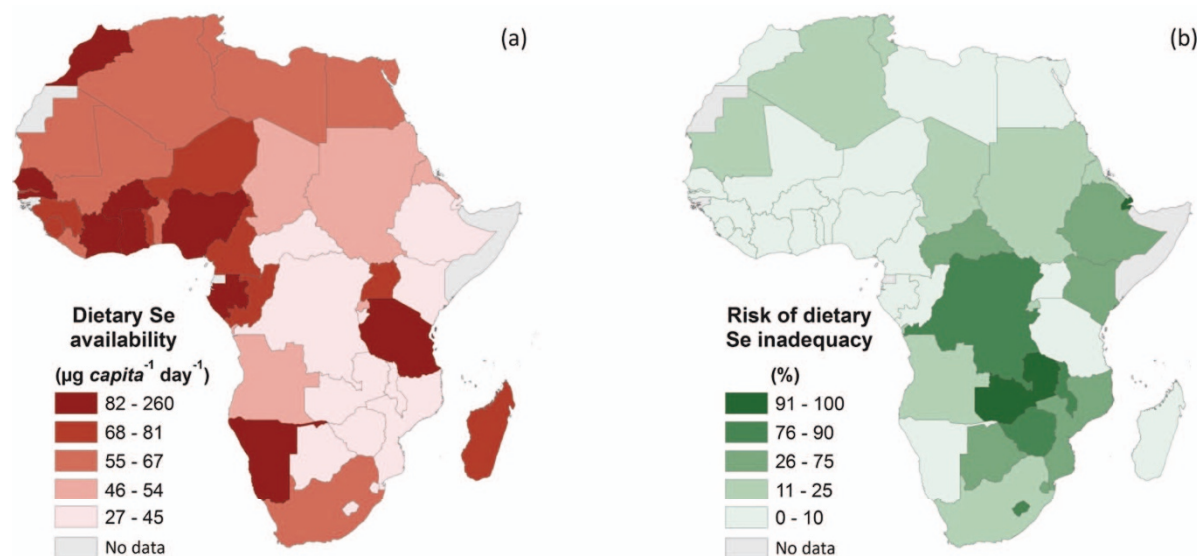
Figure 4 | Mapping dietary Se availability in Africa. (a) Mean dietary Se availability and (b) estimated risk of inadequate Se intake based on US Estimated Average Requirement (EAR) of 45 and 23 µg Se person⁻¹ d⁻¹ for those aged >10 and <10 yr, respectively, and 49 and 59 µg Se person⁻¹ d⁻¹ for pregnant and lactating women, respectively[39]. Data are calculated from national-level food balance sheets for 46 African countries in 2007 and regional food composition tables using methods described previously[19].

status[16]. All types of cereal grains, and all grain fractions are easily enriched with Se when Se(VI) forms are added to fertilisers[14–16,30,31]. Fertiliser-based strategies also avoid the significant lead-times required for crop breeding programmes and distribution of new varieties. Agronomic biofortification is therefore feasible in a Southern African context where inorganic fertilisers are used. Malawi has operated a Farm Input Subsidy Programme (FISP) since 2005/06 whereby fertiliser vouchers are distributed to farmers at a village scale through the national extension service system[32]. The FISP is therefore a potential public health intervention route if fertilisers are enriched with Se during production[15]. However, there are knowledge gaps which require further research and capacity building in the nutrition, agriculture and economics sectors to ascertain the cost/benefit of this approach, including an assessment of the ongoing costs of Se supply and monitoring of health outcomes. Another agronomic approach may be to use lime applications to increase soil-to-grain Se transfers but this approach has not yet been tested to our knowledge. However, a liming strategy could reduce the plant-availability of other essential micronutrients such as iron and zinc.

Methods

Ethical approval. Ethical approval was obtained from the National Health Sciences Research Committee, Malawi (NHSRC reference #784), prior to commencing the study.

Site selection. Two Extension Planning Areas (EPAs) in Malawi were studied: Zombwe EPA in Mzuzu Agricultural Development Division (ADD) in the north and Mikalango EPA in Shire Valley ADD in the south. Zombwe EPA is characterised by low pH soils (median 5.2, n = 11), particularly Haplic Lixisols (70% of EPA area), which have a low soil-to-maize transfer of Se and median grain Se concentration of 22 µg Se kg⁻¹ fresh weight (FW)[10]. Mikalango EPA is characterised by areas of calcareous Eutric Vertisols (median pH = 7.8, n = 16; 10% of EPA area) with a high soil-to-grain transfer of Se and a median grain Se concentration of 342 µg Se kg⁻¹ FW[10]. Six villages were selected from each EPA for participant recruitment. In Zombwe EPA, villages were located in Zombwe I Section: Bandawe Tembo, Kenani, Msekeni, Ngayiwona, Yesaya Jere, Yolamu. In Mikalango EPA, villages were located in Nyamichimba Section: Billy, Chamwaka, Chifundo, Chimkango, Moses, Mozyenti.

Participant selection. In total, a sample of 120 apparently healthy adult females aged 18–50 years were recruited to participate in the study, from six villages per EPA, most of whom were subsistence farmers. Only one volunteer *per* family was recruited to avoid multiple sampling of participants with similar dietary patterns. Exclusion criteria for participants included known pregnancy, smokers, those diagnosed with chronic physical or mental disease or long term illness requiring treatment, acute or chronic infection, those taking regular prescribed medicine including oral contraceptive pills, those taking regular dietary supplements and those who had lived in the study area for less than six months. Sensitisation visits were held in September 2010 by the scientific team and Extension Planning Officers from the Malawi Ministry of Agriculture and Food Security, and in March 2011 by the Ministry of Health and Traditional Village Authorities. Informed consent of all volunteers was obtained prior to the collection of the duplicate diet composites and tissue samples by a trained member of the Lilongwe University of Agriculture and Natural Resources study team. No payment was made for participation or the use of tissue samples. Participants were reimbursed at cost-price for the food and beverages in the duplicate diet composites taken for analyses.

Socio-economic status of participants. Background data were collected using questionnaires administered by trained interviewers in the homes. A socio-economic status (SES) index was developed for each participant based on categories used earlier in the Nutrition Collaborative Research Support Program (NCRSP[33]), and in a later study of pregnant women in rural Malawi[34]. Information collected included house quality, sanitation, water source, household size, occupation and schooling. The index has a maximum theoretical numerical value of 14 (high SES). The numerical value is based on derived numerical equivalents calculated for each of the string variables in STATA (StataCorp LP, College Station, Texas, USA). The SES index and background data on participants are given in Supplementary Table 1.

Duplicate diet composites. Duplicate diet composites were collected over a full day (24 h) for each participant. Food records and diet composites samples were collected between 13 and 16 March 2011 in Mikalango EPA, and between 20 and 23 March 2011 in Zombwe EPA. Research assistants (RAs) were recruited to reside in the household of each participant to weigh and sample all food and beverage items (including drinking water and snack items consumed away from the household) during the sampling date using a 750 mL polyethylene jug and a kitchen scale accurate to ~1 g. For each food and beverage item consumed, an exact duplicate sample was weighed and collected in a double-lined trace-element-free sealable polyethylene bag. Composites were placed in a cooler box with ice packs, transferred to a central laboratory within 24 h of collection, and blended to a homogeneous slurry using a domestic blender. Approximately 50 mL of homogenate was subsampled using a trace-element-free pipette (7017, Corning, Costar, Amsterdam, The Netherlands) and this was divided into two trace-element-free universal tubes (Bibby Sterilin, Stone, Staffordshire, UK). Homogenate subsamples were frozen at −20°C. Weighed food samples were recorded (data not shown) to provide details on the composite diet samples containing animal products, to comply with import licence requirements.

One set of frozen diet composite subsamples (n = 115) was transported to the UK on dry ice for Se analyses, and the remaining set was retained in Malawi. Subsamples were freeze-dried in their original universal tubes with muslin caps. Subsample volumes were recalculated to correspond to the loss of mass on freeze-drying plus the residual mass of solid material, assuming that the dry food had a material density of 1.0 and was wholly in suspension. The mean volume of each subsample was 24.7 mL: typically 20–23 mL and 1–4 g dry material. Freeze-dried diet composites were manually ground to homogenise the samples and crush seeds *etc*. Approximately 0.3 g of each sample was microwave-digested in 3.0 mL of 70% Trace Analysis Grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL milli-Q water (18.2 MΩ cm; Fisher

Scientific UK Ltd, Loughborough, UK). Elemental analysis was by inductively coupled plasma-mass spectrometry (ICP-MS; X-Series[II], Thermo Fisher Scientific Inc., Waltham, MA, USA). Acid digests were analysed by ICP-MS (i) using Collision Cell Technology with Kinetic Energy Discrimination (CCT-KED) mode for Na, Mg, K, Ca, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Mo, Cd, Cs, Ba, Pb and U (using Sc, Ge, Rh and Ir internal standards), and (ii) in H_2-reaction cell mode for ^{78}Se alone (using Ge and Rh internal standards) with 2% methanol added to samples to max-imise sensitivity for Se determination. Three certified reference materials (CRMs) from NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) were included: NIST 1573a (tomato leaves, certified value 0.054 mg Se kg^{-1} dry weight, DW, 123% recovery), NIST 1577c (bovine liver, 2.031 mg Se kg^{-1} DW, 96% recovery) and NIST 1567a (wheat flour, 1.1 mg Se kg^{-1} DW, 100% recovery). In addition, four inter-lab flour samples supplied by Dave Hart, Institute of Food Research, UK yielded an average recovery of 102%. Operational blanks (n = 10) were run to determine practical Limit of Detection (LOD; 3*SD) and Limit of Quantification (LOQ, 10*SD) values for Se of 0.0018 and 0.0059 mg kg^{-1} DW, respectively, in composite diet samples. These and subsequent data collected from individuals were analysed by ANOVA with 'EPA/Village' as a treatment factor and no blocking terms using GenStat (15th Edition, VSN International, Hemel Hempstead, UK).

Blood and urine samples. Whole blood was collected from consenting volunteers by trained staff from the Community Health Sciences Unit, Malawi. Samples were collected on Saturday 12 March 2011 in Mikalango EPA and Saturday 19 March 2011 in Zombwe EPA. Each volunteer was seated, their skin cleaned with alcohol at the site of the antecubital vein, and their arm restricted with a tourniquet for <1 minute. Blood was drawn into two 7 mL trace-element-free blue-top evacuated tubes (NH119 I.U. ref 367735, Becton Dickinson UK Ltd, Plymouth, UK). Disposable polyethylene powder-free gloves were used for all blood handling steps. The tourniquet was loosened during blood collection and before the needle was removed from the arm. Immediately after the blood was collected the tube was inverted 10 times to mix the contents before placing the samples in a cooler box with ice packs for transport to the nearest laboratory for processing (Zombwe EPA, Mzuzu Central Hospital; Mikalango EPA, Zomba District Hospital). For one of the 7 mL samples, plasma was separated by centrifugation at 1500 g for 10 min and transferred into trace-element-free tubes (n = 4 aliquots for each sample) and frozen at −80°C. Plasma samples were transported to the UK on dry ice for Se analysis using ICP-MS, and to determine Se-dependent glutathione peroxidase activity, as described below. The other 7 mL samples were used for whole-blood analyses (data not shown). Casual urine (i.e. samples unrelated to eating/sleeping patterns) samples were collected on the same day as the blood sampling. These were collected in sterile pre-labelled tubes (30 mL sterile universal tubes, Bibby Sterilin, UK) and placed in a cooler with ice before transfer to the nearest laboratory for processing. Two 8 mL subsamples were frozen at −20°C and transported to the UK using dry ice for ICP-MS analysis.

Selenium concentration in blood plasma was determined by ICP-MS in H_2-cell mode. Samples and standards (SPEX CertiPrep Inc., Metuchen, NJ, USA) were diluted 1-in-20 in 1% TAG HNO_3 containing 0.1% of a non-ionic surfactant ('Triton X-100' + 'antifoam-B', Sigma-Aldrich Company Ltd., Dorset, UK) and 2% methanol. Internal standards containing Ir (5 µg L^{-1}), Rh (10 µg L^{-1}), Ga (25 µg L^{-1}) and Sc (50 µg L^{-1}) were included. Calibrations were in the range 0–50 µg Se L^{-1}. Quality control CRMs included Seronorm™-1 (107 µg Se L^{-1}, 109% recovery), Seronorm™-2 (163 µg Se L^{-1}, 102% recovery) (Sero AS, Billingstad, Norway) and UTAK-66816 Lot 7528 (certified value 115 µg Se L^{-1}, 106% recovery) (UTAK Labs Inc., Valencia, CA, USA) CRMs. Sample blanks (n = 10) were run to determine LOD and LOQ values of 0.055 and 0.182 µg L^{-1}, respectively, for blood plasma. Urine Se was determined by ICP-MS in H_2-cell mode for samples diluted 1-in-10 with 2% HNO_3 and calibrated using Seronorm™-1 (certified value 58.6 µg Se L^{-1}) as a matrix-matched calibration standard. Urine assays were corrected for creatinine by dividing urine Se concentration (µg L^{-1}) by creatinine concentration (g L^{-1}). Sample blanks (2% HNO_3; n = 10) were run to determine LOD and LOQ values of 0.095 and 0.316 µg L^{-1}, respectively, for urine. Creatinine was analysed using a commercial kit on a RX IMOLA Chemistry Analyzer (Randox Laboratories, Belfast, UK) according to the manufacturer's instructions.

Quantification of plasma GPx3 activity. Plasma glutathione peroxidase (GPx3) activity was quantified using a high throughput 96 well spectrophotometric enzyme assay[35]. All plasma samples were analysed in triplicate. The enzyme assay mixture contained 100 mM Tris-HCl pH 7.4, 3 mM glutathione, 0.25 mM NADPH, 1U glutathione reductase and 0.1% triton X100 with hydrogen peroxide as the substrate. The rate of decrease in absorbance at 340 nm was measured at 10 s intervals for 15 min at 37°C using a plate reader with kinetic capability (FLUOstar Omega plate reader, BMG Labtech, Germany). The GPx3 activities were calculated from the initial rates of reaction[36], with one unit (U) defined as 1 nmol NADPH oxidised per minute.

Estimating dietary selenium supply from food balance sheets. *Per capita* supply of Se was estimated for all African countries as the product of food supply data available for human consumption at a retail level and food Se concentration using previously described methodology[19]. Food supply was based on Food Balance Sheet (FBS) data sourced from 46 Food and Agriculture Organization (FAO) member countries in Africa[19]. Food Se concentration data were sourced from published food composition tables[21,22,37,38] and three food Se concentration databases were generated: East (E) Africa, Southern (S) Africa (also used for North (N) Africa) and West (W) Africa (also

used for Mid (M) Africa). All Se concentration data are expressed as µg Se 100 g^{-1} fresh weight (FW) edible portion. Food Se concentration data, literature sources and best-fit FBS categories are shown in Supplementary Table 3. *Per capita* Se supply data were used to infer risk of dietary Se inadequacy using the EAR cut-point method[19,20]. The US Estimated Average Requirement (EAR) of 45 and 23 µg Se person^{-1} d^{-1} was used for those aged >10 and <10 yr, respectively, and 49 and 59 µg Se person^{-1} d^{-1} for pregnant and lactating women, respectively[39], with a conservative inter-individual coefficient of variation (CV) of Se intake of 25%. As discussed previously[19], the EAR cut-point method is highly sensitive to CV and also to food concentration data reported within the food composition tables. For example, Stadlymayr et al.[21] reported 810 µg Se 100 g^{-1} FW edible portion in raw coconuts, resulting in high intake estimates for some West African countries (see Results).

Estimating disability-adjusted life years (DALYs) lost due to micronutrient deficiency in Malawi. It is not straightforward to determine the existing burden of disease due to Se deficiency in any country at present because an accepted framework for Se is lacking. However, it is possible to illustrate the impact of mineral deficiencies in Malawi using zinc deficiency (ZnD) as an example. Thus, the number of DALYs lost due to ZnD in Malawi was estimated using previously developed methods[24,40–42]. Population size was taken from World Bank sources[43] and demographic data were obtained from the 2008 Population and Housing Census Results of the National Statistical Office of Malawi[44]; the crude birth rate was obtained from the 2008 Population and Housing Census Results Main Report[45]. Infant mortality rate was obtained from the 2008 Population and Housing Census Results Mortality Report[46], while the under-five mortality rate was obtained from the 2012 Malawi Population Data Sheet[47]. Average remaining life expectancies were obtained from the World Health Organization life tables[48], while stunting rates (height/age - 2 SD) were taken from the WHO Dataset on Child Growth and Malnutrition for Malawi[49]. The estimated number of episodes of diarrhoea and pneumonia in infants and children were taken from Stein et al.[40] and confirmed by local expert opinion (AA Kalimbira, Lilongwe University of Agriculture and Natural Resources). *Per capita* income figures were taken from the World Development Indicators of the World Bank[43]; DALYs were valued in accordance with established methods[50] and using a discount rate of 3%[40,41]. To convert DALYs lost to monetary terms, a standard figure for developing countries of US $1,000 was used which corresponds approximately to triple the annual *per capita* income in Malawi.

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