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Article

Unveiling the Origin of Copper Accumulation in Plasma with Aging

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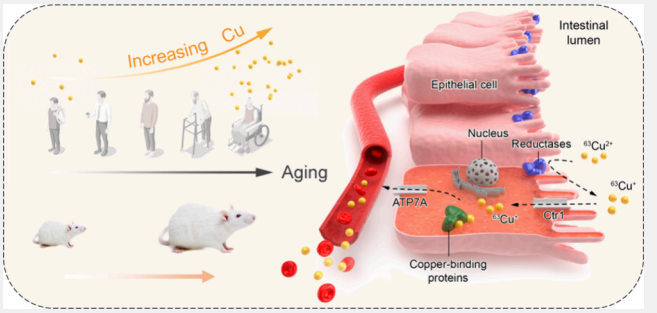
ABSTRACT: Aging is intricately linked to various diseases including cancers, neurodegenerative disorders, and metabolic irregularities. Copper (Cu) overexposure has been found to be linked to many diseases during aging, particularly neurodegenerative diseases. Meanwhile, as an essential element, Cu has been implicated in key processes associated with aging, raising questions about its role in age-related health issues. This study delves into the mechanisms behind the copper imbalance during aging. By analyzing blood copper concentrations of healthy individuals (including data from healthy subjects ($26 \leq$ age ≤ 90, $n = 62$) and publicly available data from the National Health and Nutrition Examination Survey ($18 \leq$ age < 80, $n = 1624$)) and employing C57BL/6N male mice models ($n = 22$), we reveal a consistent age-related increase in copper levels, particularly in plasma. Utilizing stable copper isotopic analysis, copper-associated protein analysis, and metabolomic analysis, we trace the sources of Cu imbalance associated with aging. Our findings reveal that aged mice had a higher copper concentrations and an enrichment of light copper isotope (^{63}Cu) in plasma compared to controls. Additionally, copper reductases and copper transporters are upregulated in the intestine tract, associated with the AMPK and mTOR signaling pathways. We suggest that aged mice have an abnormally high copper intake requirement, probably due to deregulated nutrient sensing, leading to increased expression levels of copper reductases and copper transporters for extra copper absorption in the intestines. This research provides a copper-centric perspective on the connection between deregulated nutrient sensing and aging, thus shedding light on the aspect of aging and copper overexposure.



KEYWORDS: *copper, aging, nutrient sensing, stable isotopic analysis, metabolism*

INTRODUCTION

Aging is an inevitable life process of living organisms, encompassing 12 hallmarks (i.e., genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, disabled macroautophagy, chronic inflammation, and dysbiosis).[1,2] Aging is associated with a high incidence of many diseases, including cancer, neurodegenerative diseases, arteriosclerosis, diabetes, etc.[3−7] The aging process is closely related to metabolic disorders, particularly those involving essential trace metals such as copper (Cu).[8−10] All the copper in the body is taken from the external environment, it plays a crucial role in key biological processes associated with aging, and it has been widely suspected that the copper imbalance would contribute to the aging process.[11,12] Previous studies have reported that imbalanced copper homeostasis during aging;[9,13,14] however, identifying the causes of copper imbalance during aging has been challenging due to the lack of research tools.

Compared with quantitative analysis, isotopic analysis of an element provides a new research perspective to explore the Cu variation. Cu has two naturally occurring stable isotopes (i.e., ^{63}Cu and ^{65}Cu) with relatively constant abundances, while some physical, chemical, or biological processes can cause stable isotopic fractionation as they show a (slight) preference for one of the two isotopes, thus giving rise to (small) differences in the Cu isotopic composition.[15] Thus, stable Cu isotopic composition has the potential to be used as a powerful tracer due to the unidirectional stable isotopic fractionation that occurs during a specific process.[16] Besides, Cu isotopic composition may be more sensitive than Cu concentration in responding to the subtle copper variations in the body.

Oxidant damage is a critical aspect of aging, as toxic free radicals generated by excess copper absorption slowly contribute to mitochondrial damage.[8] This extra copper absorption also increases the risks of aging-related diseases (e.g., neurodegenerative diseases, arteriosclerosis, diabetes, and

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cancers). For example, increased copper intake has been linked to increased amyloid plaques and reduced cognitive performance in a rabbit model.[17] Moreover, free copper levels have been proposed as a predictor of the annual decline in mini-mental state examination (MMSE) values in Alzheimer's disease patients.[18] Sauzeat et al. found that the Cu concentration in cerebrospinal fluid (CSF) of amyotrophic lateral sclerosis (ALS) or Alzheimer's disease (AD) patients did not change significantly compared with the control population, whereas the CSF of ALS patients showed a tendency to enrich heavy Cu isotopes.[19] Hobin et al. found that the plasma Cu isotope ratio in mice was affected by AD.[14] Concerning cancers, an enrichment of light Cu isotope in blood or tissue was observed with bladder cancer, hepatocellular carcinoma, breast cancer, colorectal cancer, ovarian cancer, hematological malignancies, and oral squamous cell carcinoma.[20−27] Therefore, there may be a similar mechanism that governs the imbalances of copper metabolism underlying the aging and aging-related diseases, which needs further exploration. Hence, clarifying the mechanisms of copper variation during aging is crucial for understanding the relationship between aging and these diseases. However, the sources of copper in plasma are quite complex, given its involvement in various important physiological processes across different organs. Until now, the mechanisms of Cu imbalance caused by aging remain unclear.

Here we initially analyzed the copper concentration in the populations and observed an age-related increase in the copper concentration in plasma. To trace the sources and explore the internal molecular mechanisms of Cu imbalance during aging, we carried out experiments using mice. We collected blood samples and typical organ samples from two groups of mice (2 months old as the young group and 14 months old as the aged group). Natural stable isotopic variation of elements provides a powerful tool to trace their sources and processes, providing a new dimension of information independent of element concentration.[20,28] The unique source tracing ability of stable isotopic analysis, well established and widely used in geochemistry, archeology, anthropology, etc.,[15,16,29,30] was employed in this study to trace the origins of copper imbalance during aging. Additionally, we applied Cu-associated protein analysis coupled with metabolomic analysis to gain an in-depth understanding of the underlying mechanisms of imbalanced copper homeostasis in the aging process. We considered kidney and liver as target organs for analysis due to their vital roles in body metabolism, with the liver serving as the main organ regulating copper status and controlling copper release into the blood. We also focused on the intestines due to their involvement in copper absorption by the body.[31]

MATERIALS AND METHODS

Chemicals and Reagents

Nitric acid and hydrochloric acid were purchased from Merck (Darmstadt, Germany) and further distilled twice via a distilling apparatus. Hydrogen peroxide (30%) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Ultrapure water (18.2 MΩ cm) was generated from a Milli-Q Gradient system (Millipore, Bedford). Anion exchange resin (AG-MP-1M) was purchased from Bio-Rad Laboratories, Inc. (Hercules, California, USA). For copper isotopic analysis, the copper isotope standard ERM-AE633 was used that was purchased from the Institute for Reference Materials and Measurements (Geel, Belgium), and CAGS-Cu was provided by the Chinese Academy of Geological Sciences (Beijing, China). For copper quantification, the element calibration standard solution for ICP-MS was purchased from Agilent (Santa Clara, CA). For metabolic analysis, methanol and acetonitrile were purchased from Merck (Darmstadt, Germany); formic acid was purchased from Thermo Fisher (Waltham, MA, USA).

Study Participants

All blood samples of healthy subjects were collected around Tianjin, China (n = 62). The healthy subjects presented no clinical evidence of diseases. Samples were collected by Ultraclean disposable devices. The blood samples were immediately separated into red blood cells (RBC) and plasma after collection by centrifugation and stored separately at −80 °C in the dark before analysis. All participants provided informed consent, and the study protocol was approved by the Ethics Committee of Tianjin Medical University (reference number: TMUhMEC2016022) and compliant with all relevant ethical regulations for studies involving human subjects.

Mice

All animal experiments were conducted with C57BL/6N male mice (2 months old as young group (n = 11) and 14 months old as aged group (n = 11)) purchased from Beijing Vital River Laboratory Animal, Inc. (Beijing, China, https://www.vitalriver.com). Ten of them were used for copper concentration analysis, copper isotope analysis, and copper-associated protein analysis, while the remaining 12 mice were used for metabolomics analysis. The mice were euthanized with carbon dioxide, and then their blood samples and organ samples were immediately collected. The blood samples were immediately separated into plasma and red blood cells (RBCs) by centrifugation after collection. The animal experiment ethics has been approved by the Animal Ethics and Welfare Committee of Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

Western Blot (WB) Assay

Protein expression levels of Ctr1, CcO, MT, SOD1, ATP7A, STEAP2, STEAP3 and STEAP4 were assessed by the WB assay. The total proteins in samples were extracted by using a Protein Extraction Kit (GenePool/GPP1815) and were denatured at 100 °C for 10 min. A certain amount of proteins was loaded on the sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, 0.22 μm). The membrane was then blocked in Milk Blocking Buffer (GenePool/GPP1819)/BSA Blocking Buffer (GenePool/GPP1818) for 1 h and then incubated with primary antibody overnight at 4 °C. Subsequently, the membrane was incubated with HRP-conjugated secondary antibody (Goat Anti-Mouse IgG, HRP or Goat Anti-Rabbit IgG, HRP) at room temperature for 50 min. GAPDH antibody (Abcam ab181602, 1:3000) was used as a loading control. Finally, the membrane was fixed on Koda film using ECL (GenePool/GPP1824) solution. The gray values of protein bands were identified by Quantity One V.4.6.2 software. The following antibodies were used in Western blot: Ctr1 (Abcam ab129067, 1:1000); CcO (Abcam ab150422, 1:1000); MT (Abcam ab235036, 1:500); SOD1 (Abcam ab183881, 1:1000); ATP7A (Thermofisher PA5−36558, 1:500); STEAP2 (Thermofisher PA5−25495, 1:500); STEAP3 (Abcam ab180770, 1:500); STEAP4 (Thermofisher PA5−106509, 1:500); Goat Anti-Mouse IgG, HRP (Abcam ab6789, 1:5000); Goat Anti-Rabbit IgG, HRP (Abcam ab6721, 1:5000). GenePool (Beijing, China, http://www.gpbiotech.net).

Sample Preparation for Copper Isotopic Analysis

The sample preparation procedures were from a previous study. Briefly, sample digestion and isolation of target element were performed as follows: 5 mL of 14 mol HNO_3 and 1 mL of 30% H_2O_2 were used for sample digestion in a microwave reaction system (Anton Paar). Teflon beaker was used for heating sample solution to dryness, and 1 mL of 8 mol HCl/0.001% H_2O_2 was used to redissolve sample. For samples purification, an anion exchange chromatographic method as reported previously was used.[32] In addition, we also examined the biological matrix effect and preparation process

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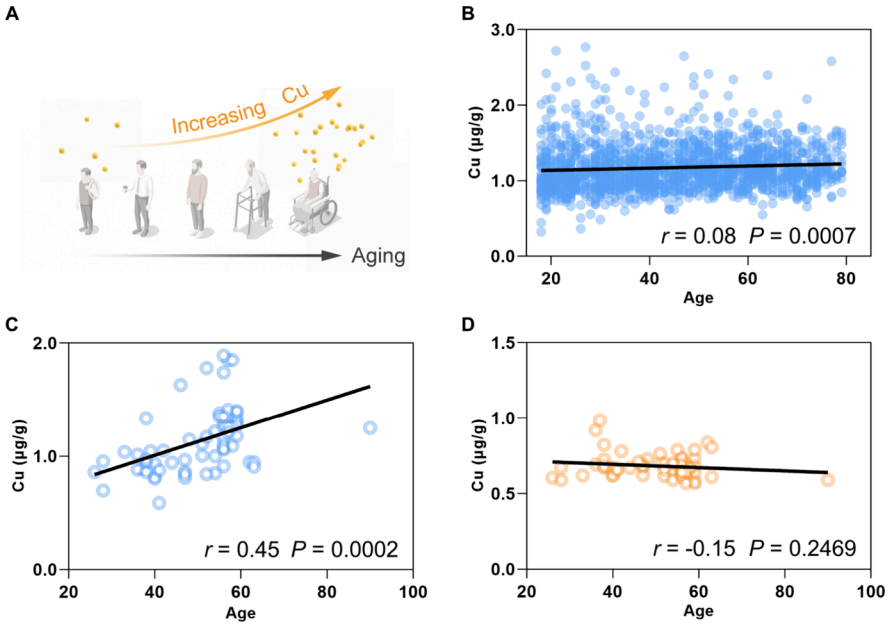

Figure 1. Variations in Cu concentration in human blood associated with aging. (A) Schematic showing the increasing trend of copper concentration with aging. Note that the yellow points only represent the variation trend of Cu concentration but no actually data points. (B) Copper concentration data from National Health and Nutrition Examination Survey (NHANES) participants (2011−2012), n = 1624. (C) Variation of Cu concentration in plasma associated with aging in a cohort in China, n = 62. (D) Variation of Cu concentration in RBC associated with aging in a cohort in China, n = 62.

interference. The results showed that the biological matrix has almost no effect on Cu isotopic analysis. The recovery of Cu during the whole sample preparation process was >95%.

Measurement of Cu Concentration and Isotopic Ratios

We used an Agilent 8800 inductively coupled plasma mass spectrometer (Santa Clara, CA, USA) for Cu concentration measurement and a Nu Plasma II MC-ICP-MS (Wrexham, UK) equipped with 16 Faraday cups for Cu isotopic composition measurement. For the Cu isotopic composition measurement, we injected samples in wet mode with a peristaltic pump. The optimized instrumental parameters and Faraday cup configuration are given in Table S1. The Cu isotopic composition (δ^{65}Cu value) was expressed relative to that of the standard material (ERM-AE633):

$$\delta^{65}\mathrm{Cu} = \left(\frac{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})_{\mathrm{sample}}}{(^{65}\mathrm{Cu}/^{65}\mathrm{Cu})_{\mathrm{sample}}} - 1 \right) \times 1000‰ \quad (1)$$

We used the standard-sample-standard bracketing (SSB) method to correct the mass bias.[33] Besides, we used two standard materials, ERM-AE633 and CAGS-Cu, to prove the method is highly accurate and precise (δ^{65}Cu value of CAGS-Cu relative to ERM-AE633 was 0.57 ± 0.1‰ (n = 3)). This value was very close to that reported in the previous literature.[34,35]

Metabolic Analysis

Metabolite Extraction. The sample was homogenized with a grinder after thawing. A solution (methanol: water = 7:3, V/V, 400 μL) containing internal standard was added in to 20 mg grinded sample, and shaken at 1500 rpm for 5 min. Then, the sample was centrifuged at 12000 rpm for 10 min (4 °C) after placing it on ice for 15 min. Afterward, we collected 300 μL of supernatant and placed in −20 °C for 30 min. The sample was centrifuged again at 12000 rpm for 3 min (4 °C). Ultimately, 200 μL supernatant were transferred for LC-MS analysis.

HPLC Conditions. All samples were acquired by the LC-MS system according to the sample sequence. The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm × 100 mm); flow rate, 0.4 mL/min; injection volume, 2 μL; column temperature, 40 °C. Solvent system, mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The column was eluted with 5% mobile phase B at 0 min followed by a linear gradient to 90% mobile phase B (0.1% formic acid in acetonitrile) over 11 min, held for 1 min, and then come back to 5% mobile phase B within 0.1 min, held for 1.9 min.

Mass Spectrometry. Analyst TF 1.7.1 Software (Sciex, Concord, ON, Canada) was used for data acquisition in the information-dependent acquisition (IDA) mode. We set the source parameters as follows: ion source gas 1, 50 psi; ion source gas 2, 60 psi; curtain gas, 35 psi; temperature, 550 °C; declustering potential, 60 V in positive mode or −60 V in negative mode; and ion spray voltage floating, 5500 V in positive mode or −4500 V in negative mode. We set the mass range as 50−1000 Da for the TOF MS scan parameters and 25−1000 Da for the product ion scan parameters. Collision energy, 30 V in positive mode or −30 V in negative mode; resolution, 35000 units; charge state, 1 to 1; intensity, 100 cps; mass tolerance, 50 mDa.

Statistical Analysis

We applied GraphPad Prism 8 (ver. 8.3.0.538) and R studio (ver.3.5.1) for statistical analysis. We employed the Mann−Whitney test for P value calculation when the data set did not conform to normal distribution and the unpaired Student two-tailed t-test for P value calculation when the data set conformed to normal distribution and passed the homogeneity test of variance. If the data set did not pass the homogeneity test of variance, we employed Welch's t-test for P value calculation.[36−38]

Data Availability

The published article includes all of the data generated and analyzed during this study. Data will be made available freely from the corresponding authors upon request. The National Health and Nutrition Examination Survey data used in this study is publicly available and downloadable from Centers for Disease Control and Prevention Web site, under National Health and Nutrition Examination Survey 2011−2012 sections (https://wwwn.cdc.gov/Nchs/Nhanes/).

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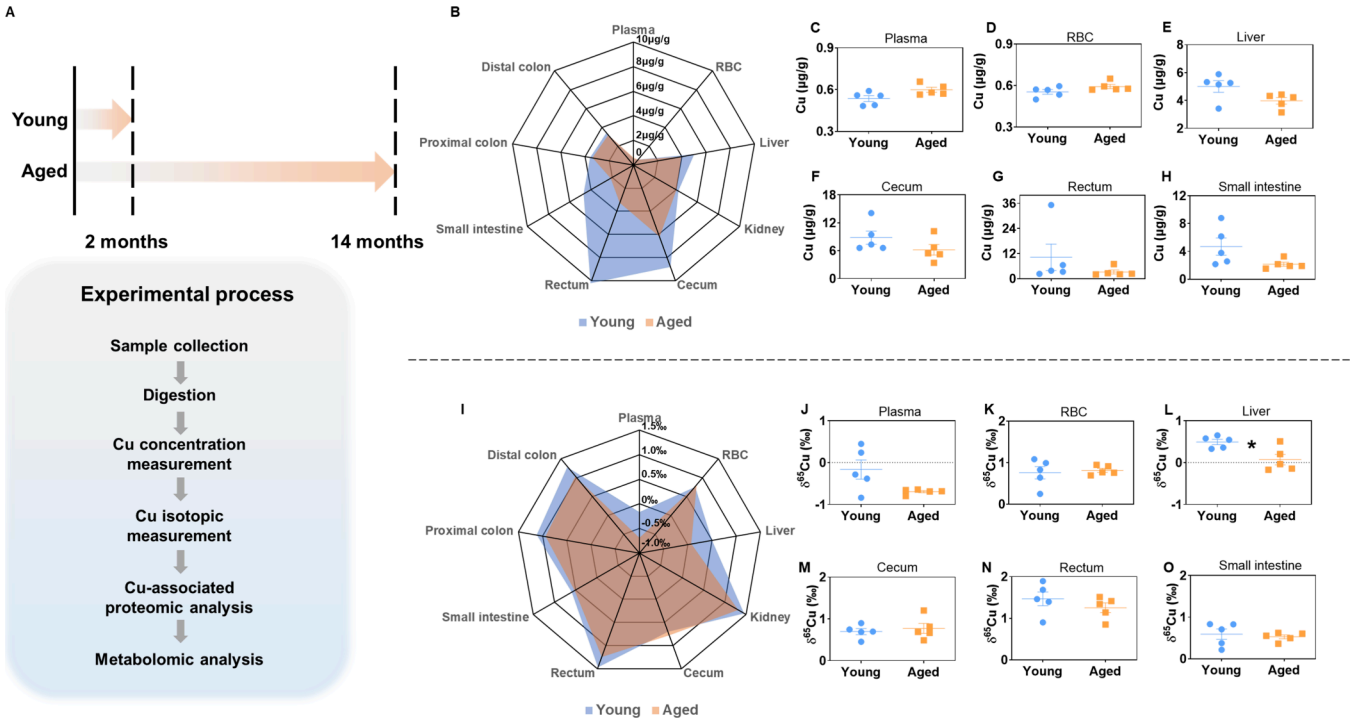

Figure 2. Variations of the Cu concentration and Cu isotopic composition (δ^{65}Cu) in mouse blood and different organs induced by aging. (A) Scheme showing the experimental procedures. (B) Radar chart intuitively showing the Cu concentration discrepancy in blood and several organs between young and aged groups. (C−H) Comparison of Cu concentration in blood and different organs between young and aged groups. Each symbol presents an individual subject. P_C = 0.0525, unpaired Student's two-tailed *t*-test. P_D = 0.1508, Mann−Whitney test. P_E = 0.0637, unpaired Student's two-tailed *t*-test. P_F = 0.1874, unpaired Student's two-tailed *t*-test. P_G = 0.2222, Mann−Whitney test. P_H = 0.1096, Welch's *t*-test. (I) Radar chart intuitively showing the Cu isotopic composition discrepancy in blood and several organs between young and aged groups. (J−O) Comparison of Cu isotopic composition in blood and different organs between young and aged groups. These tissues are consistent with Figure 2 C−H. Each symbol presents an individual subject. P_J = 0.0815, Welch's *t*-test. P_K = 0.7244, unpaired Student's two-tailed *t*-test. P_L = 0.0182, unpaired Student's two-tailed *t*-test. P_M = 0.6009, unpaired Student's two-tailed *t*-test. P_N = 0.3197, unpaired Student's two-tailed *t*-test. P_O = 0.6452, unpaired Student's two-tailed *t*-test. Data is presented as mean ± SEM, n = 5 per group.

RESULTS AND DISCUSSION

Accumulation of Copper in Human Plasma with Aging

We first analyzed the copper concentration data from publicly available data of the National Health and Nutrition Examination Survey (NHANES). Our analysis included all adult participants (age ≥18, n = 1624) from the 2011−2012 cohort, excluding pregnant women. Specifically, we excluded participants with an age recorded as 80, because individuals over 80 years old were uniformly recorded as 80 in this data package from NHANES. Our findings indicate an age-related increase in copper concentration in plasma (Figure 1B, r = 0.08, P = 0.0007), particularly in the male cohort (Figure S1A, r = 0.19, P < 0.0001). The difference in Cu variation trends between genders may be caused by physical differences between men and women, such as the effect of menstruation.[13,32,39−41] In addition, we collected blood samples from healthy subjects without clinical evidence of diseases (n = 62), ranging in age from 26 to 90, for copper concentration analysis (Figure 1C,D). We also observed a similar upward trend in the Cu concentration in plasma with age. The consistent results across different populations suggest that copper concentrations did increase with age, demonstrating universality, irrespective of regional differences.

Tracing the Sources of Aging-Associated Accumulation of Copper

To trace the sources of Cu increase and explore the effect of aging on copper homeostasis, we then carried out experiments using mice. In copper analysis based on NHANES population (see Supporting Information Figure S2), we found that males exhibited a more significant trend of copper concentration variation with age compared to females. This suggests that male mice may be a better model for exploring copper variation with aging, avoiding interference from biological factors, such as menstruation. Hence, we chose male mice as the experimental model rather than female mice. We first measured the Cu concentration in the plasma and red blood cell (RBC) of mice from the young group (controls) and the aged group (cases) (see Table S2). In plasma (Figure 2C), the aged group had a higher Cu concentration level than the young group (0.598 ± 0.040 μg/g versus 0.536 ± 0.047 μg/g, P = 0.0525), while in RBC, the Cu concentration between the aged and young group showed only a slight difference (0.593 ± 0.033 μg/g versus 0.554 ± 0.037 μg/g, P = 0.1508) (Figure 2D). The results revealed that aging led to an increase in plasma copper, which was highly consistent with the phenomena observed in humans (Figure 1B,C).[11,20,42] Furthermore, we measured the Cu concentration in several organs (i.e., liver, kidney, and intestines) (Figure 2B) and found that in most organs, the aged mice had a lower copper concentration than that of young mice, especially in liver

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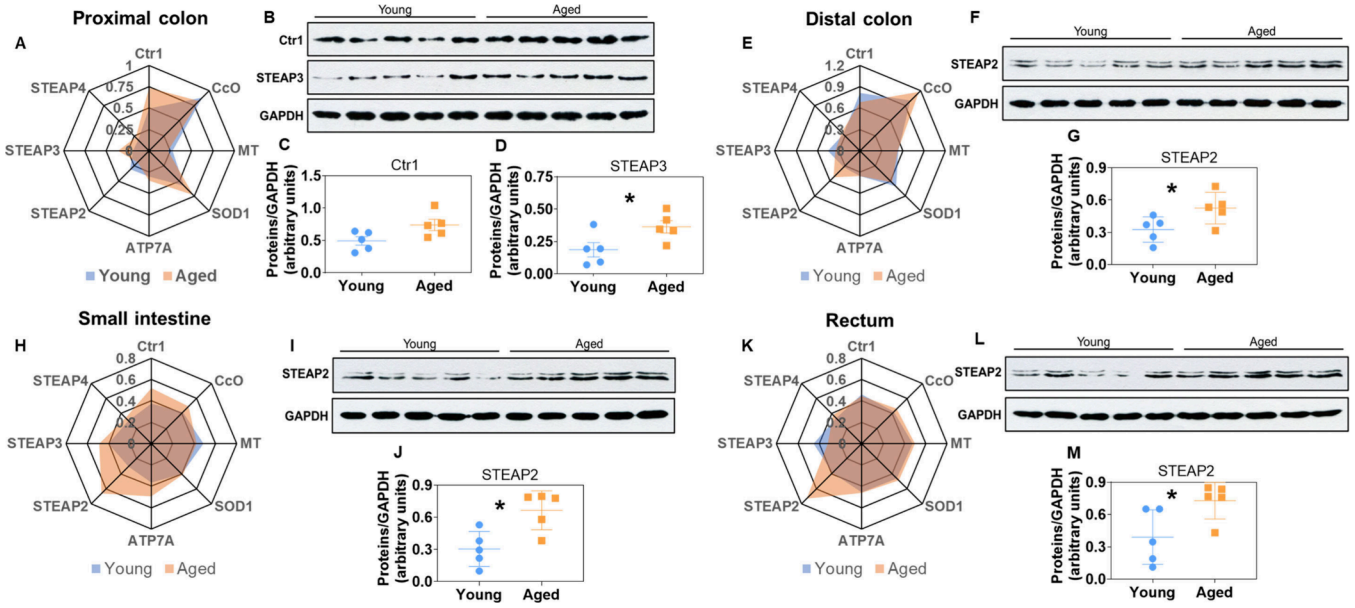

Figure 3. Western blot (WB) assay for copper-related proteins in several intestinal tissues. Eight Cu-related proteins were selected for WB assay, including copper transporter (i.e., Ctr1 and ATP7A), copper reductases (i.e., STEAP2, STEAP3 and STEAP4) and metalloproteins (i.e., SOD, CcO and MT). (A) Radar chart intuitively showing the WB results of the selected proteins in proximal colon between young and aged groups. The unit is proteins/GAPDH (arbitrary units). (**B**) The WB results of Ctr1 and STEAP3 in proximal colon. (C, D) Comparison of WB data for Ctr1 and STEAP3 in proximal colon between young and aged groups. P_C = 0.0514, P_D = 0.0407, unpaired Student's two-tailed *t*-test. (E) Radar chart intuitively showing the WB results of the selected proteins in distal colon between young and aged groups. (F) WB results of STEAP2 in distal colon. (G) Comparison of WB data for STEAP2 in distal colon between young and aged groups. P_G = 0.0465, unpaired Student's two-tailed *t*-test. (H) Radar chart intuitively showing the WB results of the selected proteins in small intestine between young and aged groups. (I) WB results of STEAP2 in small intestine. (J) Comparison of WB data for STEAP2 in small intestine between young and aged groups. P_J = 0.0108, unpaired Student's two-tailed *t*-test. (K) Radar chart intuitively showing the WB results of the selected proteins in rectum between young and aged groups. (L) WB results of STEAP2 in rectum. (M) Comparison of WB data for STEAP2 in rectum between young and aged groups. P_M = 0.0317, Mann−Whitney test. For all panels, the asterisk (*) represents $P < 0.05$. Data is presented as mean ± SEM, n = 5 per group.

(3.980 ± 0.527 μg/g versus 5.010 ± 0.932 μg/g, P = 0.0637) (Figure 2E). Copper has two natural stable isotopes (^{63}Cu and ^{65}Cu), and the Cu isotopic ratio is expressed here as δ^{65}Cu relative to a standard material ERM-AE633 (see Materials and Methods). Stable isotopic composition can bear fingerprint information about the sources or processes of an element compared with concentration, due to the unidirectional stable isotopic fractionation during a specific biological process.[20,43] For example, superoxide dismutase 1 (SOD1, a main Cu metalloprotein) tends to bind with ^{65}Cu more than metallothionein (MT, another main Cu metalloprotein) does, as the heavier isotope preferentially binds with a ligand that has stronger electronegativity (oxygen > sulfur). This leads to the results that $\delta^{65}Cu_{SOD1} > \delta^{65}Cu_{MT}$.[21,44] Therefore, here, we further carried out copper isotopic analysis in this study (see Figure S3 and Table S3).

In Figure 2J, we observed that the aged group tended to enrich the light copper isotope (^{63}Cu) compared with the young group in plasma (δ^{65}Cu = −0.693 ± 0.061 ‰ versus −0.163 ± 0.513 ‰, P = 0.0815). As mentioned above, aged mice had a lower copper concentration in the liver, suggesting that the extra copper burden in plasma was possibly predominantly released from the liver. If so, the aged group should be inclined to enrich the heavy copper isotope (^{65}Cu) in the liver compared with controls. However, this phenomenon was not observed in our experiments; on the contrary, the aged group tended to significantly enrich the light copper isotope (^{63}Cu) in the liver relative to controls (δ^{65}Cu = 0.072 ± 0.284 ‰ versus 0.492 ± 0.141 ‰, P = 0.0182) (Figure 2L). Therefore, this result indicated that the extra copper burden in plasma should not originate from the release of endogenous copper stores from the liver. Noteworthily, the difference between the aged and young group was not observed in RBC (δ^{65}Cu = 0.815 ± 0.109 ‰ versus 0.758 ± 0.331 ‰, P = 0.7244) (Figure 2K). This result was in accordance with the Cu concentration in the RBC (Figures 1D and 2D).

Copper primarily binds to three metalloproteins in the cells (i.e., MT, cytochrome c oxidase (CcO), and SOD1).[21,45] If the endogenous copper stores in these organs (e.g., liver) were not the direct origin of the extra copper burden in plasma, the expression levels of the three main metalloproteins in these organs should not differ significantly. To verify this point, we further assessed the expression levels of the major cuproproteins in these organs via the Western blot (WB) assay. The WB results showed that the expression levels of SOD1, MT, and CcO in intestine tract, liver, and kidney indeed showed no significant difference between the aged and young group (Figures S4−S6). This finding supports our deduction that the extra copper burden in plasma with aging was not directly caused by the release of endogenous copper stores.

Since the endogenous copper stores in these organs (especially the liver) were not the direct source of the extra copper burden in aged mice, it is rational to infer whether exogenous copper absorption or copper efflux by bile were responsible for the copper imbalance in blood. A previous study has demonstrated that cholestasis can induce the copper isotopic composition of mice to decline.[46] Hence, the copper efflux by bile must be taken into consideration. The copper-

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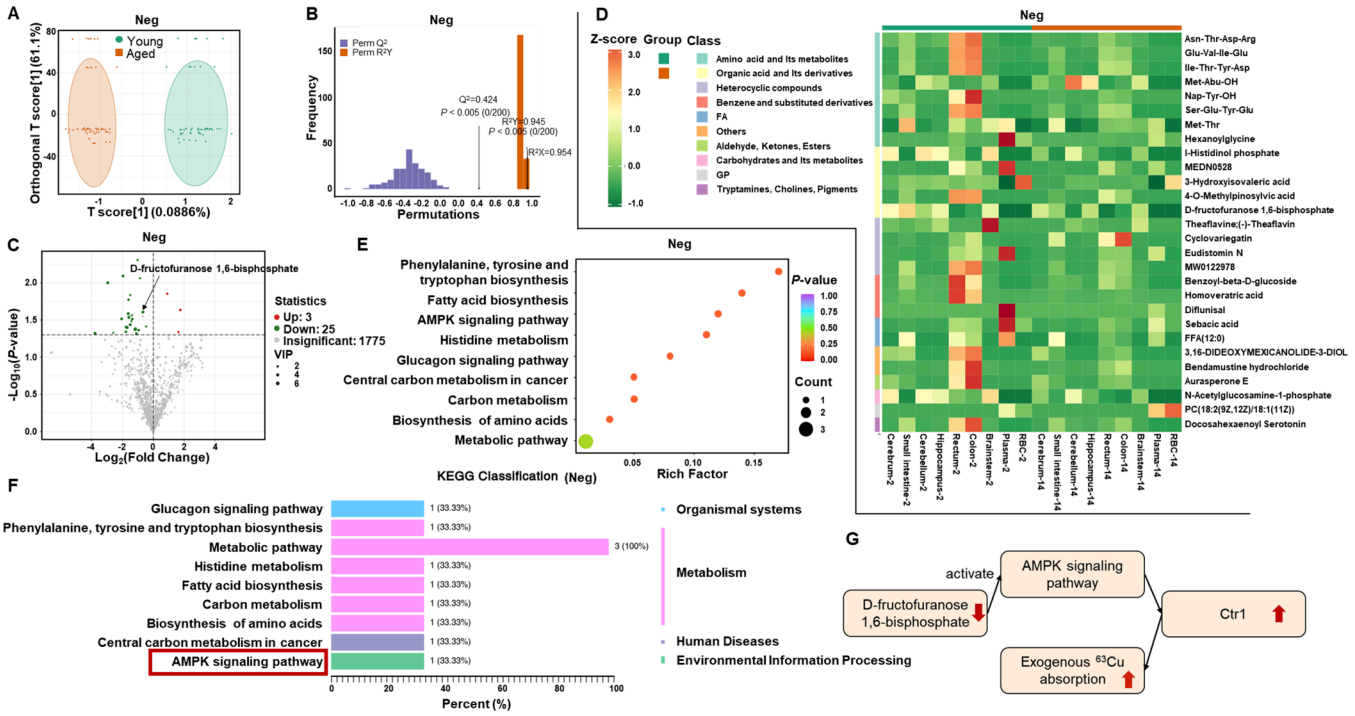

Figure 4. Metabolic analysis of mice. (A) OPLS-DA score plot of metabolites between young group and aged group. (B) OPLS-DA model validation. (C) Volcano plot of *P* values of metabolites between young group and aged group. Notably, the green represents "downregulated differential metabolites" and the red means "upregulated differential metabolites". (D) Heatmap of differential metabolites between young group and aged group. Different colors represent different values obtained after standardized treatment of different relative contents (red represents "high levels" and green represents "low levels"). Notably, MEDN0528 represents 1,2-ethanediol, 1-(3-methoxy-4-(sulfooxy)phenyl)- and MW0122978 represents (10-methoxy-1,4,14,19,19-pentamethyl-8,17-dioxo-2,7,18 trioxapentacyclo [11.9.0.03,11.05,9.014,20] docosa-3(11),4,9-trien-21-yl) acetate. (E) KEGG enrichment map of differential metabolites. The dots size indicates the number of enriched metabolic differential metabolites, and the color of dots is expressed as the *P*-value. (F) KEGG classification map of differential metabolites. (G) Diagram of increased intestinal ^{63}Cu absorption affected by D-fructofuranose 1,6-bisphosphate. The results of metabolites shown in this figure were obtained using electrospray mass spectrometry in the negative ionization mode.

related proteins in the liver we observed in this study did not show significant differences, indicating that the potential role of bile may be relatively small.

We then considered intestinal copper absorption. The comparatively low δ^{65}Cu value in aged mice, including in blood and other organs (especially the liver), implied that exogenous copper absorption should result in an enrichment of ^{63}Cu in aged mice. Exogenous copper is mainly absorbed in the intestines and is closely related to copper transporters and copper reductases in the intestines.[47−49] In an equilibrium process, the light isotope (^{63}Cu) tends to be enriched in species/counterparts with lower oxidation states and a weaker binding energy environment,[50] causing $^{63}Cu^{2+}$ to be more easily reduced to Cu^{+} by copper reductases compared with $^{65}Cu^{2+}$. The enrichment of ^{63}Cu in aged mice implied that the expression levels of copper reductases and high-affinity Cu^{+} transporter in aged mice might be higher than those in young mice. Therefore, to verify this point, we further assessed the expression levels of the main copper reductases (i.e., STEAP2, STEAP3 and STEAP4) and copper transporters (i.e., Ctr1 and ATP7A).

Notably, we found that the expression levels of copper reductases in the intestinal tract showed a significant difference between the aged and young groups (Figure 3). The aged group had significantly higher expression of STEAP3 in the proximal colon (P = 0.0407) and STEAP2 in the distal colon, small intestine, and rectum (distal colon, small intestine, and rectum, P = 0.0465, P = 0.0108, and P = 0.0317, respectively) than the young group. In addition, the expression of Ctr1 increased in the proximal colon of the aged group compared with that of the young group (P = 0.0514). These Cu-associated protein results verified our deduction from the copper isotopic analysis. That is, the increased expression levels of copper reductases (i.e., STEAP2 and STEAP3) and copper transporter (i.e., Ctr1) in the intestine tract might enhance the absorption of Cu^{+}, thus resulting in a comparatively low δ^{65}Cu value in aged mice, especially in the liver due to its crucial role as the main organ that regulates and controls the copper release in the body.[51,52]

Exploring the Role of Deregulated Nutrient Sensing in the Accumulation of Copper with Aging

Intestinal copper absorption is regulated by nutrient sensing.[53] Here, we observed abnormal copper intake in the intestines with aging, suggesting that nutrient sensing might be out of balance. In fact, deregulated nutrient sensing is a critical hallmark of aging.[1,2] Nutrient sensing plays an important role in maintaining homeostasis and adapting to a fluctuating environment,[54] which might also be greatly affected by aging. Nutrient sensors are widely present in enteroendocrine cells and absorptive enterocytes in animals.[54,55] To examine whether the aged mice really had deregulated nutrient sensing, we further carried out metabolite analysis. We chose intestinal tissues, brain tissues, and blood for metabolite analysis (Figures 4 and S7−S15). Intestinal tissues were selected mainly based on the fact that the intestinal tract is the main site of nutrient

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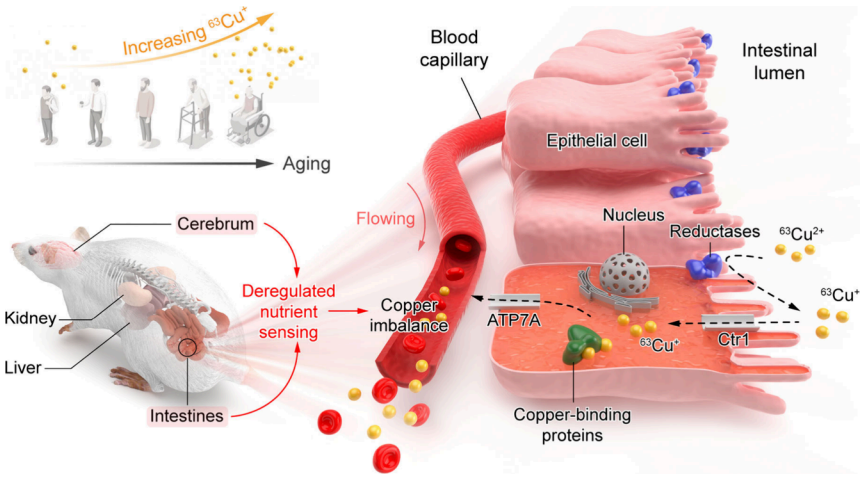

Figure 5. Scheme showing the possible mechanisms of aging-induced Cu imbalance. The higher expression levels of copper reductases (i.e., STEAP2 and STEAP3) and copper transporter (i.e., Ctr1) during aging would induce exogenous ^{63}Cu absorption increase in intestinal tract, further leading to the copper imbalance in aged mice.

sensors distribution and the primary site of copper absorption, and brain tissues were studied mainly because brain tissues constitute the central nervous system and may be involved in the regulation of copper.

We first performed Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) to assess the metabolic variances between young and aged groups (see Figures 4A,B and S7A,B). The score plots generated from OPLS-DA models indicated that clear separation of samples from different groups with no overlapping, demonstrating distinct difference in physiological and metabolic conditions between aged and young mice. Subsequently, we identified significantly differential metabolites and represented them in volcano plots (Figures 4C and S7C). Notably, we find a significantly differential metabolite (i.e., D-fructofuranose 1,6-bisphosphate), which is a crucial metabolite in the AMPK signaling pathway (see Figure 4E,F). As an important signaling pathway in nutrient sensing, AMPK signaling pathway is crucial for sensing nutrient levels and regulating metabolic balance.[56,57] As shown in Figure S14D, we found that D-fructofuranose 1,6-bisphosphate in the rectum was significantly lower in the aged group than the young group.The reduced level of D-fructofuranose 1,6-bisphosphate would activate the AMPK signaling pathway[58] and further promote Ctr1 protein expression.[59] This might subsequently facilitate the absorption of excess exogenous ^{63}Cu (in Cu^{+}), leading to a decreased $\delta^{65}Cu$ value in aged mice (see Figure 4G). These findings were highly consistent with the observed higher expression levels of copper reductases and copper transporters in the intestines of aged mice. Therefore, our results indicate that the copper imbalance might linked to the aging process through deregulated nutrient sensing, involving alterations in the expression levels of copper reductases and copper transporter in the intestine tract. (see Figure 5).

In addition, we had also screened other 6 significantly differential metabolites related to nutrient sensing pathway (i.e., AMPK signaling and mTOR signaling pathways) in various tissues and blood (see Figure S14). Results indicated that the nutrient sensing pathway was indeed affected in the aged mice. Subsequently, we conducted an in-depth analysis of other differential metabolic pathways associated with copper, with a primary focus on those displaying significant differences across multiple tissues or blood (see Figure S15). Changes in these metabolic pathways might also be correlated to copper imbalances during aging. For example, the ABC transporter metabolic pathway is associated with transmembrane transport.[60,61] The perturbation observed in the ABC transporter metabolic pathway also implied potential alterations in the transmembrane transport of copper, aligning with our earlier WB results indicating upregulation of copper transporters in the intestine. Additionally, we identified some metabolic pathways linked to the AMPK signaling pathway, including the cAMP signaling pathway and the glucagon signaling pathway.

■ ENVIRONMENTAL HEALTH IMPLICATIONS

The findings of this study shed light on the intricate relationship among aging, copper metabolism, and exposure factors. All of the copper in the body is taken up from the external environment (e.g., dietary intake, drinking water, air pollution, and occupational exposures). Note that dietary exposure is one pathway of environmental exposure. Age-related accumulation of copper in plasma suggests the possibility of increased exposure to copper over time, which is associated with various health conditions, including neurodegenerative diseases, cardiovascular disorders, and metabolic dysregulation. The copper exposure may interact with age-related changes in copper metabolism, amplifying the risk of developing age-related diseases. Deregulated nutrient sensing pathways, implicated in age-related copper imbalance, underscore the importance of environmental factors in modulating metabolic processes. Dietary patterns, environmental pollutants, and lifestyle factors can influence nutrient sensing pathways, impacting copper absorption, distribution, and metabolism. Addressing environmental determinants of nutrient sensing dysfunction is crucial for promoting healthy aging and reducing the burden of age-related diseases.

Furthermore, age-related changes in copper metabolism may interact with other environmental stressors such as heavy metals, oxidative stress, and inflammatory agents. Synergistic or antagonistic interactions between copper and environmental stressors can exacerbate cellular damage, accelerate aging processes, and increase susceptibility to diseases. Comprehensive environmental risk assessments are needed to evaluate the

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cumulative impact of multiple stressors on aging and health outcomes. Considering the complexity of factors influencing aging, future studies are needed for a comprehensive understanding of the role of copper intake from the environment during aging involving more hallmarks of aging and its implications for health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00096.

Copper concentration, copper isotopic results, Western blot assay results, and metabolic analysis results (PDF)

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Author Contributions

Q.L. and W.W. designed the research; W.W. performed most of experiments; D.L., H.Y., W.L., and Z.C. helped with the MC-ICP-MS measurements; S.S. and L.P. helped to collect blood samples of healthy subjects; G.J. supervised the project; W.W. and Q.L. analyzed the data; Q.L. and W.W. wrote the paper.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lopez-Otin, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. *Cell* **2013**, *153*, 1194−1217.

(2) Lopez-Otin, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. Hallmarks of aging: An expanding universe. *Cell* **2023**, *186*, 243−278.

(3) Harman, D. The aging process: major risk factor for disease and death. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 5360−5363.

(4) Bottazzi, B.; Riboli, E.; Mantovani, A. Aging, inflammation and cancer. *Semin. Immunol.* **2018**, *40*, 74−82.

(5) Beal, M. F. Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann. Neurol.* **1995**, *38*, 357−366.

(6) Wissler, R. W.; Robert, L. Aging and Cardiovascular Disease - A Summary of the Eighth Munster International Arteriosclerosis Symposium. *Circulation* **1996**, *93*, 1608−1612.

(7) Ahima, R. S. Connecting obesity, aging and diabetes. *Nat. Med.* **2009**, *15*, 996−997.

(8) Brewer, G. J. Risks of Copper and Iron Toxicity during Aging in Humans. *Chem. Res. Toxicol.* **2010**, *23*, 319−326.

(9) Coudray, C.; Feillet-Coudray, C.; Rambeau, M.; Tressol, J. C.; Gueux, E.; Mazur, A.; Rayssiguier, Y. The effect of aging on intestinal absorption and status of calcium, magnesium, zinc, and copper in rats: A stable isotope study. *J. Trace Elem. Med. Biol.* **2006**, *20*, 73−81.

(10) Morel, J.-D.; Sauzeat, L.; Goeminne, L. J. E.; Jha, P.; Williams, E.; Houtkooper, R. H.; Aebersold, R.; Auwerx, J.; Balter, V. The mouse metallomic landscape of aging and metabolism. *Nat. Commun.* **2022**, *13*, 607.

(11) Malavolta, M.; Piacenza, F.; Basso, A.; Giacconi, R.; Costarelli, L.; Mocchegiani, E. Serum copper to zinc ratio: Relationship with aging and health status. *Mech. Ageing Dev.* **2015**, *151*, 93−100.

(12) Brewer, G. J. Iron and copper toxicity in diseases of aging, particularly atherosclerosis and Alzheimer's disease. *Exp. Biol. Med.* **2007**, *232*, 323−335.

(13) Jaouen, K.; Gibert, M.; Lamboux, A.; Telouk, P.; Fourel, F.; Albarede, F.; Alekseev, A. N.; Crubezy, E.; Balter, V. Is aging recorded in blood Cu and Zn isotope compositions? *Metallomics* **2013**, *5*, 1016−1024.

(14) Hobin, K.; Costas-Rodriguez, M.; Van Wonterghem, E.; Vandenbroucke, R. E.; Vanhaecke, F. Alzheimer's Disease and Age-Related Changes in the Cu Isotopic Composition of Blood Plasma and Brain Tissues of the APPNL-G-F Murine Model Revealed by Multi-Collector ICP-Mass Spectrometry. *Biology* **2023**, *12*, 857.
(15) Hoefs, J.; Hoefs, J. *Stable isotope geochemistry*; Springer: 2009; Vol. *285*.
(16) Wiederhold, J. G. Metal stable isotope signatures as tracers in environmental geochemistry. *Environ. Sci. Technol.* **2015**, *49*, 2606−2624.
(17) Sparks, D. L.; Schreurs, B. G. Trace amounts of copper in water induce b-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11065−11069.
(18) Squitti, R.; Bressi, F.; Pasqualetti, P.; Bonomini, C.; Ghidoni, R.; Binetti, G.; Cassetta, E.; Moffa, F.; Ventriglia, M.; Vernieri, F.; Rossini, P. M. Longitudinal prognostic value of serum ″free″ copper in patients with Alzheimer disease. *Neurology* **2009**, *72*, 50−55.
(19) Sauzeat, L.; Bernard, E.; Perret-Liaudet, A.; Quadrio, I.; Vighetto, A.; Krolak-Salmon, P.; Broussolle, E.; Leblanc, P.; Balter, V. Isotopic Evidence for Disrupted Copper Metabolism in Amyotrophic Lateral Sclerosis. *iScience* **2018**, *6*, 264−271.
(20) Wang, W.; Liu, X.; Zhang, C.; Sheng, F.; Song, S.; Li, P.; Dai, S.; Wang, B.; Lu, D.; Zhang, L.; Yang, X.; Zhang, Z.; Liu, S.; Zhang, A.; Liu, Q.; Jiang, G. Identification of two-dimensional copper signatures in human blood for bladder cancer with machine learning. *Chem. Sci.* **2022**, *13*, 1648−1656.
(21) Balter, V.; Nogueira da Costa, A.; Bondanese, V. P.; Jaouen, K.; Lamboux, A.; Sangrajrang, S.; Vincent, N.; Fourel, F.; Telouk, P.; Gigou, M.; Lecuyer, C.; Srivatanakul, P.; Brechot, C.; Albarede, F.; Hainaut, P. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 982−985.
(22) Telouk, P.; Puisieux, A.; Fujii, T.; Balter, V.; Bondanese, V. P.; Morel, A.-P.; Clapisson, G.; Lamboux, A.; Albarede, F. Copper isotope effect in serum of cancer patients. A pilot study. *Metallomics* **2015**, *7*, 299−308.
(23) Toubhans, B.; Gourlan, A. T.; Telouk, P.; Lutchman-Singh, K.; Francis, L. W.; Conlan, R. S.; Margarit, L.; Gonzalez, D.; Charlet, L. Cu isotope ratios are meaningful in ovarian cancer diagnosis. *J. Trace Elem. Med. Biol.* **2020**, *62*, No. 126611.
(24) Hastuti, A. A. M. B.; Costas-Rodriguez, M.; Matsunaga, A.; Ichinose, T.; Hagiwara, S.; Shimura, M.; Vanhaecke, F. Cu and Zn isotope ratio variations in plasma for survival prediction in hematological malignancy cases. *Sci. Rep.* **2020**, *10*, 16389.
(25) Gordon, G. W.; Monge, J.; Channon, M. B.; Wu, Q.; Skulan, J. L.; Anbar, A. D.; Fonseca, R. Predicting multiple myeloma disease activity by analyzing natural calcium isotopic composition. *Leukemia* **2014**, *28*, 2112−2115.
(26) Vanhaecke, F.; Costas-Rodríguez, M. High-precision isotopic analysis of essential mineral elements: capabilities as a diagnostic/prognostic tool. *View* **2021**, *2*, No. 20200094.
(27) Lobo, L.; Costas-Rodriguez, M.; Carlos de Vicente, J.; Pereiro, R.; Vanhaecke, F.; Sanz-Medel, A. Elemental and isotopic analysis of oral squamous cell carcinoma tissues using sector-field and multi-collector ICP-mass spectrometry. *Talanta* **2017**, *165*, 92−97.
(28) Wang, W.; Li, Z.; Lu, Q.; Zhang, L.; Lu, D.; Yang, H.; Yang, X.; Zhang, L.; Zhang, Y.; Liu, Q.; Wang, B.; Guo, Y.; Ren, A.; Jiang, G. Natural copper isotopic abnormity in maternal serum at early pregnancy associated to risk of spontaneous preterm birth. *Sci. Total Environ.* **2022**, *849*, 157872−157872.
(29) Makarewicz, C. A.; Sealy, J. Dietary reconstruction, mobility, and the analysis of ancient skeletal tissues: Expanding the prospects of stable isotope research in archaeology. *J. Archaeol. Sci.* **2015**, *56*, 146−158.
(30) Bartelink, E. J.; Chesson, L. A. Recent applications of isotope analysis to forensic anthropology. *Forensic sciences research* **2019**, *4*, 29−44.
(31) Gupte, A.; Mumper, R. J. Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. *Cancer Treat. Rev.* **2009**, *35*, 32−46.
(32) Van Heghe, L.; Engstrom, E.; Rodushkin, I.; Cloquet, C.; Vanhaecke, F. Isotopic analysis of the metabolically relevant transition metals Cu, Fe and Zn in human blood from vegetarians and omnivores using multi-collector ICP-mass spectrometry. *J. Anal. At. Spectrom.* **2012**, *27*, 1327−1334.
(33) Yang, L. Accurate and precise determination of isotopic ratios by MC-ICP-MS: a review. *Mass Spectrom. Rev.* **2009**, *28*, 990−1011.
(34) Yuan, H.; Yuan, W.; Bao, Z.; Chen, K.; Huang, F.; Liu, S. Development of two new copper isotope standard solutions and their copper isotopic compositions. *Geostand. Geoanal. Res.* **2017**, *41*, 77−84.
(35) Li, J.; Tang, S.-h.; Zhu, X.-k.; Li, Z.-h.; Li, S.-Z.; Yan, B.; Wang, Y.; Sun, J.; Shi, Y.; Dong, A.; Belshaw, N. S.; Zhang, X.; Liu, S.-a.; Liu, J.-h.; Wang, D.; Jiang, S.-y.; Hou, K.; Cohen, A. S. Basaltic and solution reference materials for iron, copper and zinc isotope measurements. *Geostand. Geoanal. Res.* **2019**, *43*, 163−175.
(36) Zimmerman, D. W.; Zumbo, B. D. Rank transformations and the power of the Student t test and Welch t'test for non-normal populations with unequal variances. *Can. J. Exp. Psychol.* **1993**, *47*, 523−539.
(37) Skovlund, E.; Fenstad, G. U. Should we always choose a nonparametric test when comparing two apparently nonnormal distributions? *J. Clin. Epidemiol.* **2001**, *54*, 86−92.
(38) Fagerland, M. W.; Sandvik, L. Performance of five two-sample location tests for skewed distributions with unequal variances. *Contemp. Clin. Trials* **2009**, *30*, 490−496.
(39) Van Heghe, L.; Deltombe, O.; Delanghe, J.; Depypere, H.; Vanhaecke, F. The influence of menstrual blood loss and age on the isotopic composition of Cu, Fe and Zn in human whole blood. *J. Anal. At. Spectrom.* **2014**, *29*, 478−482.
(40) Michos, C.; Kalfakakou, V.; Karkabounas, S.; Kiortsis, D.; Evangelou, A. J. G. E. Changes in copper and zinc plasma concentrations during the normal menstrual cycle in women. *Gynecol. Endocrinol.* **2010**, *26*, 250−255.
(41) Toro-Román, V.; Muñoz, D.; Maynar-Mariño, M.; Clemente-Gil, S.; Robles-Gil, M. C. J. N. Sex Differences in Copper Concentrations during a Sports Season in Soccer Players. *Nutrients* **2023**, *15*, 495.
(42) Madaric, A.; Ginter, E.; Kadrabova, J. Serum copper, zinc and copper/zinc ratio in males: influence of aging. *Physiol. Res.* **1994**, *43*, 107−111.
(43) Miaou, E.; Tissot, F. L. Copper isotope ratios in serum do not track cancerous tumor evolution, but organ failure. *Metallomics* **2023**, *15*, No. mfad060.
(44) Larner, F.; McLean, C. A.; Halliday, A. N.; Roberts, B. R. Copper Isotope Compositions of Superoxide Dismutase and Metallothionein from Post-Mortem Human Frontal Cortex. *Inorganics* **2019**, *7*, 86.
(45) Kim, B.-E.; Nevitt, T.; Thiele, D. J. Mechanisms for copper acquisition, distribution and regulation. *Nat. Chem. Biol.* **2008**, *4*, 176−185.
(46) Costas-Rodriguez, M.; Van Campenhout, S.; Hastuti, A. A. M. B.; Devisscher, L.; Van Vlierberghe, H.; Vanhaecke, F. Body distribution of stable copper isotopes during the progression of cholestatic liver disease induced by common bile duct ligation in mice. *Metallomics* **2019**, *11*, 1093−1103.
(47) van den Berghe, P. V. E.; Klomp, L. W. J. New developments in the regulation of intestinal copper absorption. *Nutr. Rev.* **2009**, *67*, 658−672.
(48) Sandstead, H. H. Copper bioavailability and requirements. *Am. J. Clin. Nutr.* **1982**, *35*, 809−814.
(49) Turnlund, J. R. Human whole-body copper metabolism. *Am. J. Clin. Nutr.* **1998**, *67*, 960S−964S.
(50) Fujii, T.; Moynier, F.; Abe, M.; Nemoto, K.; Albarede, F. Copper isotope fractionation between aqueous compounds relevant

to low temperature geochemistry and biology. *Geochim. Cosmochim. Acta* **2013**, *110*, 29−44.
(51) Sternlieb, I. Copper and the Liver. *Gastroenterology* **1980**, *78*, 1615−1628.
(52) Linder, M. C.; Wooten, L.; Cerveza, P.; Cotton, S.; Shulze, R.; Lomeli, N. Copper transport. *Am. J. Clin. Nutr.* **1998**, *67*, 965S−971S.
(53) Duca, F. A.; Waise, T. M. Z.; Peppler, W. T.; Lam, T. K. T. The metabolic impact of small intestinal nutrient sensing. *Nat. Commun.* **2021**, *12*, 903.
(54) Redhai, S.; Pilgrim, C.; Gaspar, P.; van Giesen, L.; Lopes, T.; Riabinina, O.; Grenier, T.; Milona, A.; Chanana, B.; Swadling, J. B.; Wang, Y.-F.; Dahalan, F.; Yuan, M.; Wilsch-Brauninger, M.; Lin, W.-h.; Dennison, N.; Capriotti, P.; Lawniczak, M. K. N.; Baines, R. A.; Warnecke, T.; Windbichler, N.; Leulier, F.; Bellono, N. W.; Miguel-Aliaga, I. An intestinal zinc sensor regulates food intake and developmental growth. *Nature* **2020**, *580*, 263−268.
(55) Miguel-Aliaga, I. Nerveless and gutsy: intestinal nutrient sensing from invertebrates to humans. *Semin. Cell Dev. Biol.* **2012**, *23*, 614−620.
(56) Gonzalez, A.; Hall, M. N.; Lin, S.-C.; Hardie, D. G. AMPK and TOR: The Yin and Yang of Cellular Nutrient Sensing and Growth Control. *Cell Metab.* **2020**, *31*, 472−492.
(57) Herzig, S.; Shaw, R. J. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 121−135.
(58) Zhang, C.-S.; Hawley, S. A.; Zong, Y.; Li, M.; Wang, Z.; Gray, A.; Ma, T.; Cui, J.; Feng, J.-W.; Zhu, M.; Wu, Y.-Q.; Li, T. Y.; Ye, Z.; Lin, S.-Y.; Yin, H.; Piao, H.-L.; Hardie, D. G. R.; Lin, S.-C. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature* **2017**, *548*, 112−116.
(59) Zhang, X.; Jiang, Q.; Su, Y.; Bu, L.; Sun, Z.; Wu, X.; Gao, B.; Wang, L.; Lin, Y.; Xie, W.; Guo, J. AMPK phosphorylates and stabilises copper transporter 1 to synergise metformin and copper chelator for breast cancer therapy. *Br. J. Cancer* **2023**, *128*, 1452−1465.
(60) Higgins, C. F. ABC transporters: physiology, structure and mechanism - an overview. *Res. Microbiol.* **2001**, *152*, 205−210.
(61) Schinkel, A. H.; Jonker, J. W. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv. Drug Delivery Rev.* **2012**, *64*, 138−153.

https://doi.org/10.1021/envhealth.4c00096
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