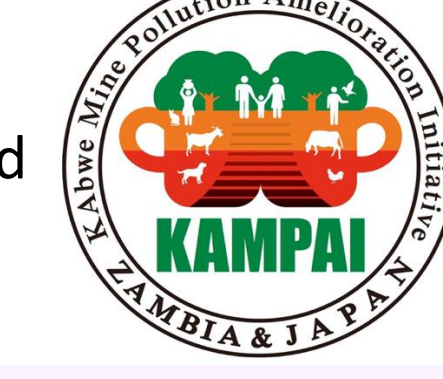


Investigating Metal-Induced Metabolic Alterations in Wild Rats Around a Historical Lead-Zinc Mining Site in Zambia

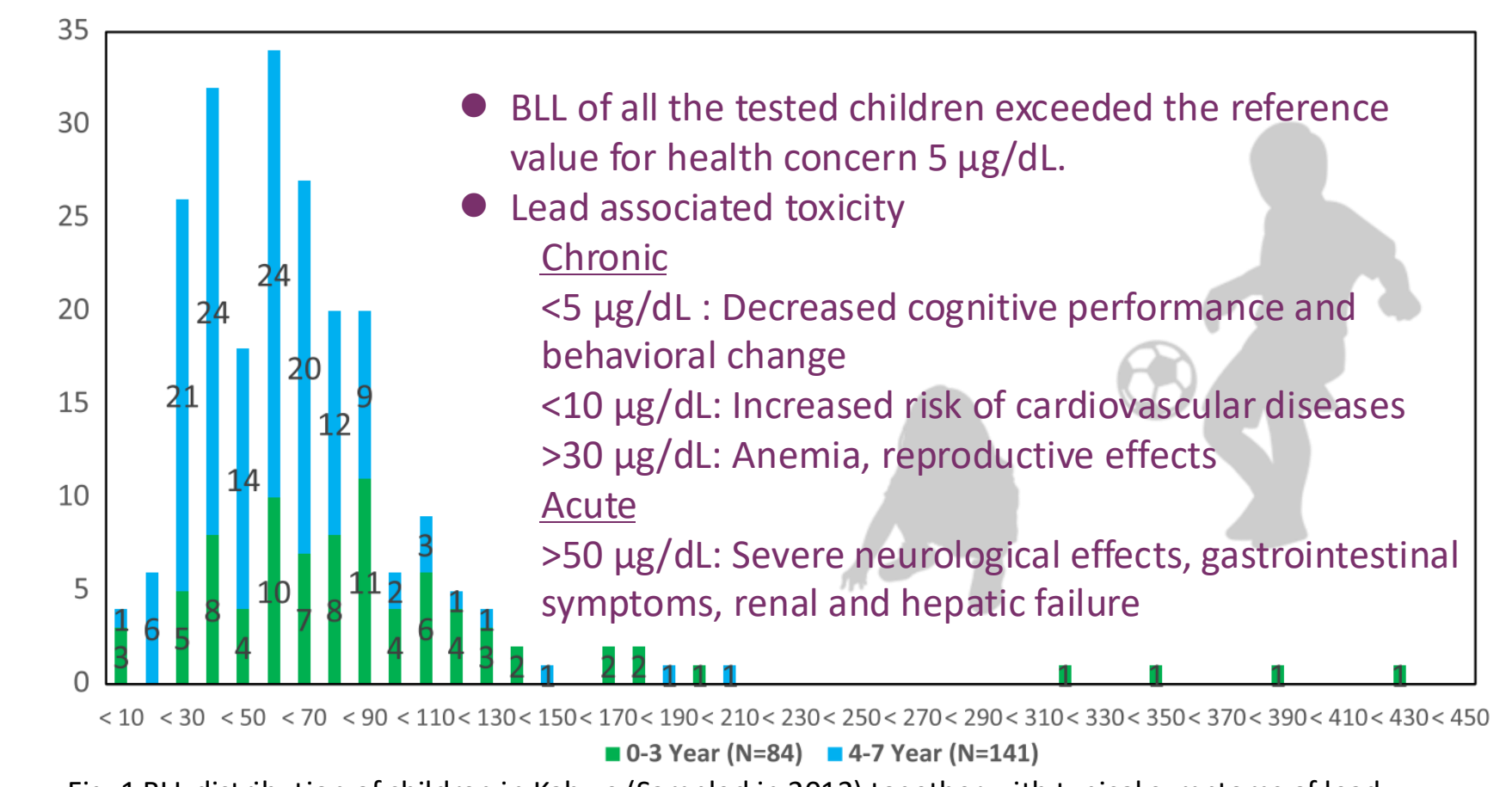
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Background: a town left with high level of lead pollution

- ✓ The current research site is in Kabwe, Republic of Zambia, which has a long history of lead and zinc mining, from 1902 up to 1994. Even after closure of the mine, the slags were left uncontrolled and remained as a significant pollution source.
- ✓ Previous researches project have revealed widespread and severe lead (Pb) pollution in the environment, animals, and residents. [Yabe et al. 2015, Nakata et al. 2017, Doya et al. 2020].
- ✓ In 2017-2021, an intensive and interdisciplinary research project between University of Zambia and Hokkaido University, **KAMPAL** produced more data and remediation insight. Based on the survey result, World Bank project has been working on remediation, blood-lead-level(BLL) testing, and treatment.



➡ Despite the high accumulation levels, lead poisoning patients with significant symptoms have not been reported. Non-typical and potentially overlooked health effects should be evaluated.



Materials and Methods: exploring invisible health effects of lead

- ✓ Sampling
 - Period and site: August 2022 in Kabwe, Zambia [Fig. 2]
 - Samples: Blood (separated into serum and clot), liver, and kidney of Black rat (*Rattus rattus*) [Table 1]
- *Samples are brought to Hokkaido University under permission of both Zambian and Japanese government
- Table 1 Rat sample overview

Area	Mutwe Wansofu (High-contamination)	Kang'omba (Low-contamination)
Male	N=15	N=17
Female	N=14	N=16
Total	N=29	N=33
- ✓ Metal analysis of blood clot, liver, and kidney
 - Sample preparation: Microwave assisted acid digestion (30% HNO_3 5 mL, 30% H_2O_2 1 mL)
 - Analysis: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Agilent 7700
- ✓ Biochemical markers in serum
 - Clinical analytical machinery DryChem: Glucose(Glu), Triglyceride(TG), Total cholesterol(T-Cho), HDL cholesterol (HDL-C), Insulin
- ✓ RNA sequencing for comprehensive gene expression analysis in liver of representative samples
 - illumina NovaSeq 6000 / PE150
 - From Mutwe Wansofu and Kang'omba, the five rats with the highest and lowest liver lead concentrations were selected, respectively. cDNAs were pooled and prepared for analysis.
- ✓ qPCR for quantitative gene expression in liver and kidney of all the samples
 - QuantStudio 12K Flex Real-Time PCR System

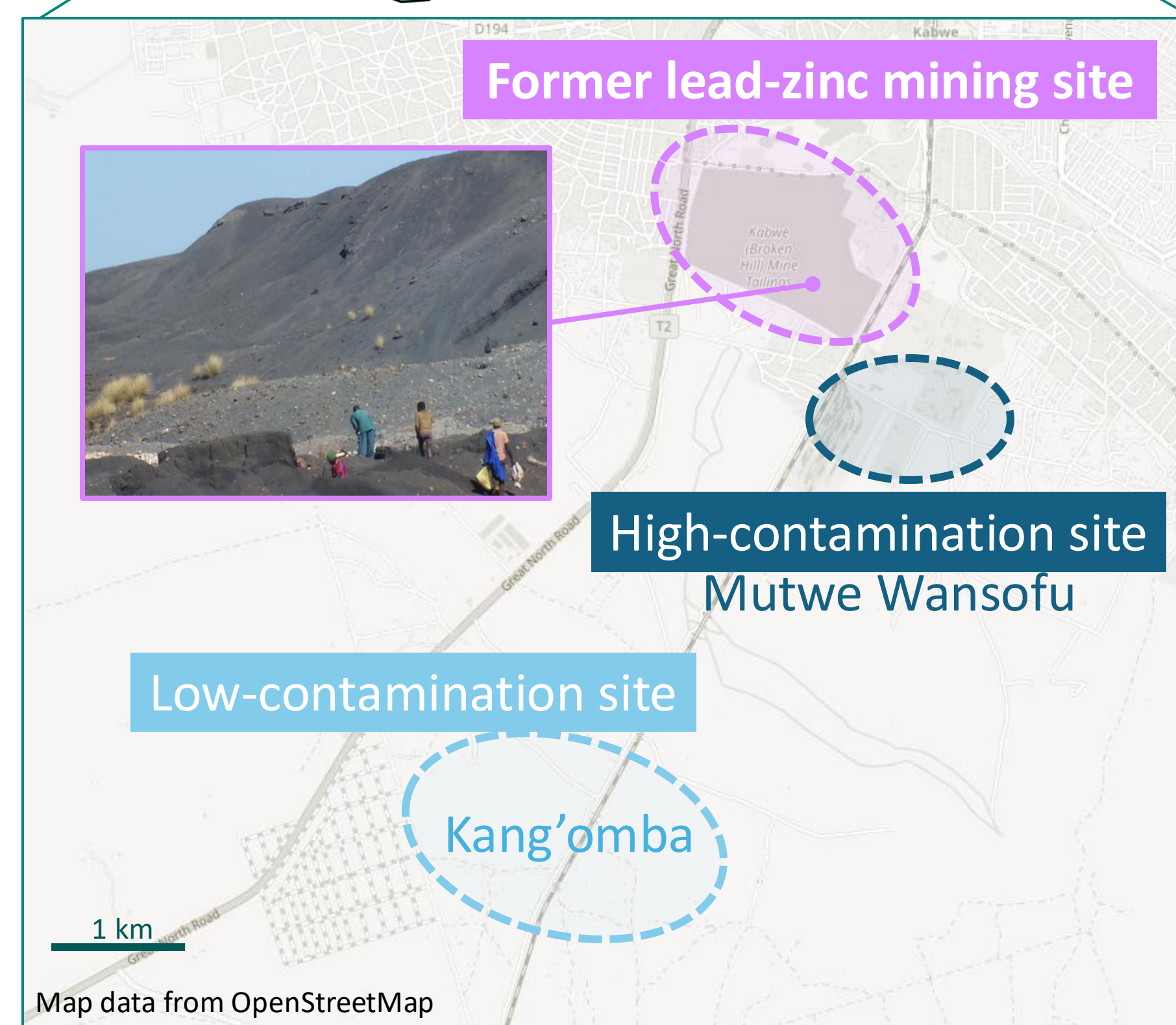


Fig. 2 Overview of sampling site. Abandoned slags are deposited mainly at the south part of the mining site. Mutwe Wansofu is a residential area adjacent to the deposit. Kang'omba is another residential area which is about 7 km away from the mining site.

Key findings: Severe lead accumulation in organs with potential metabolic alternation

1 Significant lead accumulation near the former mining site

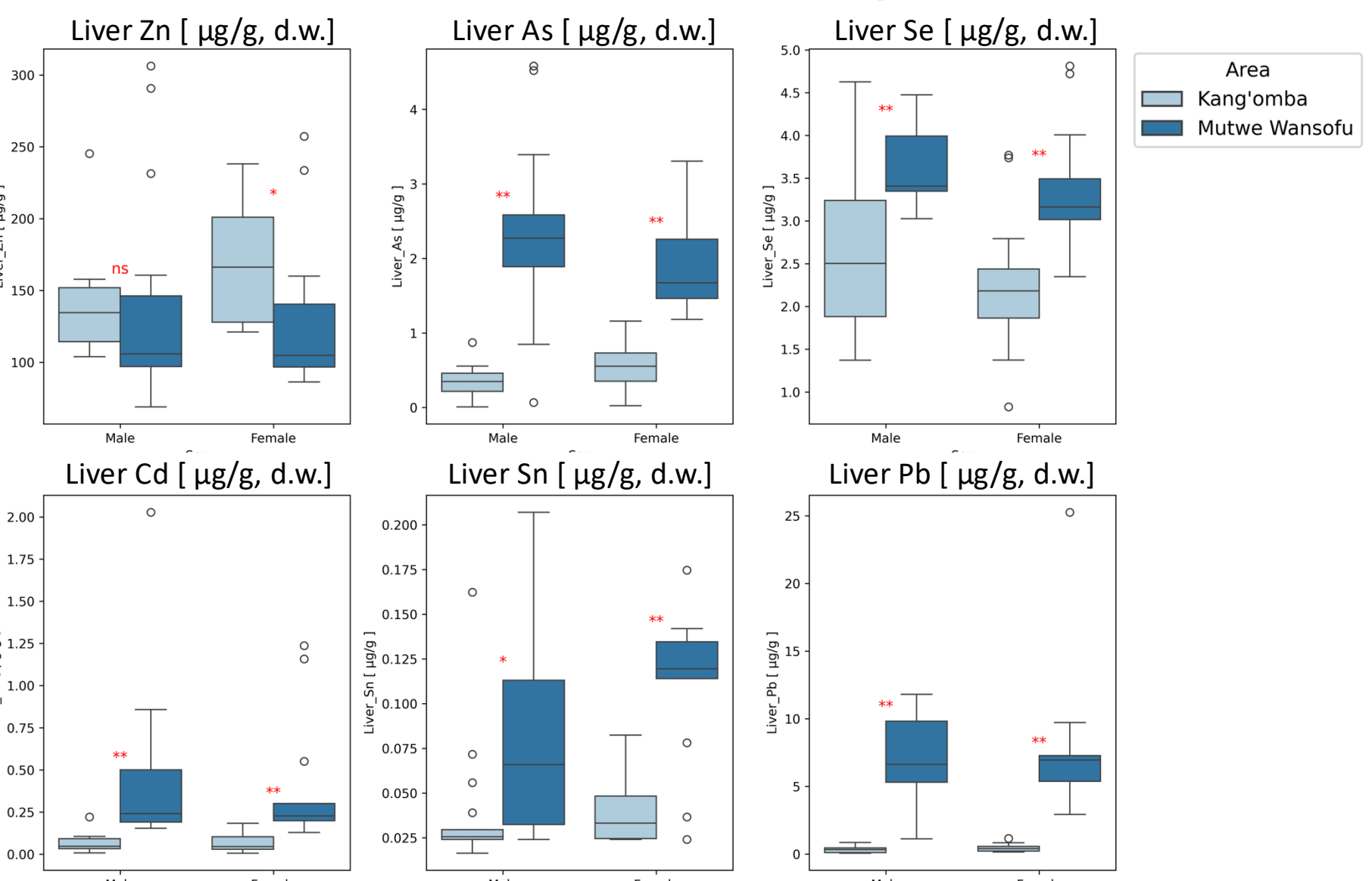


Fig. 3 Comparison of element concentrations in liver of black rats. Concentrations are expressed on a dry weight basis. [Zn: zinc, As: arsenic, Se: selenium, Cd: cadmium, Sn: tin, Pb: lead] [Wilcoxon rank sum test, * $p < 0.05$, ** $p < 0.01$, ns: $p > 0.05$]

- ✓ All the element concentrations, apart from Zn in male rats, were significantly higher in Mutwe Wansofu (high-exposure area).
- ✓ The median values of lead were comparable to the high dose sub-chronic exposure experiment (120 mg/kg, 28days) [Qadar et al. 2022]

2 Limited effects in physiological and biochemical parameters

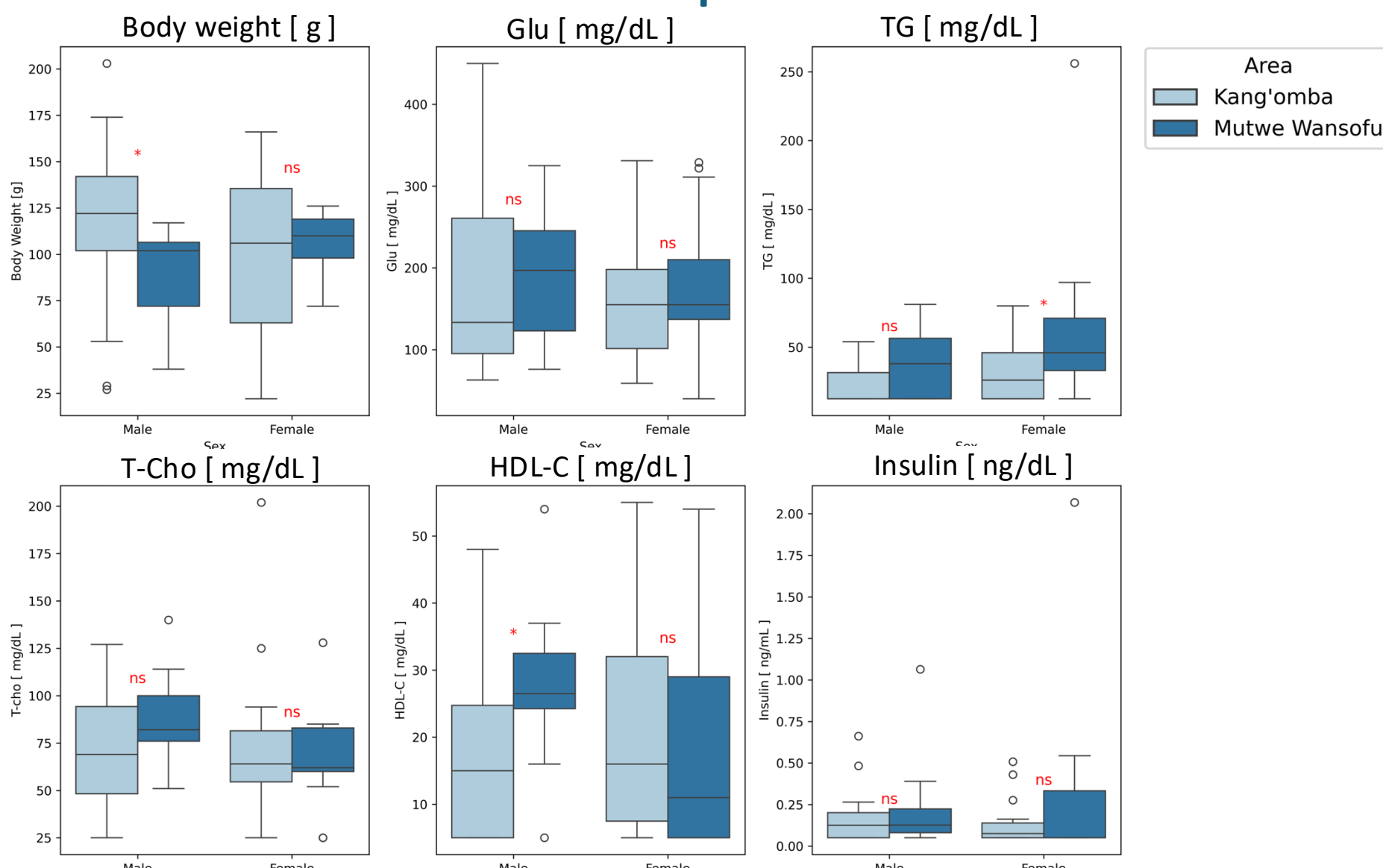


Fig. 4 Comparison of body weight and biochemical parameters in serum of black rats (Glu: Glucose, TG: Triglyceride, T-Cho: Total cholesterol, HDL-C: HDL cholesterol) [Wilcoxon rank sum test, * $p < 0.05$, ** $p < 0.01$, ns: $p > 0.05$]

- ✓ Body weight was significantly low, and HDL-C was significantly high in Mutwe Wansofu (high-exposure area).
- ✓ HDL-C ranges of all groups were within normal range.

3 Potential alternation of metabolism indicated by RNA sequencing

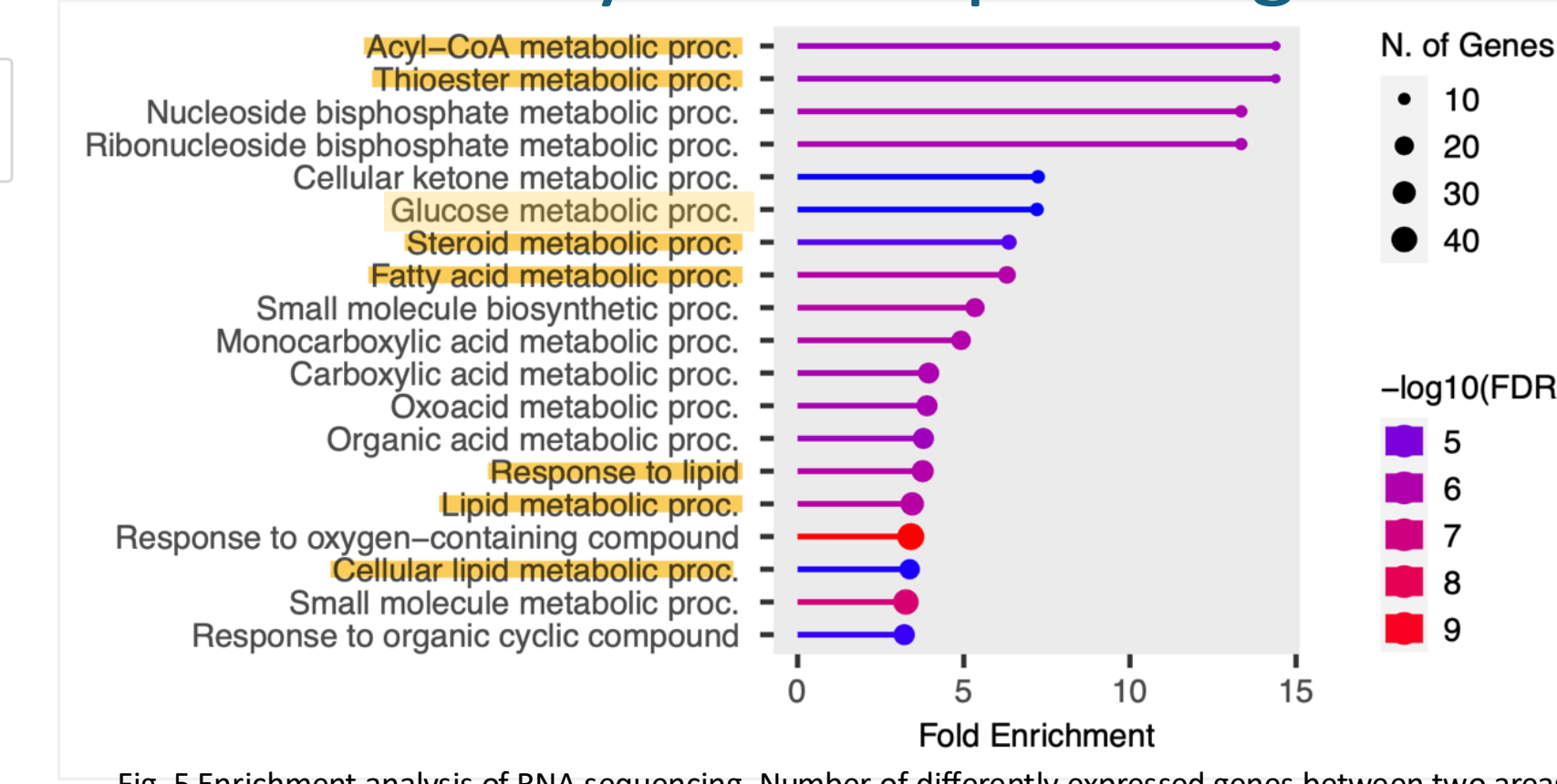


Fig. 5 Enrichment analysis of RNA sequencing. Number of differently expressed genes between two areas are indicated with corresponding biological pathways. (N = 5 in each group)

- ✓ Major pathways corresponding to differently expressed genes included glucose and lipid metabolism.
- ✓ There are arising number of researches on metabolomic alternation caused by lead. Qader et al. (2022) reported elevated blood glucose and total cholesterol level and decreased HDL-cholesterol level after sub-chronic exposure of lead to rats.
- ✓ Representative genes for those pathways are selected to quantitatively analyze their expression in the all individual.

Side topic: NCDs in Sub-Saharan Africa
 Non-communicable diseases (NCDs) refer to chronic health impairment such as cardiovascular diseases, cancers, and diabetes. In sub-Saharan Africa, cardiovascular diseases are the emerging burden on the population. The major cardiovascular risk factors include obesity, diabetes, and dyslipidemia. Common causes of those health conditions are unhealthy diets and reduced physical activities [The Lancet, 2019].
 This trend of increasing NCDs is considered to be associated with rapid urbanization in the region [Juma et al., 2019]. Air pollution is also often listed as one of the NCD risk factor. Lead and other metals/metalloids have been reported to increase risks of cardiovascular diseases and chronic inflammation. Historical pollution may further increase the risk of NCDs among local residents compared to the general population.

4 Several glucose- and lipid-metabolism-related genes might be associated with lead accumulation

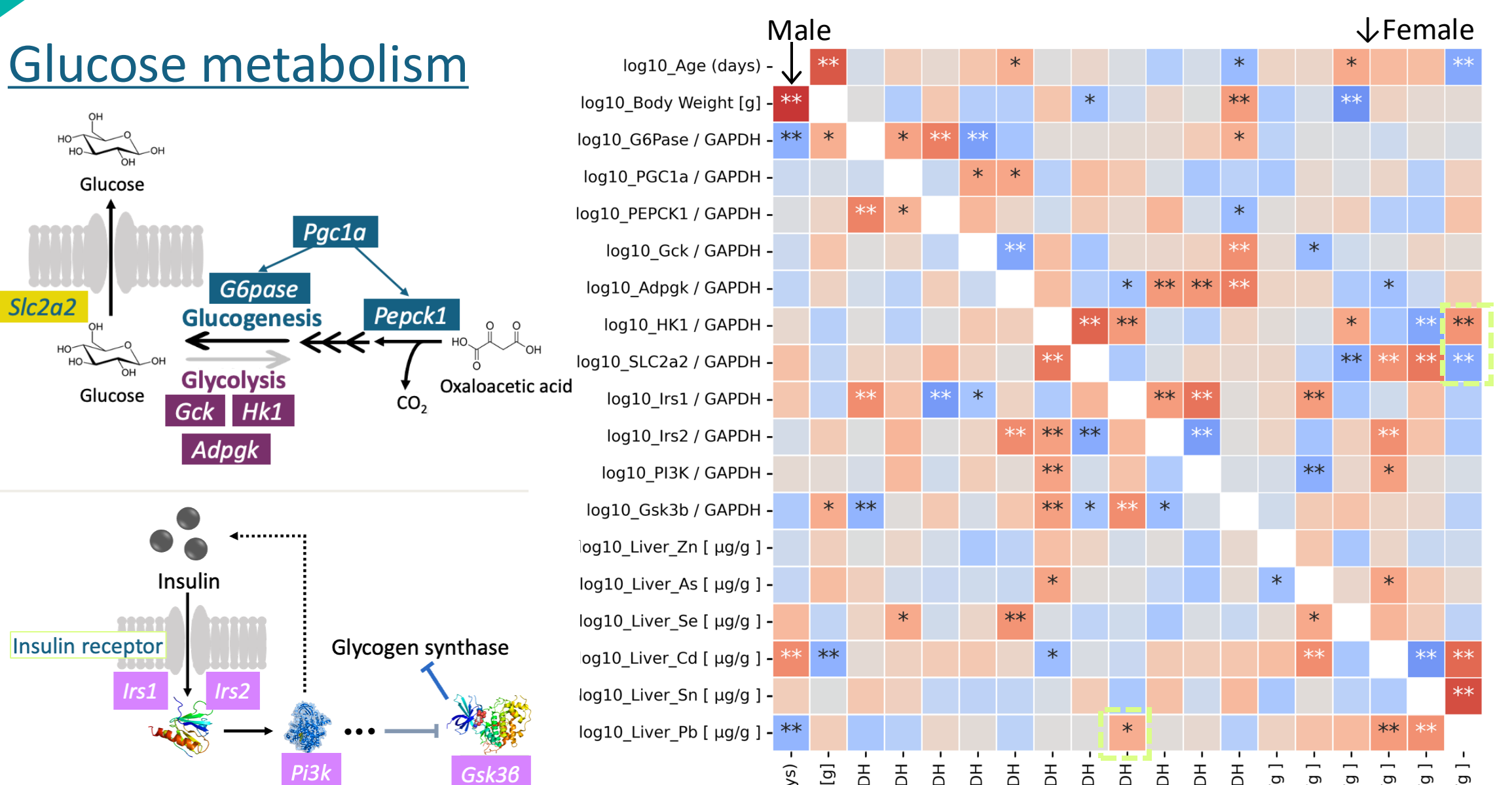


Fig. 6 Functions of target genes for qPCR related to glucose metabolism (G6pase: glucose 6-phosphatase, Pck1: phosphoenolpyruvate carboxylase, Gsk: glycokinase, Adpgk: ADP-dependent glucokinase, HK: hexokinase, Slc2a2: solute carrier family 2, member 2). e.g. glucose transporter 2, Irs: insulin receptor substrate, Pdk: phosphatidylinositol-3 kinase, Gsk: glycogen synthase kinase

- ✓ In males, only *Irs1* exhibited significant positive correlations with hepatic Pb concentrations. Pb was the only element which showed significant correlation with *Irs1*.
- ✓ In females, *Hk1* exhibited significant positive correlations with hepatic Pb concentrations, whereas *Slc2a2* showed a significant negative correlation. This may indicate the enhanced glycolysis related to higher Pb accumulation.
- ✓ Both in males and females, partial correlation coefficients were generally moderate, with the maximum absolute values of 0.394 and 0.548, respectively.

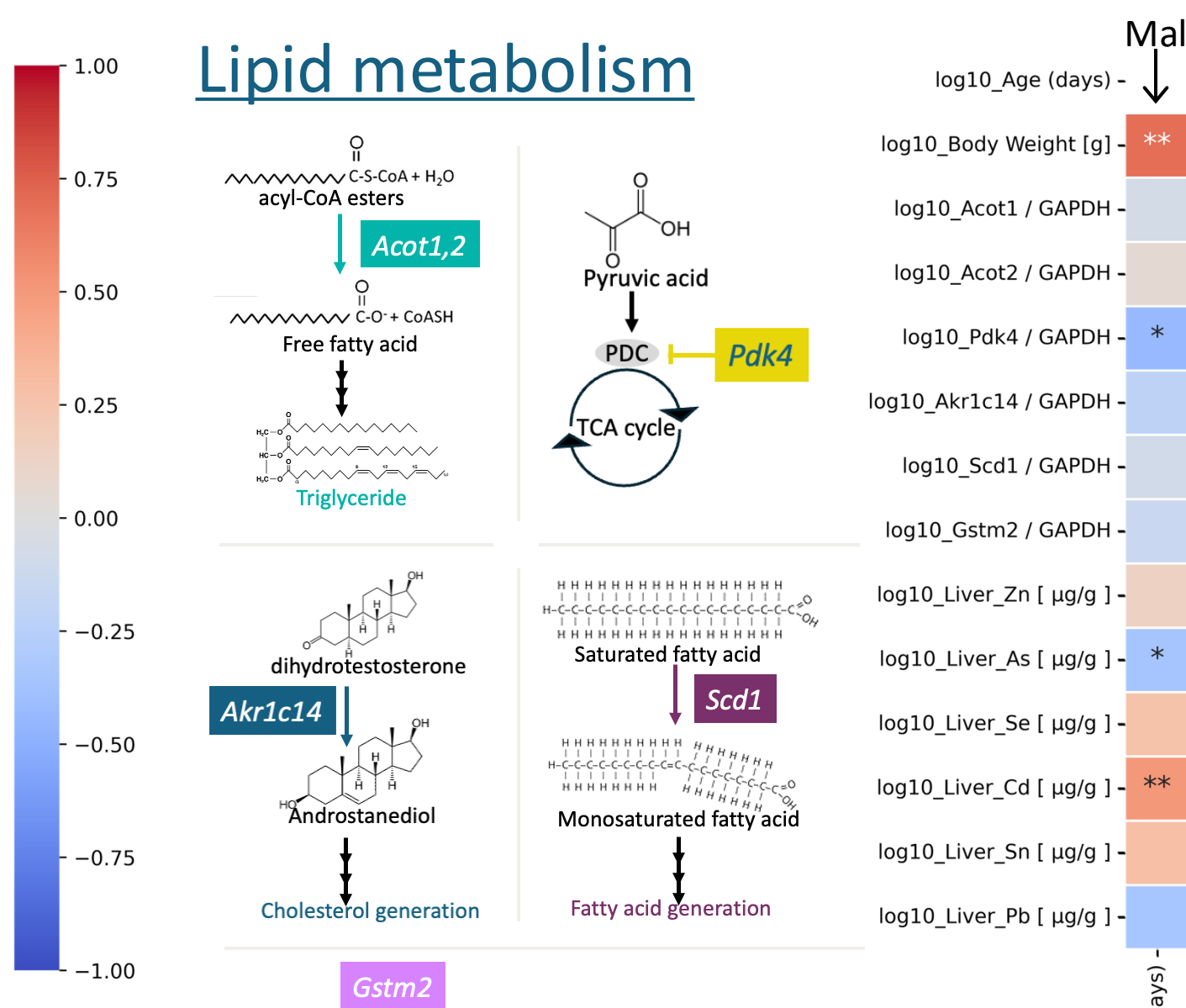


Fig. 8 Functions of target genes for qPCR related to lipid metabolism (Acot: Acyl-CoA thioesterase, Pdk: pyruvate dehydrogenase kinase, Akr1c14: aldo-keto reductase family 1 member C4, Scd: stearoyl-CoA desaturase, Gstm: glutathione S-transferase mu)

- ✓ In males, *Acot1*, *Pdk4*, *Akr1c14*, and *Gstm2* exhibited significant negative correlations with hepatic Pb concentrations, whereas *Acot2* showed a significant positive correlation. The partial correlation coefficients for *Acot1* and *Acot2* had opposite directions with respect to Pb and other metals.
- ✓ In females, *Akr1c14* and *Scd1* demonstrated significant negative correlations. Both genes are involved in the biosynthesis of lipid metabolites, such as cholesterol and fatty acids.

5 Metallothionein gene expression decreased in highly exposed group

Given the limited observed effects despite the high lead concentrations, a potential biological by Metallothioneins (MT1 and MT2), known as representative metal-binding proteins, was investigated.

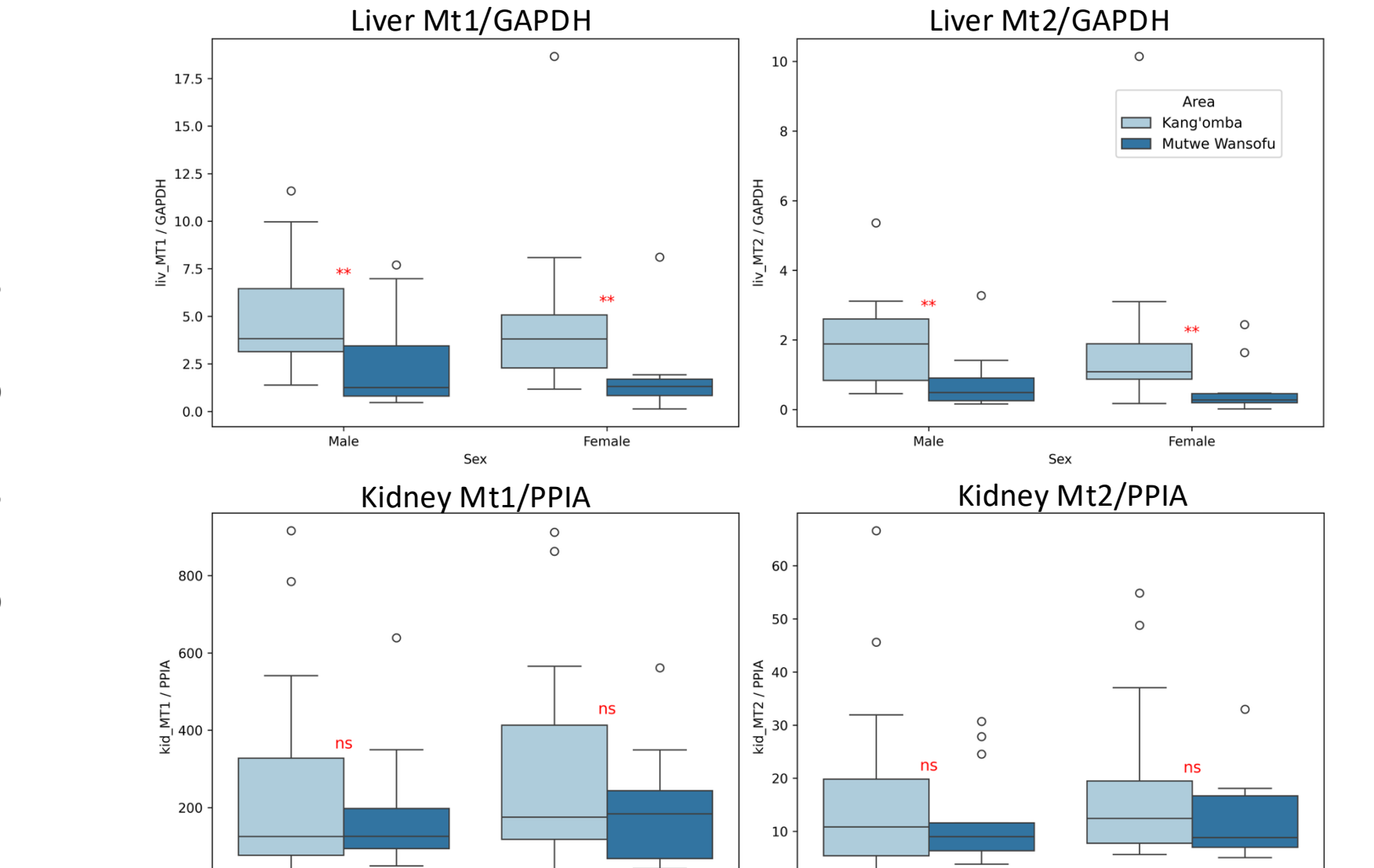


Fig. 10 Comparison of gene expression levels of metallothionein 1 and 2 in liver and kidney between two areas. [Wilcoxon rank sum test, ** $p < 0.01$, ns: $p > 0.05$]

- ✓ Expression levels of *Mt1* and *Mt2* in liver were significantly lower in Mutwe Wansofu in both male and female.
- ✓ The result was opposite with the typical response to divalent metals, such as Cd in kidney.

Summary and Take-Home Consideration

- Severe accumulation of metals and metalloids, especially lead, was observed at the high-contamination site, with concentrations comparable to those in vivo experiments that showed lipid metabolism impairment.
- Quantitative gene expression analysis identified several genes related to the insulin signaling pathway, glycolysis, and cholesterol synthesis that were altered in response to lead accumulation. To allow clearer biochemical and clinical interpretation, the reproducibility and site-independence of the results should be validated.
- An adaptive alteration of the defense mechanism was suspected; however, the pattern differed from typical responses. Potential shifts in genomic sequences and enzyme activity in the exposed population warrant further investigation.
- The effects of co-exposure to diverse metal composition in the environment should also be considered.

Upcoming project with four countries

- Enhancing laboratory capacity for strategic monitoring
- Building a network of monitoring specialists with a One Health perspective
- Promoting collaboration through shared data and expertise
- Strengthening fundamentals and motivation for sustainable activities

KAMPAL to ZAZINAMBO

- Zambia
- Zimbabwe
- Namibia
- Botswana



▲ Previous publications in KAMPAL. Updates on ZAZINAMBO will be added here soon.