Reprogramming of glycometabolism caused by low-level lead in vascular smooth muscle cells

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Abstract

Background: Lead induces cardiovascular disease in humans and animals. Changes in the structure and function of vascular smooth muscle cells (VSMCs) are the cytopathological basis of many cardiovascular diseases. The present study investigated the effects of low-level lead on oxidative stress and carbohydrate metabolism in rat VSMCs.

Materials and Results: VSMCs were cultured in media with 0 μM (control group) 0.25 μM 1 μM lead acetate for 24 h. Low-level lead exposure caused oxidative stress damage to vascular smooth muscle cells, increased level of Reaction oxygen species (ROS) and reduced glutathione (GSH) in VSMCs were detected compared with that in control group (P< 0.05). Compared with the control group, the decreased activity of PKM2, LDH and HK, the key enzymes for oxygen-free oxidation in lead-exposed group (P< 0.05) were detected. The key oxygen-oxidation enzyme PDH in the lead-exposed group was detected and the activity was reduced (P< 0.05), while the PDK1 content increased (P< 0.05). The activity of G-6-PD, a key enzyme of the pentose phosphate pathway, and the product NADPH increased (P< 0.05) in the lead-exposed group. Moreover, the Intracellular glucose content significantly increased in the lead-exposed group (P< 0.05).

Conclusion: Low-level lead exposure leads to oxidative stress damage in VSMCs and enhances pentose phosphate pathway flow by improving the metabolic efficiency of the glutathione redox cycle, while disrupts the glycometabolism of VSMCs, inhibits anaerobic and aerobic oxidative processes, causing glycometabolism reprogramming in VSMCs. These findings may be the molecular mechanism for cardiovascular disease induced by low-level lead.

Introduction

Lead is a common heavy metal environmental pollutant that can enter the human body, and environmental exposure to lead is ubiquitous. Lead was in widespread use in the production of lead-acid batteries for its relative inertness, in the construction industry as an architectural metal, in sailboat keels as ballast for its high density and resistance to corrosion, in voltage power cables as sheathing material and so on [1]. There are two forms of lead pollutants in the environment, organic lead and inorganic lead. The organic lead compounds that mainly exist as lead tetraethy1 and lead tetramethy1, are volatile and tend to be diffused into air in a large portion, particularly combustion of leaded gasoline. The results of a cohort study in the United States showed that the average blood lead level of subjects was 27.1 μg/L, 20% of which had a concentration of lead in blood of at least 50 μg/L [2]. In previous studies, the blood lead level of Chinese children aged 0-6 years was 63.15 μg/L[3] the average blood lead concentration of Chinese adult was 81.7 μg/L[4] .

Nevertheless, more and more studies show that lead has no minimum safe concentration for the body [5,6,7]. The safe threshold has varied dramatically and has been continuously lower in the past. In the early years, the safe threshold of lead exposure was established at levels of 600 μg/L in 1960s, then reduced to

400 μg/L, 300 μg/L, 250 μg/L and finally further reduced to 100 μg/L in 1991 by the Centers for Disease Control in United States [8]. Low-level lead is more concerned as a risk factor for cardiovascular disease. A cohort study showed that the prevalence of hypertension was positively correlated with blood lead in people with blood lead of 33 to 100 μg/L [9]. More recently, a large population study demonstrated that the lead level of lower than 50 μg/L could also cause cardiovascular mortality [2].

There are many studies on the mechanism of cardiovascular diseases caused by lead. Our previous studies have shown that lead can induce apoptosis and hyperacetylation of rat vascular smooth muscle cells [10]. He al[1] demonstrated the important role of telomere shortening and lipid disorders in cardiovascular diseases caused by low-level lead. Besides There is a growing evidence that lead can induce free radicals and lead to enhanced oxidative stress. In a previous study, lead could promote ROS production in kidney and cardiovascular tissues [11,12]. Jintana et al found that short-term lead occupational exposure increased SOD and CAT activity in workers, and caused hypertension [13]. Besides, it has been known that nicotinamide adenine dinucleotide phosphate (NADPH) was extremely important in the maintenance of antioxidant defenses [14]. The pentose phosphate pathway is the only way to produce NADPH, and is an important part of the glycometabolism process. We speculated that low-level lead might cause abnormal glycometabolism while causing oxidative stress. Thus, the objective of the present study was to validate and further study the effects of low-level lead on oxidative stress and glycometabolism in vascular smooth muscle cells, so as to elucidate the molecular mechanism for cardiovascular disease induced by low-level lead.

Materials And Methods

1. Animals

The current study was performed in accordance with the Guide for the Care and Use of Laboratory Animals the National Institutes of Health. The animal use protocol approved by the Institutional Animal Care and Use Committee of Hebei Medical University. Male Sprague Dawley rats (specified pathogen-free grade) weighing 120 g was purchased from Hebei Medical University Laboratory Animal Center (Shijiazhuang, China).

2. Cell culture

Take the rat thoracic and abdominal aorta under aseptic conditions, peel off the connective tissue, and rinse three times with pre-cooled PBS. Cut the artery into small segments of 2-3 cm, and peel off the adventitia under a microscope. Then cut the vascular wall longitudinally, gently scrape off the inner membrane with a blade, rinse with PBS, and cut into small pieces of about 1 mm^3. The tissue block is inoculated into a culture flask at a density of 1 to 3 blocks/cm^2, and cultured at 37°C for 2 to 4 hours to make the tissue block firmly adhere to the wall. Slowly add the culture solution along the side wall or the

upper wall, put it into the CO_2 incubator, and continue static culture at 37°C. Change the fluid every 3d. , they are passaged after the cells are confluent in a single layer

The cells were routinely cultured in Dulbecco's Modified Eagle Medium (Gibco, USA) supplemented with 10% fetal calf serum (Thermo Fisher Scientific), 100,000 IU/L penicillin and 100 mg/L streptomycin (Solarbio). The cells were digested and passaged using digestion solution containing 0.1% collagenase and 0.125% trypsin (Solarbio), and cells of passages 3 to 5 were used.

3. Western blotting

Cells were incubated for 24 h, with the different treatments in serum-free media. After the treatments described, the cells were washed with ice-cold PBS, scraped, centrifuged at 12000 rpm for 5 min at 4℃, and extracted in lysis buffer. Proteins (20 μg per lane) were equally loaded and separated on 8% SDS-PAGE, and transferred to PVDF membranes (Biosharp). Membranes were blocked with 5% freshly prepared nonfat milk-TBST (Tris-buffered saline, 0.1% Tween 20) for 2 h at room temperature, andincubated overnight at 4℃ with polyclonal antibodies against PKM2 (dilution 1/1000, Abbkine), PDK1 (dilution 1/1000, Abbkine) or β-actin, then incubated 1 h with appropriated secondary antibodies (dilution 1/10000) at room temperature. Proteins were detected using an enhanced chemilumi-nescence (ECL) kit (Affinity Bioscience)and signal intensities were normalized and quantified to ß-actin by densitometry using Image Lab 3.0software (Bio-Rad, USA).

4. Biochemical assay

Cells were incubated for 24 h, with the different treatments in serum-free medium. After the treatments described, the cells were washed with ice-cold PBS, scraped and centrifuged at 12000 rpm for 5 min at 4℃. GSH was determined using colorimetric kit by a spectrophotometer according to the instructions provided with the kits (Nanjing Jiancheng Institute of Bioengineering). G-6-PD activity was measured by its ability to reduce 1 μM of nicotinamide-adenine dinucleotide phosphate ($NADP^+$) in the presence of glucose-6-phosphate, as previously described[15], using colorimetric kits spectrophotometer according to the instructions provided with kits (AAT Bioquest).The PDH, HK, LDH, glucose, and NADPH assay procedures are performed in strict accordance with the kit instructions.

5. Determination of reactive oxygen species levels

Intracellular ROS levels were determined using 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime) as described previously[16]. Inside the cells, DCFH-DA is cleaved by non-specific esterases to form DCFH, which is in a non-fluorescent form and is oxidized to the fluorescent compound 2'7'-dichlorofluorescein by ROS. The ROS level was read by the fluorescence intensity of the photo taken by the microscope. The specific operation is as follows: The cells were seeded into a 24-well plate, and

DCFH-DA was diluted with serum-free culture medium at a ratio of 1:1000 to make the final concentration of 10μmol/L. After the cells are collected, they are suspended in 200μl diluted DCFH-DA and incubated for 20 minutes in a 37°C cell incubator. Then, it was washed three times with PBS and observed under a fluorescence microscope.

6. Statistical analysis

One-way ANOVA was employed to determine the effects and their interactions using the general linear model procedure of SPSS (Version 21.0). Differences were considered significant at P< 0.05.

Results

1. Rat vascular smooth muscle cell culture

Under inverted phase contrast microscope, vascular smooth muscle cells showed good growth, long fusiform shape, vortex growth, and the growth convergence was typical of “peak-valley”, with typical smooth muscle cell characteristics.

2. Oxidative stress levels

The fluorescence intensity of Radical oxygen species (ROS) in VSMCs treated with lead acetate increased compared with control group(P< 0.05) (Fig.1) and the level of reduced glutathione (GSH) increased in lead-exposed group compared with that in the control group (P< 0.05).

3. Pentose phosphate pathway flow and glucose concentration

Compared with the control group, the content of NADPH and G-6-PD activity increased (P< 0.05) in low-level lead exposure groups (Fig.2) moreover, the intracellular glucose content significantly increased in lead-exposed group. (P< 0.05).

4. Effect on key enzymes of the aerobic oxidation pathway

The key aerobic oxidation enzyme PDH and PDK1 was detected. Compared with the control group, the activity of PDH was reduced (P< 0.05), while the PDK1 protein expression was increased (P< 0.05) (Fig.3).

5. Effect on key enzymes of anaerobic oxidation pathway

Compared with the control VSMCs group, the activity of LDH and HK decreased in lead-treated VSMCs group ($P < 0.05$) (Fig.4), the PKM2 protein expression increased in lead-treated VSMCs group ($P < 0.05$). However, there was no significant difference in activity of PKM2 between control VSMCs group and 0.25 μM lead-treated VSMCs group ($P > 0.1$).

Discussion

Previous studies have shown that lead is toxic to the nervous system, hematopoietic system and digestive system[17]. However, recent animal studies and epidemiological surveys have shown that long-term lead exposure also increases the risk of cardiovascular disease[18,19,20]. For example, blood lead was found to be associated with left ventricular hypertrophy and peripheral arterial disease[21]. Lead exposure can cause elevated homocysteine level, oxidative stress, endothelial injury and inflammation, which can promote hypertension and cardiovascular diseases[11].

Under physiological conditions, the body will produce active oxygen such as H_2O_2, MDA, as well as antioxidant substances such as GSH, SOD. The body's oxidation system and antioxidant system maintain a dynamic balance[22]. The concentrations of ROS (such as superoxide anions, H_2O_2 and hydroxyl radicals) exceeding the antioxidant protection levels of cells can cause widespread damage to DNA, proteins and endogenous lipids[23]. Our results show that low level lead exposure can increase the concentration of ROS. At the same time, we found that the GSH concentration increased in the lead-exposed groups. GSH is one of the predominant endogenous antioxidants responsible for the detoxification of ROS, removal of lipid peroxides, and repair of oxidatively damaged proteins through a reaction catalysed by GPx[24]. Our results indicated that lead can cause oxidative stress damage to VSMCs and abnormal ROS production caused a compensatory increase in GSH.

The level of antioxidant defense system such as intracellular GSH mainly depends on the redox efficiency of the cell living environment. NADPH can meet the cellular needs for reductive biosynthesis and maintain the levels of GSH. The pentose phosphate pathway is the only way to produce NADPH, and G-6-PD is the first and rate-limiting enzyme involving in the control of the flux of glucose-6-phosphate through the pentose phosphate pathway[25]. Studies have shown that increased G6PD activity can weaken the effect of oxidative stress[26,27]. In the present study, the up-regulation of G-6-PD levels was detected in low level lead exposure groups but not in control groups. Meanwhile, the NADPH concentration increased in low-level lead exposure groups. These results show that low-level lead exposure increased the flow of the pentose phosphate pathway compared with the control group.

Although the flux of the pentose phosphate pathway increased, the results of the glucose test showed that glucose accumulation occurred. In addition to the pentose phosphate pathway, carbohydrate metabolisms also include aerobic and anaerobic oxidation. Therefore, we conducted further research on aerobic and anaerobic oxidation pathways.

During glycolysis, glucose is converted to glucose-6-phosphate by HK, and the activity of HK is mainly affected by the content of ADP and glucose-6-phosphate. Inhibition of HK activity will directly affect mitochondrial oxidative phosphorylation and anaerobic oxidation. In our study, lead was found to decrease the activity of HK in low-level lead exposed groups compared with the control groups. PKM converts phosphoenolpyruvate to pyruvate while releasing energy. PKM has four isoenzymes, in which PKM1 is expressed in normal cells, and PKM2 is highly expressed in proliferating cells, especially in abnormally proliferating tumor cells[28]. It is worth noting that the PKM2 concentration decreased in the low level lead exposure groups. The weakening of glycolytic process has been proved to be an important pathogenesis of cardiovascular disease[29]. In our study, the decreases in HK activity and PKM2 content suggest that low-level lead may result in inhibition of the glycolysis process.

Pyruvic acid is a pivotal substance associated with aerobic and anaerobic oxidation. Interfering with the progress and direction of pyruvate conversion will affect the relative flow of aerobic and anaerobic oxidation. Another central player in glycolysis is LDH, which is responsible for the conversion of pyruvate to lactate at the end step of the glycolytic process[30,31,32]. Our results show the low-level lead reduced the activity of LDH. This suggests that low levels lead exposure may result in a decrease in anaerobic oxidation levels. On the other hand, pyruvic acid forms acetyl-CoA into the aerobic oxidation moiety under the action of PDC (pyruvate dehydrogenase complex). PDH is an important component of PDC and is the rate-limiting enzyme in aerobic oxidation processes. The activity of PDH determines the proportion of oxidative phosphorylation in glucose metabolism. One of a central player in aerobic oxidation is PDKs, which negative regulate the activity of pyruvate dehydrogenase complex (PDC), an important gatekeeper enzyme that catalyzes the conversion of pyruvate to acetyl-CoA and links to the tricarboxylic acid cycle[33,34,35]PDKs has four isoenzymes, PDK1 and PDK2 are distributed in the heart, and the expression range of PDK3 and PDK4 is relatively limited[36]. In the present study, the low-level lead reduced the activity of PDH and increased PDK1 activity compared with the control groups. This means that low-level lead exposure can inhibit the aerobic oxidation of VSMCs.

Glucose is a common substrate for aerobic, anaerobic oxidation and pentose phosphate pathways. The glucose concentration increased in low-level lead exposure groups compared with the control groups, and it was found that low-level lead decreased aerobic and anaerobic oxidation and increased pentose phosphate pathway flow. Therefore, we speculate that because the inhibitory effect on aerobic and anaerobic oxidation is stronger than the enhancement effect on pentose phosphate pathway, it eventually leads to glucose accumulation. More importantly, the energy supply of cells mainly comes from aerobic oxidation and anaerobic oxidation. The inhibitory effect of low-level lead on them will inevitably lead to energy supply disorders of VSMCs.

Glucose, together with lipids and proteins, are the three major nutrients in the human body and maintain dynamic balance. Accumulation of glucose can cause disorders in the lipid and proteins metabolism of VSMCs. These are in agreement with a previous study, in which stage III heart failure patients had elevated levels of fatty acid oxidation and reduced glycolysis[29]. Li[37] et al. confirmed that cancers cells

transformed their mitochondria into synthesis machines supported by augmented glutaminolysis to supply lipid production, amino acid synthesis, and the pentose phosphate pathways, causing cancerous metabolic reprogramming. Polyzos[38] et al. also demonstrated that astrocytes in each brain region adapt by metabolically reprogramming their mitochondria to use endogenous, non-glycolytic metabolites as an alternative fuel, and mitochondria reprogramed by oxidizing fatty acids as an energy source but at the cost of escalating reactive oxygen species (ROS)-induced damage. The effect of low-level lead on lipid metabolism and protein metabolism of VSMCs deserves further study.

Interestingly, when the same study was performed on the 0.25 μM and 1 μM dose groups in this experiment, it was found that the GSH content in the 1 μM group increased compared with that in the control group, but the increase was less than that in the 0.25 μM group. Similarly, the content of glucose and NADPH in the 1 μM group increased compared with that in the control group, but the increase was less than that in the 0.25 μM group. HK activity in the 0.25 μM group was significantly reduced compared to that in the control group, but there was no significant change in HK activity in the 1 μM group. In addition, compared with the control group, there was no significant change in the expression of PKM2 protein in the 0.25 μM group, but its expression increased significantly in the 1 μM group. The mechanism of this anomalous result requires further study.

In conclusion, the results of the present study indicate that the low-level lead exposure leads to oxidative stress damage in VSMCs and enhances pentose phosphate pathway flow by improving the metabolic efficiency of the glutathione redox cycle, while disrupts the glycometabolism of VSMCs, inhibits anaerobic and aerobic oxidative processes, causing reprogramming of glycometabolism in VSMCs. These findings may be the molecular mechanism for cardiovascular disease induced by low-level lead.

Abbreviations

G-6-PD: glucose-6-phosphate dehydrogenease

GSH: reduced glutathione

HK: hexokinase

LDH: Lactic dehydrogenase

NADPH: nicotinamide adenine dinucleotide phosphate

PBS: phosphate buffer saline

PDH: pyruvate dehydrogenase

PDK1: Pyruvate Dehydrogenase Kinase Isozyme 1

PKM2: Pyruvate kinase isozyme type M2

ROS: Reaction oxygen species

VSMCs: vascular smooth muscle cells

Declarations

The author contributions

Tong-jia Zhang, Ya-shu Liu, Jin-sa Zhou, Wen-xiu Hu, Huan Zhang and Wan-ying Shi conducted the experiment. Li-hui Xu,Huan-ren Duan and Yu Zhang worte the manuscript. Su-ju Sun revised the manuscript.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Statements and Declarations

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References

1. He, L., Chen, Z., Dai, B., Li, G., Zhu, G., 2018. Low-level lead exposure and cardiovascular disease: the roles of telomere shortening and lipid disturbance. J. Toxicol. Sci. 43, 623-630.
2. Lanphear, B.P., Rauch, S., Auinger, P., 2018. Low-level lead exposure and mortality in US adults: a population-based cohort study. Lancet Public Health. 3, e177-e184.
3. Lv, Y., 2014. A study of current situations and countermeasures on blood lead level of children in China. Nanhua University. R179.
4. Zhang, M., Gu, X., 2014. Analysis of blood lead detection results in 600 adults. Chongqing Medical. 43, 480-481.
5. Emmanuel, O.-G., 2020. Cumulative Effects of Low-Level Lead Exposure and Chronic Physiological Stress on Hepatic Dysfunction-A Preliminary Study. INT J MED SCI. 8(3).
6. Jonaina, F., Maylla, R.S., Bruna, F.D.A., Rogério, F.R.J., Leonardo, D.S., Alessandra, S.P., Dalton, V.V., 2020. Increased endothelial nitric oxide production after low level lead exposure in rats involves activation of angiotensin II receptors and PI3K/Akt pathway. Toxicology. 443.
7. Lanphear, B.P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D.C., Canfield, R.L., Dietrich, K.N., Bornschein, R., Greene, T., 2005. Low-level environmental lead exposure and children's

intellectual function: An international pooled analysis. Environ. Health Perspect.. 113, 894.

8. Vorvolakos, T., Arseniou,S., Samakouri, M., 2016. There is no safe threshold for lead exposure: A literature review. Psychiatriki. 27, 204-214.
9. Gambelunghe, A., Sallsten, G., Borné, Y., Forsgard, N., Hedblad, B., Nilsson, P., Fagerberg, B., Engström, G., Barregard, L., 2016. Low-level exposure to lead, blood pressure, and hypertension in a population-based cohort. Environ. Res. 149, 157-163.
10. Xu, LH., 2015. Lead Induces Apoptosis and Histone Hyperacetylation in Rat Cardiovascular Tissues. PLoS One. 10(6):e0129091.R114.
11. Vaziri, N.D., 2008. Mechanisms of lead-induced hypertension and cardiovascular disease. Am. J. Physiol.-Renal. 295, H454-465.
12. Gonick, H.C., Ding, Y., Bondy, S.C., Ni, Z., Vaziri, N.D., 1997. Lead-Induced Hypertension Interplay of Nitric Oxide and Reactive Oxygen Species. Hypertension. 30, 1487-1492.
13. Sirivarasai, J., Kaojarern, S., Chanprasertyothin, S., Panpunuan, P., Petchpoung, K., Tatsaneeyapant, A., Yoovathaworn, K., Sura, T., Kaojarern, S., Sritara, P., 2015. Environmental Lead Exposure, Catalase Gene, and Markers of Antioxidant and Oxidative Stress Relation to Hypertension: An Analysis Based on the EGAT Study. *Biomed* Res. Int. 2015, 856319.
14. Yang, H.-C., Wu, Y.-H., Liu, H.-Y., Stern, A., Chiu, D.T., 2016. What has passed is prolog: New cellular and physiological roles of G6PD. Free Radic. Res. 50, 1047–1064.
15. Peiró, C., Romacho, T., Azcutia, V., Villalobos, L., Fernández, E., Bolaños, J.P., Moncada, S., Sánchez-Ferrer, C.F., 2016. Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. Cardiovasc. Diabetol. 15, 82.
16. Zhang, H., Chan, Y., Li, Y., Yang, L., Wang, J., Wang, T., 2014. Medium-chain TAG attenuate hepatic oxidative damage in intra-uterine growth-retarded weanling piglets by improving the metabolic effificiency of the glutathione redox cycle. Brit. J. Nutr. 112, 876–885
17. Lopes, A.C.B.A., Peixe, T.S., Mesas, A.E., Paoliello, M.M.B., 2016. Lead Exposure and Oxidative Stress: A Systematic Review. Rev. Environ. Contam. T. 236, 193-238.
18. Yang, W.-Y., Zhang, Z.-Y., Thijs, L., Cauwenberghs, N., Wei, F.-F., Jacobs, L., Luttun, A., Verhamme, P., Kuznetsova, T., Nawrot, T.S., Staessen, J.A., 2017. Left Ventricular Structure and Function in Relation to Environmental Exposure to Lead and Cadmium. J. Am. Heart Assoc. 6, e004692.
19. Khalil, N., Wilson, J.W., Talbott, E.O., Morrow, L.A., Hochberg, M.C., Hillier, T.A., Muldoon, S.B., Cummings, S.R., Cauley, J.A., 2009. Association of blood lead concentrations with mortality in older women: A prospective cohort study. Environmental Health-Glob. 8, 15.
20. Kaewboonchoo, O., Morioka, I., Saleekul, S., Miyai, N., Chaikittiporn, C., Kawai, T., 2010. Blood Lead Level and Cardiovascular Risk Factors among Bus Drivers in Bangkok, Thailand. Ind. Health. 48, 61-65.
21. Ana, N.-A., Elizabeth, S., A.Richey, S., Emma, C.-A., Ellen, S., Eliseo, G., 2004 Lead, cadmium, smoking, and increased risk of peripheral arterial disease. Circulation. 109:3196–201.

22. Durak, I., Bingöl, N.K., Avci, A., Cimen, M.Y., Kaçmaz, M., Karaca, L., Oztürk, H.S., 2000. Acute effects of smoking of cigarettes with different tar content on plasma oxidant/antioxidant status. Inhal. Toxicol. 12, 641–647.
23. Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74, 139–162.
24. Fang, Y.-Z., Yang, S., Wu, G., 2002. Free radicals, antioxidants, and nutrition. Nutrition. 18, 872–879.
25. Cramer, C.T., Cooke, S., Ginsberg, L.C., Kletzien, R.F., Stapleton, S.R., Ulrich, R.G., 1995. Upregulation of glucose-6-phosphate dehydrogenase in response to hepatocellular oxidative stress: studies with diquat. J. Biochem. Toxicol. 10, 293–298.
26. Zhang, Z., Yang, Z., Zhu, B., Hu, j., Liew, C. W., Zhang, Y., Leopold, A J., Handy, E D., Loscalzo, J., Stanton, C R., 2017. Increasing glucose 6-phosphate dehydrogenase activity restores redox balance in vascular endothelial cells exposed to high glucose. Plos One. 7(11).
27. Sanz, N., Díez-Fernández, C., Valverde, A.M., Lorenzo, M., Benito, M., Cascales, M., 1997. Malic enzyme and glucose 6-phosphate dehydrogenase gene expression increases in rat liver cirrhogenesis. Br. J. Cancer. 75(4):487-492.
28. Zhang, D., 2016. The research of Glycolysis of Hepatocellular Carcinoma To Growth and Proliferation. Fujian Medical University. R735.7.
29. Paolisso, G., Gambardella, A., Galzerano, D., D'Amore, A., Rubino, P., Verza, M., Teasuro, P., Varricchio, M., D'Onofrio, F., 1994. Total-body and myocardial substrate oxidation in congestive heart failure. Metabolism. 43, 174-179.
30. Zhang, S.-L., He, Y., Tam, K. Y., 2018. Targeting cancer metabolism to develop human lactate dehydrogenase 5 (hLDH5) inhibitors. Drug Discov. Today.
31. Reshma, R., Vinit, K., 2016. Recent update on human lactate dehydrogenase enzyme 5 (hLDH5) inhibitors: a promising approach for cancer chemotherapy J. Med. Chem., 59 (2016), pp. 487-496.
32. Hou, X.-M., Yuan, S.-Q., Zhao, D., Liu, X.-J., Wu, X.-A., 2019. LDH-A promotes malignant behavior via activation of epithelial-to-mesenchymal transition in lung adenocarcinoma. Biosci. Rep.. 39(1).
33. Zhang, S.-L., Hu, X., Zhang, W., Yao, H., Tam, K. Y., 2015. Development of pyruvate dehydrogenase kinase inhibitors in medicinal chemistry with particular emphasis as anticancer agents. Drug Discov. Today. 20(9):1112-9.
34. Zhang, W., Zhang, S.-L., Hu, X., Tam, K. Y., 2015. Targeting tumor metabolism for cancer treatment: is pyruvate dehydrogenase kinases (PDKs) a viable anticancer target? Int. J. Biol. Sci.. 11(12):1390-400.
35. Sun, W. X., Xie, Z., Liu, Y., Zhao, D., Wu, Z., Zhang, D.D., Lv, H., Tang, S., Jin, N., Jiang, H., Tan, M., Ding, J., Luo, C., Li, J., Huang, M., Geng, M., 2015. JX06 Selectively inhibits pyruvate dehydrogenase kinase PDK1 by a covalent cysteine modification. Canc. Res.. 75(22):4923-36.
36. Zhong, Y., 2016. Upregulated glycolysis regulates the stemness phenotypes of prostate cancer cell. Zhengzhou University. R737.25.

37. Li, C., Zhang, G., Zhao, L., Ma, Z., Chen, H., 2016. Metabolic reprogramming in cancer cells: glycolysis, glutaminolysis, and Bcl-2 proteins as novel therapeutic targets for cancer. World. J. Surg. Oncol. 14, 15.
38. Polyzos, A.A., Lee, D.Y., Datta, R., Hauser, M., Budworth, H., Holt, A., Mihalik, S., Goldschmidt, P., Frankel, K., Trego, K., Bennett, M.J., Vockley, J., Xu, K., Gratton, E., McMurray, C.T., 2019. Metabolic Reprogramming in Astrocytes Distinguishes Region-Specific Neuronal Susceptibility in Huntington Mice. Cell Metab. 29, 1258-1273. e11.

Figures

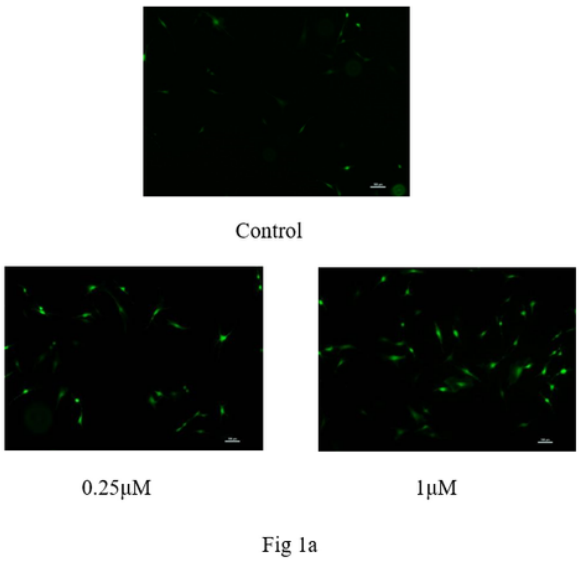

Fig 1a

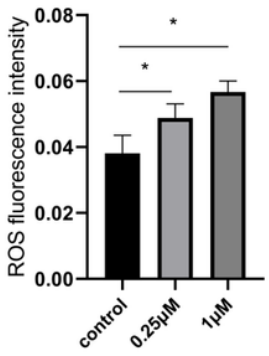

Fig 1b

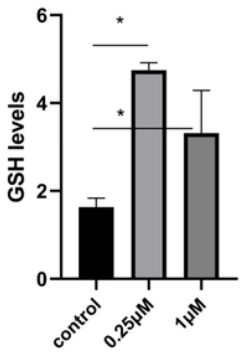

Fig 1c

Figure 1

Low-level lead promoted the production of ROS and GSH. a: ROS fluorescence imaging of VSMCs in control group and the low-level lead exposure groups. b: Statistical legend of ROS fluorescence intensity. c: Statistical legend of reduced glutathione content. Data are shown as the mean ± SD , *P< 0.05.

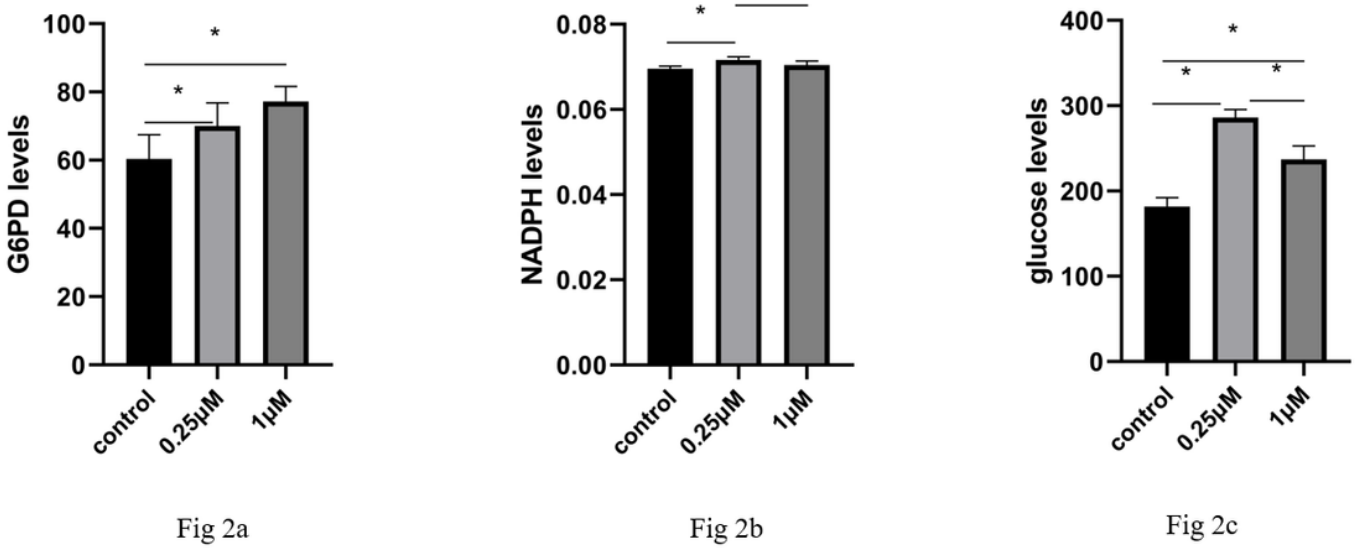

Fig 2a Fig 2b Fig 2c

Figure 2

Low-level lead increased pentose phosphate pathway flux and caused glucose accumulation. a: Statistical legend of the G6PD activity of VSMCs in the control group and the low-level lead exposure groups. b: Statistical legend of the NADPH content of VSMCs in the control group and the lead exposure groups. c: Statistical legend of the glucose content of VSMCs in the control groups and the lead exposure groups. Data are shown as the mean ± SD, *P< 0.05.

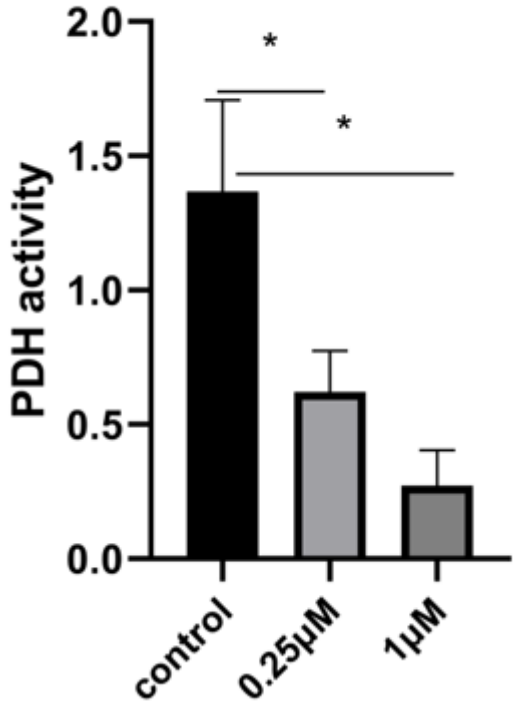

Fig 3a

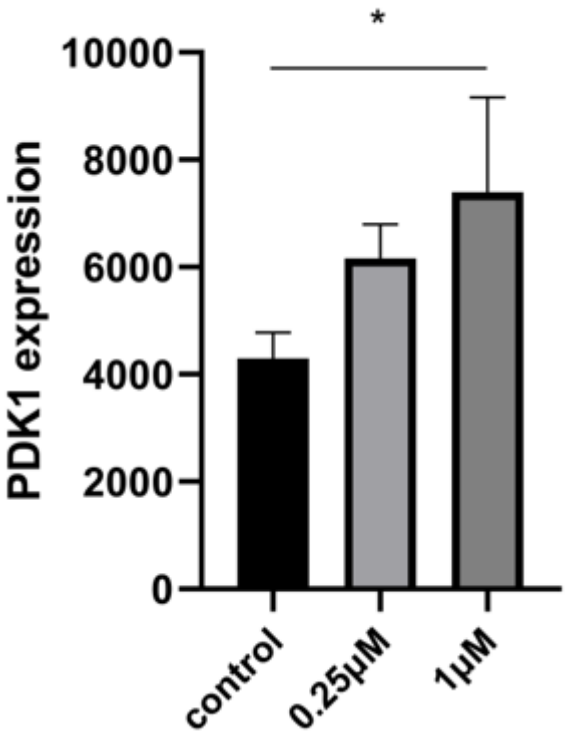

Fig 3b

Figure 3

Low-level lead inhibited aerobic oxidation of VSMCs. a: Statistical legend of PDH activity of VSMCs in control group and lead exposure groups. b: The changes of PDK1 during VSMCs by Western-blot. Data are shown as the mean ± SD, *P< 0.05.

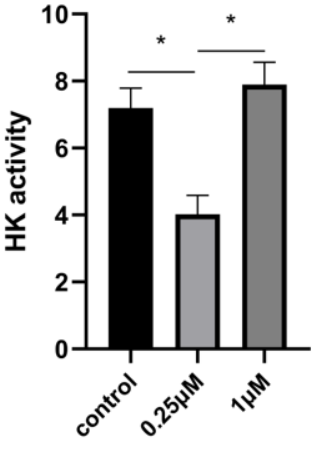

Fig 4a

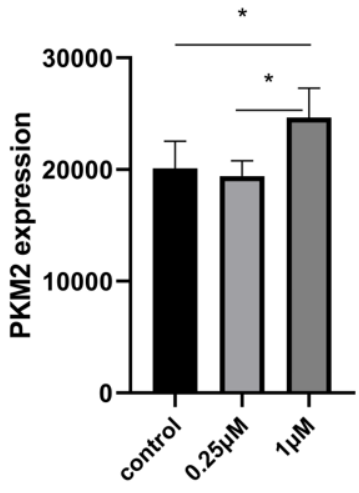

Fig 4b

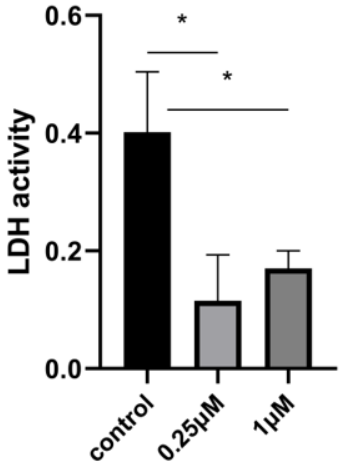

Fig 4c

Figure 4

Low-level lead inhibited anaerobic oxidation of VSMCs. a Statistical legend of HK activity of VSMCs in control group and lead exposure groups. b: The changes of PKM2 during VSMCs by Western-blot. c: Statistical legend of LDH activity of VSMCs in control group and lead exposure groups. Data are shown as the mean ± SD, *P< 0.05.

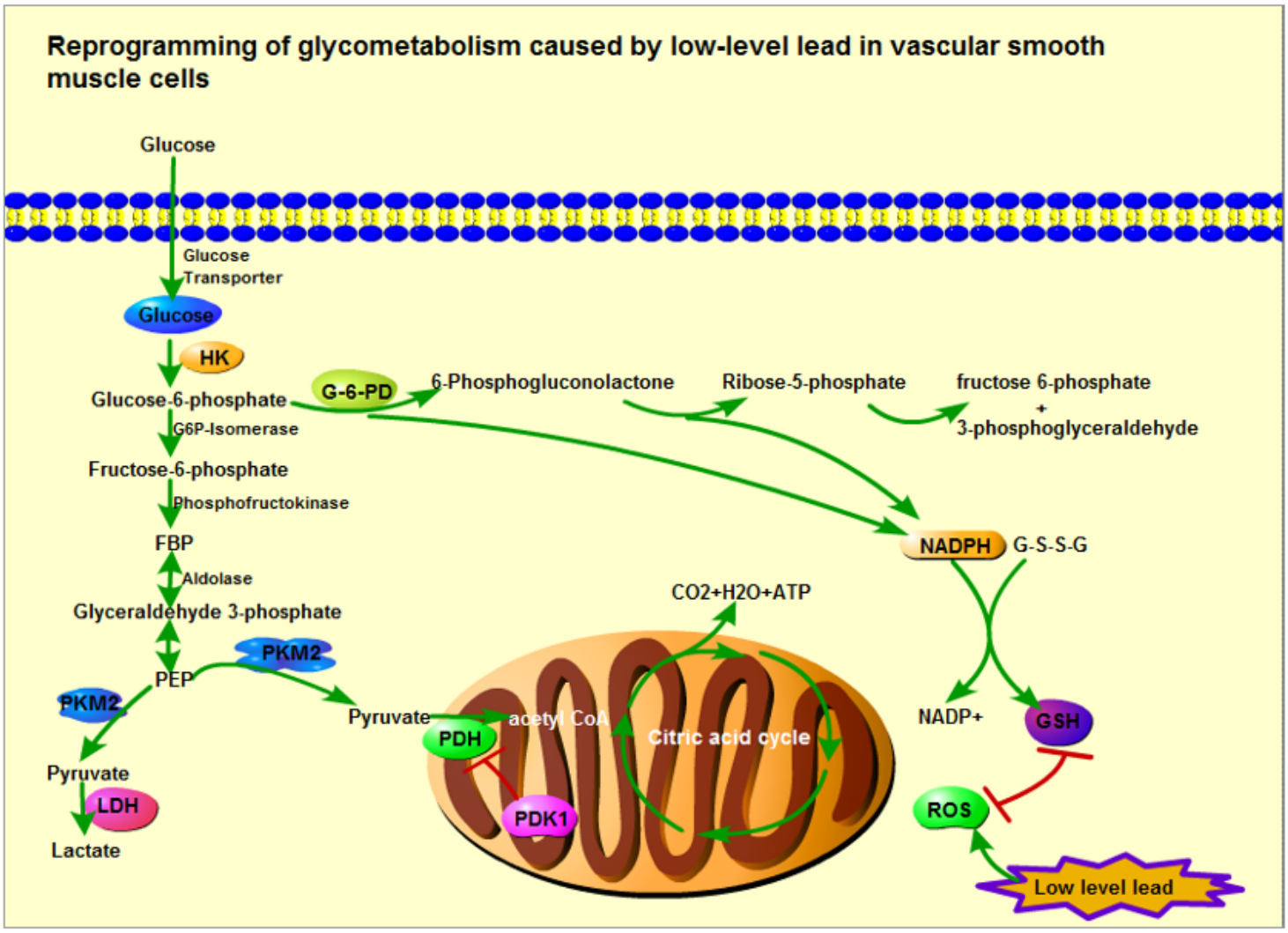

Figure 5

Schematic diagram about reprogramming of glycometabolism in vascular smooth muscle cells. The molecules studied in this article are marked with colored molecular markers. Compared with the control group, low-level lead exposure caused oxidative stress in VSMCs, which increased the intracellular ROS and GSH content. At the same time, low levels of lead inhibited the activity of HK and LDH, reduced the expression of PKM2, and significantly inhibited glycolysis and anaerobic oxidation. In addition, low level lead exposure decreased the PDH activity and increased PDK1 expression level, significantly inhibiting the aerobic oxidation process. The pentose phosphate pathway is promoted to increase the production of NADPH by increasing the activity of G6PD to accelerate the reduction of GSSH to produce GSH to participate in anti-oxidation.