DOI: https://doi.org/10.53350/pjmhs22162603

**ORIGINAL ARTICLE**

# Effect of Lead on the Bioenergetics of the Isolated Human Monocytes

ULFAT M. OMAR[1], EKRAMY M. ELMORSY[2,3]
*[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia*
*[2]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University, Mansoura, Egypt, and [3]Pathology Department, Faculty of Medicine, Northern Border University, Arar, Saudi Arabia*
*Corresponding authors: Ekramy Elmorsy, Email: ekramyelmorsy@mans.edu.eg, Cell: 00966501275835*

## ABSTRACT

**Objectives**: Lead (Pb) is widely-occurring metals with known immunotoxic properties that are frequently found in the environment. The current study was conducted to evaluate the lead induced bioenergetic disruption as underling mechanism of its immunotoxic effect on the isolated human peripheral blood monocytes (hPBM).

**Methods:** The cytotoxic effect of Pb on hPBM was evaluated by MTT assay using concentration range from o.1 to 1000 µM. The effect of lead on the phagocytic activity of the isolated cells was evaluated. Finally, the effect of lead on the bioenergetics of hPBM was evaluated by studying its effect on ATP production as well as the activities of mitochondrial complex I and III. Finally the protective effect of co-enzyme Q10 on the hPBM phagocytic activities was studied.

**Results:** Data showed that Pb was toxic to the isolated cells in a concentration and exposure duration dependent pattern with parallel decreases in the phagocytic activities of the isolated hPBM. Lead was shown to cause significant decreases in ATP production in the exposed hPBM with significant inhibitory effect on the mitochondrial complex I and III activities. Interestingly, co-enzyme Q10 showed significant protective effect on the isolated cells phagocytic activities.

**Conclusion:** It can be concluded that some of the immunotoxic effects that the metals Pb can impart on the functionality of isolated hPBM (i.e., phagocytic activity,) may be secondary to an induced disruption of hPBM cell bioenergetics.

**Keywords:** Heavy metals, monocytes, lead, mitochondria, immunotoxicity

## INTRODUCTION

Heavy metals, such as lead (Pb), are able to deposit in tissues and cause a variety of toxicities [1]. In some cases, the toxicities can be produced as a result of interactions between the metals and various proteins [2]. The immune system is complex and has become adapted to respond in precise manners to various antigens, pathogens, and the like. Because metals, in some cases as a result of their interactions with immune response proteins, can positively or negatively influence the functions of various immune cells [3], it is thus not surprising that immunotoxic manifestations like hypersensitivity and autoimmunity have been repeatedly observed in workers and in animal models following exposures to exposed to heavy metals [3-5] . In other cases, there is an immunosuppression that is induced. For instance, workers exposed to Pb were found to have a higher incidence of common cold and influenza infections - accompanied by lower blood levels of immunoglobulins - when compared to non-metal-exposed persons[6]. While higher levels of immunoglobulin E, which plays a major role in the allergic symptoms, was reported among children with high exposure to Pb[7].

Mitochondria play a key role in regulating the responses of monocytes and macrophages to pathogens and tissue injury. Environmental metals, such as lead, cadmium, and mercury were shown to be accumulated in mitochondria in higher rates than the other cellular organelles[8,9] . Interestingly, monocytes mitochondria disruption was suggested to play a role in pathogenesis of certain systemic diseases as atherosclerosis and chronic kidney disease (CKD) via induction of chronic inflammatory response [10,11]. Therefore, the current study had evaluated the cytotoxic effect of Pb on the isolated human monocytes. The effect Pb on cells phagocytic activity of the cells was studied. Then Pb effect on the isolated cells mitochondrail complexes I and III was evalted HMs induced immunotoxicity on the mitochondria of the isolated cells was evaluated using different assays.

## MATERIALS AND METHODS

**Peripheral blood mononuclear cells (PBMC) isolation and culture:** Human peripheral blood was obtained by venipuncture under aseptic conditions at the university medical center from one of the authors. Peripheral blood mononuclear cells (PBMC) from each collected sample were isolated by centrifugation (400 x g, 30 min) of each over a Ficoll-Paque Plus density gradient (Amersham Biosciences, Little Chalfont, UK), as described previously [12]. Finally, the harvested cells from each flask were then washed again and cell viability then was ascertained using Trypan blue exclusion (routinely, viability was ≥ 95%). Thereafter, the cells were pooled for use in the assays outlined below.

**Metals effects on isolated human monocyte viability (MTT assays):** The overall toxicity of the test lead compounds on the isolated monocytes was evaluated using MTT (3-(4, 5-dimethylthiazol, 2-yl)-2, 5-diphenyl tetrazolium bromide) assay. The pooled cells were seeded into 96-well plates at a density of 5 x $10^4$ cells/well and incubated overnight at 37°C. Thereafter, the medium was removed from each well and the cells then treated with lead nitrate ($Pb[NO_3]_2$) (Sigma, St Louis, MO, USA) in concentrations (e.g., 0.1, 1, 10, 100, and 1000 µM) in dedicated wells. Here, the plates were then incubated at 37°C with the test metals (or medium control) for 3, 6, 12, 24, 48 or 72 hr. Dedicated plates were generated for each time point and for each assay to be performed. At each timepoint, the MTT assay was conducted on a plate as

outlined by Elmorsy et al. (2014)[13]. All values in wells in each assay were measured in an MRX microplate reader (Dyne Technologies, VA, USA) at 590 nm. Blank wells (without cells) were subtracted from all wells' readings before further analysis. The experiments were conducted in triplicates

**Phagocytosis assay:** The pooled monocytes were cultured at density of $10^6$ cells/ml in 6-well plates and then exposed for 6 hr to the PB and Cd at concentrations of 0, 0.1, 1, 10, and 100 µM. Then the phagocytic ability of the treated cells was evaluated using the method of Schroeder and Kinden (1983)[14]. The fluorescence of the cells was measured at an excitation/emission wavelength at 440/485 nm, respectively. Trypan blue exclusion assay was used to follow monocytes viability during phagocytosis. Viability was estimated to be 95 ± 5% throughout the experiment.

**Effect of Pb on ATP production:** The isolated hPBM were seeded in the 96-well plate and treated with Pb as shown in MTT assay. The effect of Pb on ATP production was conducted using a commercial kit , following the manufacturer protocol (Abcam, Cambridge, MA, USA). Sample luminescence in each well was detected by MRX microplate reader (Dyne Technologies, VA, USA). Basal values of the medium luminescence were subtracted from each well estimated value then monocytes ATP content in each well was represented as a percentage from control wells readings.

**Effects of Pb on mitochondrial complex activity**: Effects of the Pb on the mitochondrial complexes I and III in the isolated monocytes were assessed. Cells were incubated for 24 hours with tested metals at concentration of 0.1, 1, 10, and 100 µM.

For complex I assay, monocytes mitochondrial enriched fraction (for complex I assay) was prepared following Spinazzi et al. (2012)[15]. The mitochondrial solution was subjected to three cycles of freeze-thawing in liquid nitrogen to disrupt the mitochondrial membranes just before use. For complex III assay, cells lysate (for complex III assays) was prepared according to (Spinazzi et al. 2012) protocol[15]. Protein concentration was measured using Bradford assay.

Before complex I assay, the mitochondrial enriched fraction was exposed to three cycles of liquid nitrogen freeze-thawing to disrupt the mitochondrial membranes. NADH (100 µM) and ubiquinone (60 µM) were used as terminal electron acceptors for complex I assay, while Decylubiquinol (100 µM) and cytochrome c (75 µM) were the electron acceptors for complex III assay. The absorbance was read at 340 and 550 nm wavelength for complexes I and III, respectively. Rotenone (10 µM) and potassium cyanide (300 µM) were used as specific inhibitors for calibration of the activity of the mitochondrial complexes I and III, respectively. The specific complex enzyme activity was calculated using the equation (nmol min-1 mg-1) = (Δ Absorbance/min × 1,000)/[(extinction coefficient × volume of sample used in ml) × (sample protein concentration in mg ml-1)]. Extinction coefficient was calculated as 6.2 mM-1 Cm-1 for NADH for complex I activity and 18.5 2 mM-1 Cm-1 for reduced cytochrome c for complex III activity. Experiments were repeated at least 5 times for data robustness.

**Effect of co-enzyme Q10 (CO-Q10) on mitochondrial bioenergetics:** To test if co-enzyme Q10 (CO-Q10), previously reported to significantly enhance mitochondrial bioenergetics parameters[16], might provide similar protections in the treated monocytes, cells were seeded into 6-well plates as shown in the phagocytosis assay section and then received medium containing 1 µM water-soluble Co-Q10 formulation (Q-ter) (Scharper Therapeutics, Milan, Italy) . After incubation at 37°C for 24 hr, the test metal solutions were added at concentrations of 0, 0.1, 1, 10, or 100 µM and the plate incubated for a further 24 hr. In some cases, the CO-Q10 was removed at the time the metals were introduced; in other, the CO-Q10 remained over the course of the metal exposure. At that point, phagocytosis assays was performed.

**Statistical analysis**: One-way or two-way analysis of variance (ANOVA) with a Dunn's or Bonferroni multiple comparisons post-hoc test were used when appropriate. An unpaired Student's t-test was used for comparisons of data from two different groups. All statistical analyses were performed using Prism 5 (GraphPad, San Diego, CA). Statistical significance was defined as $p < 0.05$.

## RESULTS

The current work was conducted to study the cytotoxic effect of Pb on the isolated human monocytes and their functions related to phagocytic activities. Then, the effect of the studied metals on the isolated cells mitochondrial bioenergetics was evaluated. Firstly, MTT assay showed that Pb and Cd were cytotoxic to the isolated human monocytes in concentrations and exposure duration dependent patterns (Figure 1). MTT showed that Pb was not cytotoxic in concentration of 0.1 µM at all tested time-points, while Pb was cytotoxic in (1 µM) 48 hours post-exposure.

Functionally, the effect of Pb on the phagocytic capacity of the isolated human monocytes was evaluated. Results showed a significant decrease in the phagocytosis of indicator test particles in concertation and time durations dependent patterns (Figure 2). Data of two-way ANOVA are shown in table S1.

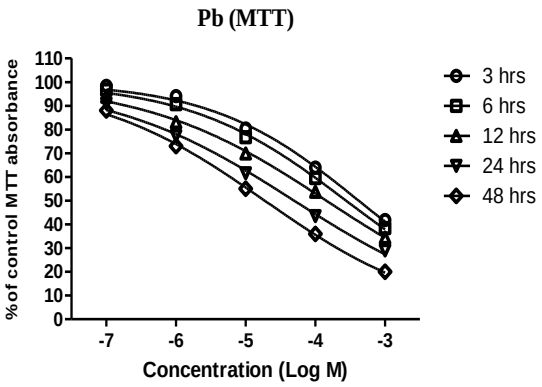


Figure 1: Effect of lead (Pb) on isolated human peripheral blood monocyte (hPBM) survival in culture. Cells were treated with indicated doses of lead nitrate or cadmium dichloride for 3, 6, 12, 24, or 48 hr. All assays were performed in triplicate, with each metal concentration tested in 3 wells/experiment.

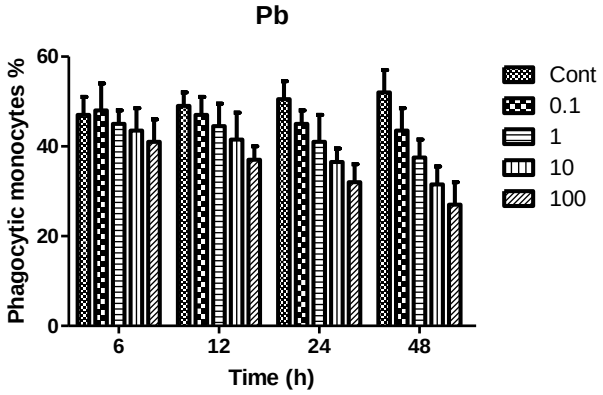


Figure 2: Effect of Pb on hPBM phagocytic capacity. The assay measured the uptake of test fluorescent latex 1-µm beads in presence or absence of Pb or Cd at 0, 0.1, 1, 10, or 100 µM after 6 hours exposure. Experiments were conducted in triplicate. Two-way ANOVA was used to assess combined effects of concentration and exposure duration. *p < 0.05, **p < 0.01, ***p < 0.0001.

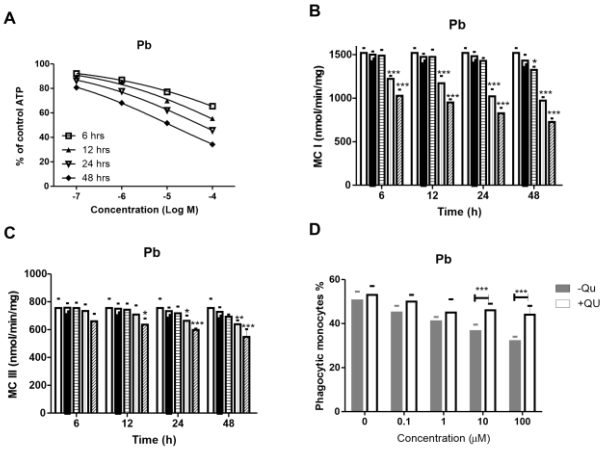


Figure 3: Effect of Pb on bioenergetics of the human peripheral blood isolated monocytes. 3A shows the effect of Pb on ATP production. Cells were treated with test agents at 0, 0.1, 1, 10, 100, or 1000 µM for 3, 6, 12, 24, and 48 hr. 3B and 3C show Effect of Pb on hPBM mitochondrial complex I and III activities and cell lactate production. Cells were exposed to the test agents at 0, 0.1, 1, 10, or 100 µM for 6, 18, 24, and 48 hr. 3D shows Protective effect of Co-enzyme Q10 (CO-Q10) against metal-induced functional disruption in the hPBM. All Experiments were conducted in triplicate. *p < 0.05, **p < 0.01, ***p < 0.0001.

Table (s1): Effects of Pb on secretory and phagocytic functions of the human monocytes.

| Cytokines | Two-way ANOVA Source of Variation | Pb | |
|---|---|---|---|
| | | % of total variation | P value |
| MCI | Interaction | 3.03 | < 0.0001 |
| | Concentration | 87.84 | < 0.0001 |
| | Exposure duration | 5.11 | < 0.0001 |
| MCIII | Interaction | 4.55 | 0.0717 |
| | Concentration | 50.08 | < 0.0001 |
| | Exposure duration | 9.66 | < 0.0001 |

Table (s.2): Effects of Pb on bioenergetics and mitochondrial parameters in the monocytes

| Cytokines | Two-way ANOVA Source of Variation | Pb | |
|---|---|---|---|
| | | % of total variation | P value |
| Phagocytosis | Interaction | 10.65 | < 0.0001 |
| | Concentration | 48.94 | < 0.0001 |
| | Exposure duration | 10.94 | < 0.0001 |

Lead was found to significantly decreased the isolated cells ATP production in a concentration and exposure duration dependent pattern (Figure 3A). Regarding complexes' assays, results showed that Pb (in 10 and 100 µM) significantly decreased complexes I activities as early as 6 hours post exposure (Figure 3B). At 1 µM concentration, Pb showed a significant inhibitory effect in the treated monocytes at 48 hours' time point. In addition, Pb showed lowered complex III activity in concentration 100 µM 12 hours after exposure to variable degrees, while PB showed significant inhibitory effect 24 hours after exposure at 10 µM concentration (Figure 3C). Data of two-way ANOVA are shown in table S2 to show the effect of concentration, exposure duration and the interaction between both variables. Interestingly co-enzyme Q10 significantly (P-value <0.0001) protect the phagocytic activity of Pb-exposed hPBM (Figure 3D)

## DISCUSSION

The current study was conducted to evaluate the effects of widely-encountered environmental metal Pb on the bioenergetics of isolated human monocytes. The effect of the metals on the bioenergetics of the isolated human monocytes was investigated using wide range of concentrations, which is covering their reported normal reference and toxic blood and serum levels [17,18]. As these metals are known to be non-degradable and accumulated in the human tissues, higher concentrations were used which may mimic the effect of their chronic prolonged exposure within the limited time frame of the in vitro studies. Pb was shown to significantly decrease monocytes cells mitochondrial complexes activities.

The observed inhibitory effects of the Pb on monocyte bioenergetics was in accordance with results from previous studies. Hon et al. (2017)[19] showed that Pb could affect cellular respiration, the electron transport chain, and mitochondrial morphology as part of the mechanisms underlying Pb-induced toxicities like encephalopathy in children. Other studies reported that Pb was able to inhibit the mitochondrial electron transport chain and cause mitochondrial transition pores to open, resulting in cytochrome c release, caspase activation, and apoptosis[20]. Moreover, Al-Ghafari et al. (2019)[21] reported that Cd and Pb were each inhibitors of mitochondrial complexes I and III in human osteoblasts.

The current data are clinically significant as it highlights the hazardous effect of the naturally occurring metals Pb as immunotoxic agents. In addition, the present study showed that metals can inhibit mitochondrial bioenergetics significant decrease in ATP production which has very crucial impact on the functions of the monocytes in health and disease[22]. In parallel, the mitochondrial impairment is expected to stimulate signaling pathways for

oxidative damage and apoptosis which is expected to seriously affect the innate immunity mechanisms[23]. Also, the current data showed that the effect of metals on mammalian mitochondria from different tissue is generic in different species. In addition, the current findings highlight the importance of bioenergetics study to evaluate the harmful effect of the different environmental pollutants.

## CONCLUSION

Lead is cytotoxic to hPBM cells in a concentration and exposure duration dependent manner. Pb-induced bioenergetics disruption which is suggested to play a major role in Pb- induced immunotoxic effect. Further in-vivo and in-vitro studies are recommended to evaluate the current study findings.

**Conflict of interest:** The authors declare no conflict of interest regarding the published results.

## REFERENCES


1. Kabir MT, Uddin MS, Zaman S, Begum Y, Ashraf GM, Bin-Jumah MN, Bungau SG, Mousa SA, Abdel-Daim MM. Molecular mechanisms of metal toxicity in the pathogenesis of Alzheimer's disease. Molecular Neurobiology. 2021 Jan;58(1):1-20.
2. Ali S, Awan Z, Mumtaz S, Shakir HA, Ahmad F, Ulhaq M, Tahir HM, Awan MS, Sharif S, Irfan M, Khan MA. Cardiac toxicity of heavy metals (cadmium and mercury) and pharmacological intervention by vitamin C in rabbits. Environmental Science and Pollution Research. 2020 Aug;27:29266-79.
3. Marth EG, Jelovcan SA, Kleinhappl BA, Gutschi AN, Barth SO. The effect of heavy metals on the immune system at low concentrations. International journal of occupational medicine and environmental health. 2001 Jan 1;14(4):375-86.
4. Ebrahimi M, Khalili N, Razi S, Keshavarz-Fathi M, Khalili N, Rezaei N. Effects of lead and cadmium on the immune system and cancer progression. Journal of Environmental Health Science and Engineering. 2020 Jun;18(1):335-43.
5. Pesce G, Sesé L, Calciano L, Travert B, Dessimond B, Maesano CN, Ferrante G, Huel G, Prud'Homme J, Guinot M, Soomro MH. Foetal exposure to heavy metals and risk of atopic diseases in early childhood. Pediatric Allergy and Immunology. 2021 Feb;32(2):242-50.
6. Ewers U, Stiller-Winkler R, Idel H. Serum immunoglobulin, complement C3, and salivary IgA levels in lead workers. Environmental research. 1982 Dec 1;29(2):351-7.
7. Lutz PM, Wilson TJ, Ireland J, Jones AL, Gorman JS, Gale NL, Johnson JC, Hewett JE. Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. Toxicology. 1999 May 3;134(1):63-78.
8. Alkharashi NA, Periasamy VS, Athinarayanan J, Alshatwi AA. Cadmium triggers mitochondrial oxidative stress in human peripheral blood lymphocytes and monocytes: Analysis using in vitro and system toxicology approaches. Journal of Trace Elements in Medicine and Biology. 2017 Jul 1;42:117-28.
9. Ommati MM, Jamshidzadeh A, Heidari R, Sun Z, Zamiri MJ, Khodaei F, Mousapour S, Ahmadi F, Javanmard N, Yeganeh BS. Carnosine and histidine supplementation blunt lead-induced reproductive toxicity through antioxidative and mitochondria-dependent mechanisms. Biological trace element research. 2019 Jan 1;187(1):151-62.
10. Hénaut L, Candellier A, Boudot C, Grissi M, Mentaverri R, Choukroun G, Brazier M, Kamel S, Massy ZA. New insights into the roles of monocytes/macrophages in cardiovascular calcification associated with chronic kidney disease. Toxins. 2019 Sep;11(9):529.
11. Orekhov AN, Nikiforov NN, Ivanova EA, Sobenin IA. Possible role of mitochondrial DNA mutations in chronification of inflammation: Focus on atherosclerosis. Journal of clinical medicine. 2020 Apr;9(4):978.
12. Reis SR, Sampaio AL, Henriques MD, Gandini M, Azeredo EL, Kubelka CF. An in vitro model for dengue virus infection that exhibits human monocyte infection, multiple cytokine production and dexamethasone immunomodulation. Memorias do Instituto Oswaldo Cruz. 2007;102:983-90.
13. Elmorsy E, Elzalabany LM, Elsheikha HM, Smith PA. Adverse effects of antipsychotics on micro-vascular endothelial cells of the human blood–brain barrier. Brain research. 2014 Oct 2;1583:255-68.
14. Schroeder F, Kinden DA. Measurement of phagocytosis usinf fluorescent latex beads. Journal of biochemical and biophysical methods. 1983 Aug 1;8(1):15-27.
15. Spinazzi M, Casarin A, Pertegato V, Salviati L, Angelini C. Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. Nature protocols. 2012 Jun;7(6):1235-46.
16. Bergamini C, Moruzzi N, Sblendido A, Lenaz G, Fato R. A water soluble CoQ10 formulation improves intracellular distribution and promotes mitochondrial respiration in cultured cells. PloS one. 2012 Mar 14;7(3):e33712.
17. Geraldine M, Venkatesh T. Lead poisoning as a result of infertility treatment using herbal remedies. Archives of gynecology and obstetrics. 2007 Apr;275(4):279-81.
18. Chakraborty S, Dutta AR, Sural S, Gupta D, Sen S. Ailing bones and failing kidneys: a case of chronic cadmium toxicity. Annals of clinical biochemistry. 2013 Sep;50(5):492-5.
19. Hon KL, Fung CK, Leung AK. Childhood lead poisoning: an overview. Hong Kong Med J. 2017 Dec 1;23(6):616-21.
20. Liu Z, Li D, Zhao W, Zheng X, Wang J, Wang E. A potent lead induces apoptosis in pancreatic cancer cells. PloS one. 2012 Jun 20;7(6):e37841.
21. Al-Ghafari A, Elmorsy E, Fikry E, Alrowaili M, Carter WG. The heavy metals lead and cadmium are cytotoxic to human bone osteoblasts via induction of redox stress. PLoS One. 2019 Nov 22;14(11):e0225341.
22. Pence BD, Yarbro JR. Aging impairs mitochondrial respiratory capacity in classical monocytes. Experimental gerontology. 2018 Jul 15;108:112-7.
23. Singh G, Guibao CD, Seetharaman J, Aggarwal A, Grace CR, McNamara DE, Vaithiyalingam S, Waddell MB, Moldoveanu T. Structural basis of BAK activation in mitochondrial apoptosis initiation. Nature Communications. 2022 Jan 11;13(1):1-5.