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## Review Article

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# Cardiovascular effects of lead exposure

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Several epidemiological and clinical studies have found a link between chronic lead exposure and elevated blood pressure. In addition, a few population studies have shown possible connection between lead exposure and other cardiovascular disorders including ischaemic coronary heart disease, cerebrovascular accidents, and peripheral vascular disease. The causal link between chronic lead exposure and hypertension (HTN) has been confirmed by several studies in experimental animals. In addition, the effects of lead on the heart and vascular function have been explored in a limited number of *in vivo* and *in vitro* studies. The *in vivo*, *ex vivo* and *in vitro* studies conducted in laboratory animal, cultured cells and isolated tissues have helped to elucidate many of the mechanisms by which lead exposure can cause HTN and cardiovascular disease. This review is intended to provide an overview of the epidemiology and the underlying mechanisms of lead-associated HTN and cardiovascular disease.

**Key words** Atherosclerosis - cardiovascular disease - coagulation - HTN - inflammation - lead exposure - thrombosis

### Epidemiology of lead-associated hypertension and cardiovascular disease

*Association of blood lead concentration with hypertension (HTN):* Several epidemiological and clinical studies have found a link between chronic lead exposure and elevated blood pressure<sup>1</sup>. Interest in the positive association between blood lead levels and blood pressure in environmentally lead-exposed individuals stems from the initial survey by Pirkle *et al*<sup>2</sup>, utilizing the database from the second National Health and Nutrition Examination Survey, conducted from 1976 to 1980 (NHANES II), which examined 543 white males aged 40-59 yr. The choice of the narrow age range, sex and race was made deliberately to minimize the effects of variations in these factors on blood pressure. In this study a multivariate adjustment disclosed

significant relationships between blood lead and both systolic and diastolic blood pressure. Publications following this initial survey<sup>3,4</sup>, covering the expanded NHANES II database from ages 12-74 yr, indicated a significant relationship between blood lead and systolic and diastolic blood pressure in both men and women; the relationship for men was independent of other variables. Since that time, multiple additional surveys have been conducted in Canada, Great Britain, Belgium, Denmark, Italy, France, Germany, Taiwan, and the US<sup>5-29</sup>, the majority of which confirmed the relationship noted by Pirkle *et al*<sup>2</sup>. In some of the surveys all individuals were included, *i.e.*, not restricted by sex, age, or race; in others, the population was divided by sex, by age, or by race<sup>3-6,9-12,15-17,26,27,29,30</sup>. The strongest associations were noted between black males and blood pressure. Staessen and co-workers, based on population surveys in

Belgium, initially cast doubt on the inter-relationship between blood lead and blood pressure<sup>27,30,31</sup>. This group<sup>27,32</sup> have more recently decided to pursue meta-analyses on the accumulated data. In their most recent meta-analysis of 31 studies, including 58,518 subjects<sup>32</sup>, they find that for a doubling of blood lead levels there is a 1.0 mm rise in systolic blood pressure and a 0.6 mm rise in diastolic blood pressure. The relationship remained statistically significant. A potential drawback of this meta-analysis is that occupational as well as nonoccupational sources of lead exposure were included. Yet animal studies have indicated that low lead, but not high lead exposure leads to the development of HTN<sup>33</sup>. What may be more to the point, however, is that the relationship between blood lead and blood pressure has persisted between the NHANES II and the NHANES III survey, which was conducted between 1988 and 1994, comprising 14,952 individuals aged 1 to 74 yr. During the time between studies, the geometric mean blood lead had decreased from 12.8 to 2.8 µg/dl, but a multivariate-adjusted relationship between blood lead and blood pressure was shown to persist in black men and women, not in Caucasians<sup>6,18</sup>. A further decline in blood lead occurred between NHANES III and the first phase of NHANES IV (1999 to 2002) by which time the geometric mean blood lead level had declined from 2.76 to 1.64 µg/dl. Despite this overall precipitous drop in blood lead from NHANES II to NHANES IV, attributed to the removal of lead from gasoline, lead-based house paint and soldering of cans, significant multivariate-adjusted relationships persisted between blood lead and blood pressure in the non-Hispanic black and Mexican-American populations<sup>34</sup>. These groups, however, tended to have higher blood lead levels than Caucasians (three times more likely to have blood lead levels of >10 µg/dl).

Thus, although the relationship between blood lead and blood pressure has withstood the impact of declining blood lead levels, additional efforts have been made to expand on this relationship, inasmuch as it has become evident that over 99 per cent of lead in blood resides in the red cell and less than 1 per cent in plasma<sup>35</sup>. Attention, therefore, has turned to plasma lead, which was thought to more precisely reflect the dynamic turnover between the circulation and bone, the long-term repository for lead (half-life of lead is 27 yr in cortical bone and 16 yr in cancellous bone)<sup>36</sup>. Several attempts have been made to measure either plasma or serum lead in environmentally or industrially exposed individuals. The majority of

measurements have employed inductively coupled mass spectrometry (ICP-MS) on plasma, which has the drawbacks of contamination by lead in heparin and variable degrees of haemolysis<sup>37</sup>. Plasma lead varied between 0.32 and 0.35 per cent in environmentally-exposed populations. Manton *et al*<sup>37</sup> employed stable isotope dilution to measure serum lead. They found serum lead to be 0.24 per cent of the lead in whole blood, a lower value than that reported using ICP-MS. Partly due to the uncertainties inherent in measuring plasma lead, no attempt has been made as yet to explore relationships between plasma or serum lead and blood pressure.

*Association of the bone lead content with HTN:* An indication of the potential validity of bone lead measurements as a tool for establishing the relationship between lead exposure and HTN comes from a study by Hernandez-Avila *et al*<sup>38</sup>, who examined 26 residents of Mexico City with no history of occupational lead exposure. They measured whole blood and plasma lead by ICP-MS and tibia and patella lead contents by K-X-Ray fluorescence. In a multivariate regression model of plasma lead values, patella lead remained an independent predictor of plasma lead. Several studies have indicated that bone lead measurements by X-ray fluorescence are predictive of HTN. In 1996 Hu *et al*<sup>39</sup> published a study of tibia and patella lead levels in a group of 590 men in the Normative Aging Study of the Veterans Administration. Their ages ranged from 21-80 yr upon enrollment, with a mean of 42 yr. Blood lead levels were low, ranging from <1 to 28 µg/dl and averaging 6.3 µg/dl. Mean levels of lead in both tibia and patella were significantly higher in hypertensive than in normotensive subjects. Furthermore, an increase from the midpoint of the lowest quintile of tibia lead (8 µg/g bone mineral) to the midpoint of the highest quintile (37 µg/g bone mineral) was associated with a 50 per cent increased risk of HTN (odds ratio of 1.5). In a follow up study of the same group in 2001 Cheng and co-workers<sup>40</sup> examined 833 participants, 337 of whom were normotensive, 182 had borderline HTN, and 314 had definite HTN (>160/95). Seventy four new cases of HTN were found in those who were free of definite HTN at baseline. In the group of 519 subjects with no definite HTN at baseline, there was a significant association between tibia lead and systolic blood pressure. In the group of 74 patients who had developed HTN, increases in both tibia lead and patella lead levels from the lowest quintile to the highest quintile were associated with the development of HTN. No

association was seen with blood lead. In 1999 Korrick *et al*<sup>41</sup> examined 214 cases of HTN in middle-aged women and 475 controls selected from Boston-area women in the Nurses Health Study of whom 297 completed the study. The mean age was 58.7 yr. Blood lead levels ranged from <1 to 14 µg/dl and mean tibia and patella lead levels were 13.3 and 17.3 µg/g, respectively. After multivariate adjustment, only patella lead was associated with HTN. Rothenberg and associates<sup>42</sup> examined the effect of lead on pregnancy-related HTN in 668 women, aged 15-44 yr. Tibia and calcaneus bone lead as well as blood lead were measured in the third trimester and post-partum. Calcaneus lead content was associated with an increased risk of HTN during pregnancy.

*Association of lead exposure with heart disease:* As described in an elegant review by Navas-Acien and associates<sup>1</sup>, general population studies have revealed a positive association between lead exposure and cardiovascular events including peripheral arterial disease and mortality from coronary heart disease and stroke. In some studies these associations were observed at surprisingly low blood lead concentrations (< 5 µg/dl). Schwartz<sup>43</sup> published a study of the incidence of left ventricular hypertrophy (LVH) in a re-analysis of the NHANES II data set in individuals aged 25-74 yr. The odds ratio for LVH was 1.33 for each 10 µg/dl increase in blood lead. Cheng *et al*<sup>44</sup> later analyzed electrocardiograms in 775 men from the Normative Aging Study. Patella and tibia lead levels were significantly correlated with the rate-adjusted QT and QRS intervals in men under 65 yr. In addition, tibia lead was significantly correlated with an increased odds ratio of intra-ventricular conduction defect (2.23 for every 10 µg/g increase in tibia lead). However, blood lead measurements did not correlate with these electrocardiographic parameters.

In the mortality study<sup>45</sup> of the NHANES II cohort subjects, 424 deaths had occurred among 4,190 men between the ages of 30 to 74 yr. They were divided into 3 groups according to their blood lead levels (10, 10-19 and 20-29 µg/dl). The risk of death from cardiovascular disease was significantly greater among those with the highest than those with the lowest lead levels. Thus both HTN and heart disease appear to be related to increased body stores of lead.

### **Mechanisms of lead-induced HTN and cardiovascular disease**

Studies conducted by several laboratories have shown that chronic exposure to low levels of lead causes

HTN in experimental animals. In addition, numerous *in vivo* and *in vitro* studies have explored the underlying mechanisms by which lead exposure can raise arterial pressure. These studies have identified involvement of oxidative stress, functional nitric oxide (NO) deficiency, inflammation, heightened central sympathetic activity, and dysregulations of various vasoregulatory and other factors in lead-induced HTN in animals. An overview of these findings is provided here.

*Role of oxidative stress and diminished NO availability:* Long-term exposure to lead causes oxidative stress in animals and cultured endothelial and vascular smooth muscle cells (VSMC). There is mounting evidence that oxidative stress plays a critical part in the development and maintenance of lead-induced HTN.

In an earlier study Khalil-Manesh *et al*<sup>46</sup> demonstrated that HTN in their lead-exposed rats was accompanied by diminished plasma cGMP concentration. They showed that chelation therapy with dimethyl succinic acid (DMSA) rapidly lowered arterial pressure and restored plasma cGMP in these animals before significantly altering the body lead burden. Since DMSA possesses strong antioxidant properties, they reasoned that rapid reduction of blood pressure and the rise in cGMP with DMSA administration may be due to alleviation of oxidative stress. Gonick and associates<sup>47</sup> subsequently reported a significant increase in plasma and tissue markers of oxidative stress in rats with lead-induced HTN. In another study<sup>48</sup> they showed that the reduction of blood pressure in response to L-arginine, infusion was much greater in lead-exposed rats than in the DMSA-treated rats with lead-induced HTN or control animals. These findings, illustrated the potential role of NO deficiency in the pathogenesis of HTN in this model. Vaziri *et al*<sup>49</sup> treated rats with lead-induced HTN with a lazaroid compound, a potent non-chelating antioxidant. The untreated lead-exposed rats exhibited HTN and reduced urinary excretion of NO metabolites indicating diminished NO bioavailability. Antioxidant therapy with this compound, improved HTN (without changing blood lead concentration) and hence, provided compelling evidence for the role of oxidative stress in the pathogenesis of functional NO deficiency and HTN caused by exposure to lead. The reduction in NO availability observed in rats with lead-induced HTN (lead acetate, 100ppm in drinking water for 12 wk) used in the above studies, was confirmed by others later<sup>50-52</sup>.

We<sup>53</sup> have shown that the reduction in NO availability in rats with lead-induced HTN is

paradoxically associated with a significant upregulation of nitric oxide synthase (eNOS and iNOS) in the kidney and cardiovascular tissues. In addition, *in vitro* incubation experiments revealed no significant change in the activity of purified eNOS in the presence of lead. The study further showed that administration of high doses of vitamin E and ascorbic acid lowered eNOS and iNOS abundance and paradoxically raised NO availability in rats with lead-induced HTN. Similar findings were observed in a subsequent study<sup>54</sup> in lead-exposed rats treated with the superoxide-scavenger drug, tempol and in lead-treated cultured human endothelial cells<sup>55</sup>.

Taken together, the above data prove that the reduction in NO bioavailability by lead exposure is mediated by oxidative stress as opposed to the reduced abundance or direct inhibition of NOS activity. Instead, inactivation of NO by reactive oxygen species (ROS) is, in part, responsible for the reduction of NO availability in animals with lead-induced HTN. This supposition was confirmed by studies which showed extensive accumulation of nitrotyrosine (a marker of NO oxidation by ROS) in the kidney, brain and cardiovascular tissues of untreated rats with lead-induced HTN and its reversal by antioxidant therapy using high doses of vitamin E and vitamin C<sup>56</sup>. Reduction of nitrotyrosine with antioxidant therapy was accompanied by significant attenuation of HTN and marked improvement of NO availability in rats with lead-induced HTN. These observations support the notion that exposure to lead causes functional NO deficiency, in part, by ROS-mediated NO inactivation.

The reduction of NO bioavailability contributes to development of HTN by several mechanisms including: (i) attenuation of NO-mediated vasodilation; (ii) reduction of NO-mediated inhibition of central sympathetic outflow; (iii) attenuation of NO-mediated diuresis culminating in extracellular volume expansion; and (iv) vascular remodeling which is normally inhibited by NO and facilitated by ROS. It should be noted that in addition to lead-induced HTN oxidative stress and the consequent disruption of NO metabolism are found in various other models of acquired and hereditary HTN<sup>57-59</sup>.

**Role of cGMP deficiency:** Most functions of NO are mediated by cGMP which is made by soluble guanylate cyclase (sGC) and serves as the NO receptor in vascular smooth muscle cells and other cell types. Activation of sGC by NO triggers production of cGMP which, in turn, mediates biological actions of NO such as

vasorelaxation. Khalil-Manesh *et al*<sup>33</sup> were the first to demonstrate significant reduction in plasma and urinary cGMP in rats with lead-induced HTN. Marques *et al*<sup>60</sup> found significant reductions of sGC abundance and cGMP production in the aorta of lead-exposed rats. These abnormalities were corrected by antioxidant therapy with ascorbic acid. These findings point to diminished sGC as an additional mechanism by which lead causes endothelial dysfunction via an ROS-mediated process. In confirmation of the *in vivo* studies, Courtois *et al*<sup>61</sup> showed a concentration-dependent reduction of the sGC and increased superoxide production in the normal rat aorta after incubation in the lead-containing media for 24 hours. Based on the findings cited above, in addition to limiting NO availability, lead exposure reduces sGC abundance and cGMP production in the vascular tissue via an oxidative stress-mediated process.

**Sources and nature of ROS in lead-induced HTN:** NAD(P)H oxidase catalyzes the single electron reduction of molecular oxygen to superoxide ( $O_2 + e^- \rightarrow O_2^{\circ-}$ ). This enzyme is a major source of ROS in the kidney and cardiovascular tissues. Prototypical (phagocytic) and tissue-specific forms of NAD(P)H oxidase are expressed in the kidney and cardiovascular tissues. Up-regulation and/or activation of these enzymes is involved in production of ROS and elevation of arterial pressure in angiotensin-induced HTN as well as many acquired and hereditary forms of HTN. We found upregulation of gp91<sup>phox</sup> subunit of NAD(P)H oxidase in the brain and to a lesser extent in the kidney and left ventricle of the lead-exposed animals<sup>54</sup>. We further found normalization of arterial pressure with infusion of superoxide-scavenger, tempol, in lead-exposed rats enforcing the role of oxidative stress in the pathogenesis of HTN in this model. In a subsequent study Ni and associates<sup>62</sup> found a transient rise in superoxide production followed by a sustained increase in  $H_2O_2$  production in human coronary endothelial and vascular smooth muscle cells cultured in lead-containing media. This was associated with upregulation of NAD(P)H oxidase and SOD but diminished or unchanged catalase and glutathione peroxidase. The latter findings provided the basis for the temporal changes in ROS production in lead exposed cells.  $H_2O_2$  is the substrate for production of hydroxyl radical ( $H_2O_2 + e^- \rightarrow \cdot OH + OH^-$ ). Thus, accumulation of  $H_2O_2$  in tissues of lead-exposed animals may raise production of  $\cdot OH$ . In fact, increased hydroxyl radical production was confirmed in rats with

lead-induced HTN<sup>63</sup> as well as in lead-treated cultured endothelial cells<sup>64</sup>.

*Lead-induced renal tubulo-interstitial inflammation:* As noted above lead exposure results in oxidative stress in animals as well as cultured vascular cells. Oxidative stress activates nuclear factor Kappa B (NF<sub>κ</sub>B) which is the general transcription factor for numerous cytokines, chemokines and adhesion molecules. Thus via activation of NF<sub>κ</sub>B, oxidative stress can cause inflammation. Conversely, inflammation can cause oxidative stress via generation and release of ROS by activated immune cells. It is therefore intuitive that by promoting ROS production, lead may trigger a perpetual cycle of oxidative stress and inflammation in the target tissues. This supposition has been confirmed by Rodriguez-Iturbe *et al*<sup>65</sup>, who found NF<sub>κ</sub>B activation, renal tubulo-interstitial infiltration of T-cells, macrophages and angiotensin-II-positive cells, increased apoptotic cells and nitrotyrosine accumulation in the kidneys of rats with lead-induced HTN. We wish to point out that renal tubulo-interstitial inflammation is present and plays a major part in the pathogenesis of HTN in various other forms of HTN in experimental animals<sup>58,59</sup>. The role of inflammation in the pathogenesis of lead-induced HTN was recently confirmed by studies which showed that attenuation of inflammation by immunosuppressive drug, mycophenolate mofetil, ameliorates HTN and oxidative stress in lead-treated rats<sup>66</sup>.

*Effect of lead exposure on adrenergic system:* A number of clinical and animal studies have focused on the sympathetic system as a possible mediator of lead-induced HTN and cardiovascular disease. Chang *et al*<sup>67</sup> found elevated plasma norepinephrine (NE) but normal plasma dopamine and epinephrine levels in a group of lead-exposed workers pointing to increased central sympathetic activity. Similarly Chang *et al*<sup>68</sup> and Tsao *et al*<sup>69</sup> found elevated plasma norepinephrine in lead treated rats which also exhibited significant reductions of β- adrenergic receptor density and isoproterenol (β agonist)- stimulated cAMP production in the heart and aorta and their elevation in the kidneys. The ability of lead to rapidly stimulate sympathetic nervous system activity was confirmed by Lai *et al*<sup>70</sup> using intra-theal (IT) injection of PbCl<sub>2</sub> in rats Together these findings provide evidence for the stimulatory effect of lead on the sympathetic nervous system and its contribution to the cardiovascular effects of lead exposure.

*Effect of lead on renin-angiotensin and kininergic systems:* In a meta-analysis of the earlier published

studies, Vander<sup>71</sup> found increased plasma renin activity and renal tissue renin content in young rats after several weeks of lead exposure. Similar results were found in rats exposed to lead *in utero* and for one month after birth. In contrast, plasma renin activity and renal renin contents were generally unchanged or even reduced in older rats whose lead exposure had commenced *in utero*. In another study, Carmignani *et al*<sup>72</sup> showed a significant increase in plasma angiotensin converting enzyme (ACE), kininase II, kininase I and kallikrein activities in the rats exposed to lead for 10 months beginning at early age. In a subsequent study Sharifi *et al*<sup>73</sup> found significant rises in plasma, aorta, heart and kidney ACE activities peaking at 2-4 wk followed by a decline to subnormal values by eight weeks coinciding with onset of significant HTN. They concluded that elevated ACE activity is involved in the induction, but may not be necessary for maintenance of HTN. Finally, recently, Rodriguez-Iturbe and colleagues<sup>65,66</sup> demonstrated a marked increase in the number of angiotensin-II positive cells in the kidneys of rats with lead-induced HTN pointing to heightened intra-renal angiotensin-II generation in this model. These data point to activation of renin-angiotensin system at some point in the course of lead-induced HTN.

*Effect of lead exposure on prostaglandins, endothelin (ET) and atrial natriuretic peptide (ANP):* Cardenas *et al*<sup>74</sup> and Hotter *et al*<sup>75</sup> found a significant increase in urinary excretion of the metabolite of vasoconstrictive prostaglandin, thromboxan (TXB<sub>2</sub>) when compared with values found in the control workers. In addition, Dorman and Freeman<sup>76</sup> demonstrated that lead promotes *in vitro* release of arachidonic acid by vascular smooth cells via activation of phospholipase A<sub>2</sub>.

Khalil-Manesh *et al*<sup>33,46</sup> studied the effect exposure to low and high levels of lead (100 vs 5000 ppm) in the drinking water for 1-12 months in rats on plasma endothelin. Rats exposed to low (but not high) levels of lead exhibited HTH and a significant increase in plasma ET-3 concentration. Similarly, Gonick *et al*<sup>47</sup> demonstrated a significant elevation of plasma and urinary ET-3 in rats with lead-induced HTN. In a recent study Molero *et al*<sup>77</sup> provided evidence that lead exposure can increase ET activity in rat vascular tissue.

ANP is a vasodilator and a natriuretic agent which is produced and secreted by cardiac myocytes. Plasma ANP rises with volume expansion and declines with volume contraction. Giridhar and Isom<sup>78</sup> studied rats treated with IP injection of lead acetate (0.0-1.0 mg/kg/

twice weekly for 30 days). Their lead-exposed animals exhibited fluid retention which was coupled with a paradoxical dose-dependent decline in plasma ANF concentration. They attributed dysregulation of ANP to the potential cardiovascular toxicity of this metal.

*Effect of lead on vascular reactivity:* The rapid action of lead on vascular reactivity *in vitro* seems to vary depending on the type of the vessel used, the lead concentration employed, and the animal species being studied. For instance, addition of lead acetate to the bathing medium has been shown to cause a concentration-dependent vasoconstriction in isolated rabbit mesenteric artery via activation of PKC<sup>79</sup>. Similarly, a concentration-dependent vasoconstrictive response to lead acetate (0.1-3.1 mM) was seen in the both intact and endothelium-denuded Wistar rat thoracic aorta rings<sup>80</sup>. In contrast to the latter studies, lead acetate did not cause contraction in the rat aorta rings used in a study reported by Shelkovnikov and Gonick<sup>81</sup>. Moreover, lead acetate did not modify the response to norepinephrine, phorbol ester or isoproterenol but it did augment the contractile response to sub-maximal concentrations of calcium.

In an effort to discern possible effect of lead exposure on vascular reactivity to various agonists, Purdy *et al*<sup>82</sup> compared Sprague-Dawley rats with lead-induced HTN. They found no significant difference in vasoconstrictive response to norepinephrine and phenylephrine or vasodilatory response to acetylcholine or nitroprusside in the aorta rings. In contrast, Marques *et al*<sup>60</sup> showed a significant reduction in vasodilatory response to both acetylcholine and nitroprusside in rats with lead-induced HTN. It should be noted that Wistar rats used in the former study had received 5 ppm lead acetate in the drinking water for one month whereas rats used in the study by Purdy *et al*<sup>82</sup> had been given a higher dosage (100 ppm) for a longer period (3 months). Therefore, the magnitude and duration of exposure may account for the differences observed between the two reports. In addition, the effect of lead on vascular reactivity may vary from one tissue to the next. This is exemplified by the studies of Oishi *et al*<sup>83</sup> using rats exposed to lead acetate for 3 months. They found a significant reduction of acetylcholine-mediated vasorelaxation in presence of NOS inhibitor, L-NAME, in mesenteric artery but not in the aorta of the same animals. These observations suggest that lead exposure may impair endothelium dependent hyperpolarization in the rat mesenteric artery but not the aorta.

*Lead-calcium interaction in vascular tissue:* Changes in cytosolic Ca<sup>2+</sup> are intimately involved in regulation of vascular tone and vascular smooth muscle contraction. Several studies have revealed that lead can potentially compete with Ca<sup>2+</sup> for the transport by channels and pumps involved in movements of ions particularly Ca<sup>2+</sup> across the cell membrane and between cytoplasm, endoplasmic reticulum and mitochondria<sup>84,85</sup>. In addition, lead can serve as a substitute for calcium in Ca<sup>2+</sup>-dependent signaling pathways by interacting with calmodulin, PKC and calcium-dependent potassium channels<sup>84-88</sup>. Thus, interactions of lead with cellular Ca<sup>2+</sup> via these complex mechanisms in the vascular cells may contribute to alterations of vascular resistance and HTN.

*Cardiotoxicity and atherogenesis:* Acute lead exposure has been reported to affect cardiac function and chronic exposure has been linked to atherosclerosis and increased cardiovascular mortality<sup>1</sup>. Using *in vitro* perfusion of isolated rat heart, Prentice and Kopp<sup>89</sup> showed that perfusion of heart for up to 60 min with a solution containing 30  $\mu$ M lead acetate prolonged AV node and His bundle conduction times, reduced coronary blood flow, lowered heart rate and altered cardiac energy metabolism pointing to the direct cardiac toxicity of lead. Using cadmium-treated male white pigeons Revis *et al*<sup>90</sup> showed that long-term low level lead exposure (0.8 ppm in drinking water) results in a significant rise in arterial pressure and doubling the number of atherosclerotic plaques in the aorta pointing to the atherogenic effects of chronic lead exposure.

*Effects of lead on endothelial cells:* Endothelial damage or dysfunction results in atherosclerosis, thrombosis and tissue injury. Chronic lead exposure has been shown to promote atherosclerosis in experimental animals<sup>90</sup>. Using cultured endothelial cells, Kaji *et al*<sup>91</sup> showed that lead nitrate causes denudation of endothelial monolayer, reduces proliferation and attenuates basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF) induced growth in cultured bovine aorta endothelial cells<sup>92</sup>. In addition, other workers<sup>93-95</sup> showed that lead acetate (1-100  $\mu$ M) causes a concentration- and time-dependent inhibition of angiogenesis. In addition, lead has been shown to interfere with the synthesis of glycosamino-glycans, heparan sulphate proteoglycan and various proteoglycans in cultured endothelial cells<sup>96-98</sup>. Given the critical role of proteoglycans in many aspects of endothelial and vascular function, their impaired production by lead plays a major part in the adverse actions of lead exposure.

*Effect of lead on vascular smooth muscle cells (VSMC):* Lead stimulates proliferation of bovine aorta vascular smooth muscle cells (VSMC), augments bFGF-induced VSMC proliferation<sup>98</sup> and promotes their transformation from the spindle or ribbon shape to cobblestone shape which simulates the neo-intimal cell morphology<sup>99</sup>. In addition, Yamamoto *et al*<sup>100</sup> have found that lead causes a significant inhibition of tissue plasminogen activator (t-PA) release in both cultured human aorta VSMC and foetal lung fibroblasts as well as a significant increase in PA inhibitor-1 (PAI-1) release in cultured fibroblasts. Thus, lead exposure appears to evoke a negative effect on fibrinolytic process in the cellular constituents of the sub-endothelial tissue.

In conclusions, by promoting oxidative stress and inflammation, disturbing NO signaling pathways, altering major vasoregulatory systems, damaging endothelial lining, promoting VSMC proliferation and transformation, and inhibiting fibrinolysis, chronic low level exposure to lead can contribute to development of HTN and cardiovascular disease.

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