Magnesium, calcium and lipoproteins in male workers with occupational exposure to lead: preliminary correlation and regression studies

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Zusammenfassung

Im Rahmen unserer Studien über Beziehungen zwischen Risikofaktoren, deren Einfluß auf den Magnesium-Stoffwechsel und dem Auftreten von Herzkreislauferkrankungen wurden bei 59 männlichen Arbeitern mit berufsbedingter Bleiexposition die folgenden Parameter gemessen: Blut-Blei-Gehalt (B-Pb), Protoporphyrin, HDL- und Gesamtcholesterin, Triglyceride, die Apolipoproteine B und A_I (Apo B; Apo A_I), Magnesiumkonzentrationen in Plasma und Erythrozyten (Pl-Mg; Erc-Mg) sowie das Plasma-Calcium (Pl-Ca). Aufgrund vermuteter Interaktionen zwischen diesen Variablen wurden einfache und multiple Korrelationen als auch schrittweise Regressionen untersucht; Es fand sich eine positive Korrelation zwischen Pl-Mg und Apo-B ($p < 0{,}01$).
Bleiexposition — auch in niedriger Konzentration — war im untersuchten Kollektiv mit erhöhtem Gesamtcholesterin, speziell mit Apo B, vergesellschaftet, wodurch sich für diese Arbeiter eine mittelhohe Risikowahrscheinlichkeit für Herzkreislauferkrankungen ergibt; Pl-Mg und Erc-Mg blieben jedoch unbeeinflußt.

Summary

Within the scope of our studies of relationships between risk factors and their implications for magnesium metabolism in cardiovascular diseases, blood lead (B-Pb), protoporphyrin, HDL- and total-cholesterols, triglycerides, apolipoproteins B and A_I (Apo B, Apo A_I), plasma and erythrocyte magnesium (Pl-Mg, Erc-Mg) and plasma calcium (Pl-Ca) were determined in 59 male workers with occupational exposure to lead. In view of supposed interactions between these variables, simple and multiple correlations as well as stepwise regression equations were searched for: a single positive correlation between Pl-Mg and Apo B ($p < 0.01$) was found; the most representative variables of Pl-Mg level were total-cholesterol and Apo B ($p < 0.01$).
Low-level lead exposure was sufficient to raise total-cholesterol and, especially, Apo B concentrations in these workers, situating them in a median zone of probability of cardiovascular risk but having no effect on Pl-Mg and Erc-Mg concentrations.

Résumé

L'étude des relations entre les facteurs de risque des maladies cardiovasculaires et le métabolisme du magnésium a été poursuivie par l'analyse du plomb sanguin (Sg-Pb), de la protoporphyrine, des cholestérols total et HDL, des triglycérides, des apolipoprotéines B et A_I (Apo B, Apo A_I), des magnésiums plasmatique et érythrocytaire (Pl-Mg; Erc-Mg) et du calcium plamatique (Pl-Ca) chez 59 travailleurs masculins, professionnellement exposés au plomb. Les corrélations simples et multiples ainsi que les équations de régression pas à pas ont été calculées: le Pl-Mg était positivement corrélé à l'Apo B ($p < 0{,}01$); le Pl-Mg a été expliqué par le cholestérol total et l'Apo B ($p < 0{,}01$).
L'imprégnation saturnine légère a été suffisante pour élever les concentrations de cholestérol total et surtout d'Apo B chez ces travailleurs, les situant dans une zone de probabilité moyenne de risque cardiovasculaire, restant toutefois sans effet sur les concentrations de Pl-Mg et Erc-Mg.

Introduction

Cardiovascular disease (CVD) is the leading cause of death among men in most industrialized countries [12]. However, only a few reviews on chemical exposures in relation to CVD morbidity have been published [10, 11, 14]. Although some evidence indicates that exposure to certain pesticides might affect lipoprotein metabolism, there are no consistent data to support the view that atherosclerosis is related to metal exposure in man [11, 12]. Our previous work in rabbits showed that very low-lead doses raised plasma cholesterol and triglyceride concentrations and modified concentrations of circulating magnesium [20]. Moreover, by comparison with control groups (men or women), it was found that lead concentrations were increased in three heart samples (right, left and infarcted left ventricle) and in the aorta after acute myocardial infarction [16]. Therefore, in continuing our studies of the relations between CVD risk factors and magnesium metabolism [18, 22], we have determined blood lead (B-Pb), protoporphyrin, HDL- and total-cholesterols, triglycerides, apolipoproteins B and A_I (Apo B; Apo A_I), plasma and erythrocyte magnesium (Pl-Mg; Erc-Mg) and plasma calcium (Pl-Ca) in workers with occupational exposure to lead. Various correlations were searched for between these variables as well as stepwise regression equations.

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Patients and methods

I. Populations

The study, carried for one year, concerned 59 white-coloured male workers 25 to 40 years of age with prolonged occupational exposure to lead. All were residents of the Loire-Atlantique Region in the western part of France, an area supplied with soft tap water. These subjects were neither obese nor suffering from known metabolic diseases and made moderate use of alcohol (not more than half a liter of wine per day) and tobacco (not more than 10 cigarettes per day).

II. Assay techniques

Plastic ware was cleaned by soaking for 24 h in 25 % (V/V) nitric acid, rinsed with demineralized water and dried in a dust-free environment. About 5 ml of blood were collected from each fasting subject between 0800 and 0900 h by venipuncture in lead-free polystyrene tubes containing lithium heparin. These tubes were sealed immediately and then transported to the laboratory. Precautions were taken during sample collection and analysis to prevent any form of contamination. One ml of this blood was used for lead and protoporphyrin analysis (by fluorescence) [8]. The remaining 4 ml were centrifuged at 3 500 × g for 8 min at 10 °C.

Determination of lead was performed by flameless atomic absorption spectrometry according to a method already described [2]. Magnesium and Ca were analyzed by flame atomic absorption spectrometry using a Hitachi 180-80 model with Zeeman effect according to operating procedures described elsewhere [19]. HDL- and total-cholesterols and triglycerides were determined by previously reported Boehringer enzymatic methods [18, 19]. Apo A_1 and Apo B were assayed by an immunonephelemetric method [21].

III. Statistical analysis

The normality of the distribution of results was checked by the chi-square test. Simple (r) and multiple (R) correlation coefficients were estimated between series of normal distributions; tests of the significance of correlation coefficients were performed respectively by the t-test and the F-test. The Spearman correlation coefficient (r_s) was used in case of non-normality of distributions [19]. Stepwise regression equations were also calculated [5] with B-Pb, Pl-Mg and Erc-Mg chosen as dependent variables. Calculations were performed using Systat (Evanston, Il, USA) software.

Results

Our results are given in Table 1 (mean and one standard deviation of the population). Protoporphyrin was not found to be normally distributed. Only significant ($p < 0.05$) correlation coefficients are indicated. There was only one positive correlation re-

Tab. 1: Means (1 SD) of results obtained in 59 male workers with occupational exposure to lead

	Blood Pb µmol/l (1)	Proto-porphyrin µmol/l (2)	HDL-cholesterol mmol/l (3)	Total-cholesterol mmol/l (4)	Trigly-cerides mmol/l (5)	Apo B g/l (6)	Apo A_1 g/l (7)	Pl-Mg mmol/l (8)	Erc-Mg mmol/l (9)	Pl-Ca mmol/l (10)
	1.83 0.86	0.87[a] 1.22	1.47 0.42	5.92 1.09	1.47 0.76	1.21 0.33	1.50 0.32	0.78 0.08	2.23 0.27	2.08 0.15
Spearman correlation	$r_s = 0.70^{**}$ 1,2									
Simple correlations			$r = -0.42^{****}$ 3,6 $r = 0.57^{****}$ 3,7	$r = 0.54^{****}$ 4,5 $r = 0.65^{****}$ 4,6	$r = 0.37^{***}$ 5,6	$r = 0.33^{**}$ 6,8 $r = -0.26^{*}$ 6,10				
Multiple correlations				$R = 0.80^{****}$ 4(1,3...10)	$R = 0.55^{*}$ 5(1,3,4...10)	$R = 0.78^{****}$ 6(1,3,4,5...10)				

[a]Non-normal distribution

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$
**** $p < 0.001$

Stepwise regression equations

y(B-Pb) = 3.113 − 0.216 total-chol. ($R = 0.273$; $p < 0.05$) (protoporphyrin being excluded)
y(Pl-Mg) = 0.748 − 0.020 total-chol. + 0.120 Apo B ($R = 0.394$; $p < 0.01$) (protoporphyrin and Erc-Mg being excluded)
y(Erc-Mg) = constant (protoporphyrin and Pl-Mg being excluded)

lative to Mg: between Pl-Mg and Apo B ($p < 0.01$).
The stepwise regression equation showed that the B-Pb concentration could only correspond to that of total-cholesterol, both variables being negatively correlated ($p < 0.05$). These results were confirmed by nonhierarchical cluster analysis. In fact, the subgroup of men (n = 11) presenting the most elevated B-Pb concentration (3.10 ± 0.72 µmol/l) had the lowest total-cholesterol level (4.92 ± 0.74 mmol/l) as well as the lowest triglycerides (0.99 ± 0.42 mmol/l) and Apo B (1.09 ± 0.42 g/l). The most representative variables of Pl-Mg level were total-cholesterol and Apo B ($p < 0.01$), whereas Erc-Mg concentration had a completely atypical behavior which did not correspond to that of any of the other variables.

Discussion

On the basis of a previous report [8], the B-Pb concentration found in our study (1.83 ± 0.86 µmol/l or 379 ± 178 µg/l) was sufficiently elevated to be considered as a cause of negative biological effects. In fact, lead is a toxic substance with no known physiologic function. Isotopic studies have shown that the body lead burden is 500 times as high as that of preindustrial humans [10, 13, 14]. Since Pb is poorly excreted, continued exposure causes accumulation [8, 12], with most Pb leaving the blood pool by tissue deposition.
In our study, as in that of Kromhout et al., no significant correlation was found between B-Pb and total- or HDL-cholesterol [10]. However, the stepwise regression equation indicated that B-Pb could only be significantly accounted for by the total-cholesterol concentration [marked by a sign (−)]. Moreover, B-Pb was the most discriminating variable for separating the population into 3 subgroups. It is of importance to note that for the intermediate subgroup (n = 27), in which B-Pb concentration (1.73 ± 0.40 µmol/l) was close to that of the mean of the total population, the concentrations of total-cholesterol (7.47 ± 0.43 mmol/l), triglycerides (2.37 ± 0.72 mmol/l) and Apo B (1.49 ± 0.21 g/l) were the most elevated. It may thus be hypothesized that increase in B-Pb above a lead-exposure threshold induces a decrease in the concentration of total-cholesterol and triglycerides because of their internalization in the artery [17, 20].
In terms of total-cholesterol concentration, this population was situated in a median risk zone (> 5.70 mmol/l). Triglycerides were at the upper limit of normal and closely correlated with total-cholesterol and Apo B, which would account in part for the significant multiple (R) correlations found for these 3 variables.
HDL-cholesterol and Apo A_I concentrations were elevated and closely correlated with each other ($p < 0.001$) [3, 4, 6]. In a previous study [9], it was also found that HDL-cholesterol concentration in male manual workers with occupational exposure to Pb was higher than that of male office workers without exposure. However, the predictive value of HDL-cholesterol and Apo A_I concentrations is considered to be uncertain, and markedly inferior to that of Apo B, the major protein constituent of atherogenic lipoproteins [1, 3, 6]. Apo B binds to cells by means of specific receptors and is favorable to cholesterol deposition on artery walls. An increase in Apo B constitutes a factor predictive of cardiovascular risk even when cholesterol level is normal [1]. in our study, the mean Apo B concentration in the 59 male workers was too elevated, situating them in a median zone of probability of cardiovascular risk, as confirmed by calculation of the Apo B/Apo A_I ratio = 0.81 (normal = < 0.68).
Despite the elevation in B-Pb concentration and its incidence on increased cardiovascular risk, the Pl-Mg and Erc-Mg levels of the 59 male workers remained close to our reference values [19]. The same was true for the 3 subgroups (n = 11, 27 and 21) after nonhierarchical cluster analysis, which made it possible to separate them according to the discriminating B-Pb values. The nature of relationships between circulating Mg and plasma lipids is quite controversial [15]. Apparently, a positive correlation between Pl-Mg and Apo B ($p < 0.01$) has not been previously reported. Moreover, total-cholesterol and Apo B were the most representative variables of Pl-Mg. Blood lead or Pl-Ca showed no significant interaction on Pl-Mg or Erc-Mg, which is contrary to previously reported results [7, 12].
Considering that maximum precautionary measures were taken during analysis to avoid contamination of samples with environmental Pb, it may be concluded that low-level Pb impregnation in our male workers led to lipid disturbances sufficient to define a probability of median cardiovascular risk; however, this impregnation had no effect on Pl-Mg and Erc-Mg concentrations. These findings both confirm and call in question our previous experimental results [20].

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