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Low Plasma Chromium in Patients with Coronary Artery and Heart Diseases

MONIQUE SIMONOFF,* YVAN LLABADOR, CHARLES HAMON, ARTHUR MACKENZIE PEERS, AND GABRIEL NICOLAS SIMONOFF

Chimie Nucléaire, ERA 144, CENBG, 33170 Bordeaux-Gradignan, France

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ABSTRACT

Plasma chromium concentrations have been determined for 150 patients. These were subjected to coronary artery cineangiography and thereby divided into three groups: group CAD (those with coronary artery disease), group HD (those with heart disease, but no CAD) and group N (those with no CAD and no HD). Weighted, average chromium levels for these groups were 1.05, 1.72, and 8.51 ng/mL, respectively. The distributions of plasma chromium levels for the three groups suggest that an upper limit for plasma chromium may be established (6 ng/mL in this work) beyond which CAD may be considered to be extremely unlikely, thus eliminating the need for a certain number of cineangiographic examinations.

Index Entries: Chromium, in human plasma; human plasma, chromium in; plasma, chromium in human; heart disease, plasma chromium in; coronary artery disease, plasma chromium in.

INTRODUCTION

The possible implication of chromium in the etiology of cardiovascular disease is based on experimental and epidemiological evi-

*Author to whom all correspondence and reprint requests should be addressed.

dence. Rats fed a chromium-deficient diet develop a diabeteslike syndrome (fasting hyperglycemia, glycosuria, raised cholesterol levels) and show increased incidence of aortic plaques (*1*). Chromium concentrations in aortas of subjects dying from atherosclerotic heart disease (*2*) are found to be significantly lower than in aortas of an unselected population (accident victims). Parenteral chromium, on the other hand, has been shown to promote the regression of atherosclerotic plaques in cholesterol-fed rabbits (*3*).

The foregoing results do not establish a cause–effect relationship for the role of chromium in atherogenesis, but constitute presumptive evidence, reinforced by the role of chromium in the metabolism of saccharides and lipids.

Trivalent chromium is an essential trace element when combined in an organic complex termed Glucose Tolerance Factor (GTF). Mertz (*4*) postulated that GTF acts as a cofactor for insulin, facilitating the binding of the latter to its receptor sites and amplifying all its known effects. GTF is indispensable for normal glucide metabolism (*5*). Preformed, readily available GTF is found in numerous foods. It can also by synthesized in vivo, using dietary inorganic chromium which is, however, very weakly absorbed.

GTF has also been reported to be effective in the maintenance of serum lipids at normal levels (*6*). It raises the level of high-density lipoprotein (*7*), which is thought to have a protective action against the formation of atheroma (*8, 9, 10*). Serum lipid levels and glucose tolerance are factors widely recognized as playing a role in the determination of cardiovascular risk.

The probable interrelations between the physiological role of chromium and atherogenesis induced us to measure plasma chromium levels for patients who were subjected to coronary artery cineangiography.

Proton-induced X-ray emission (PIXE) analysis was employed to measure the chromium concentrations in blood plasma from 150 patients.

MATERIALS AND METHODS

Patients

The 150 subjects of this study were referred to the Division of Cardiology for selective coronary artery cineangiography. All of them suffered angor and the cineangiography was employed to distinguish those with true coronary artery disease from those with normal arteries.

Each of the subjects with known heart disease was subjected to both right and left catheterization. The others were subjected to left catheterization only.

Most of the heart disease patients had been treated and followed by the same department for several years and had recently developed angor

in addition to their usual symptoms. This group of patients suffered from aortic insufficiency, mitral insufficiency, aortic stenosis with insufficiency, aortic insufficiency with mitral stenosis and myocardiopathy.

On the basis of the cineangiographic studies, the heart disease patients were divided into two groups: those with coronary artery disease (CAD) and those with heart disease, but normal arteries (HD). Most of these patients had been under treatment with digitalin and diuretics over a prolonged period. The remaining subjects (no HD) fell into group CAD or were shown to have normal arteries (group N).

Detailed questionnaires and clinical data concerning the 150 patients showed that it was not possible to form groups characterized by levels of cholesterol, triglycerides, blood pressure, obesity or tobacco consumption, so that we could not attempt to correlate any of these factors with plasma chromium levels.

Blood Collection

The subjects were brought to the Cardiac Catheterization Laboratory in the fasting state and two or four blood collections were made in heparinized polyethylene test tubes.

Left Catheterism (Coronarography)

After local anesthesia, a preformed Teflon-covered catheter is introduced through a femoral artery. The tip is positioned in the distal aorta and 10–20 mL of arterial blood are sampled for chromium analysis, the first 40 mL being rejected or stored for other purposes. Left ventricular and selective coronary artery cineangiography is then carried out with the same catheter. Again, 10–20 mL of blood are collected when the catheter is in the left ventricle.

Right Catheterism

The catheter is introduced through a femoral vein and 10–20 mL of venous blood are taken for chromium analysis. The catheter is then placed in the right ventricle and 10–20 mL of ventricular blood are collected. (The right catheterism is employed for the global cardiac evaluation of subjects with cardiac problems other than CAD).

Chromium Enrichment Procedure

We elaborated an extractive procedure (*11*) for the determination of trace chromium in blood plasma by PIXE, using less than 0.1 ng carrier-free ^{51}Cr for recovery determinations.

Wet ashing of 5–10 mL of plasma was carried out with perchloric and sulfuric acids. Complete oxidation from Cr(III) to Cr(VI) was made by $KMnO_4$ (1%). Chromium VI was complexed with ammonium pyrrolidine dithiocarbamate–methyl isobutyl ketone at pH 2.4. After evaporation of the organic phase, the metallic residue was dissolved in

nitric and hydrochloric acids. A known volume of a 50 ng/L nickel solution was added as an internal standard for quantitative PIXE determinations and the solution reconcentrated. The few residual microliters were deposited on a polycarbonate foil which constituted the target. Recovery for this preconcentration step was measured by adding radiochromium (^{51}Cr from the "Commissariat à l'Energie Atomique" C.E.A., Saclay).

Satisfactory values were found (*11*) for chromium in three NBS Standard Reference Materials (tomato leaves, oyster tissue, water). The total recovery on the target varies considerably (usually 40–90%), owing to the difficulty of collecting and depositing the final few microliters on the target foil. The recovery determinations for chromium were accurate to within ±4%. The detection limit was ~0.3 ng/mL of plasma.

PIXE Method

The detailed PIXE analytical method used for this work has been published elsewhere (*12*).

A beam of 2.5 MeV protons was employed, using the 4 MV Van de Graaff accelerator at the "Centre d'Etudes Nucléaires" in Bordeaux. The interaction between protons and target elements gives rise to X-rays with energies which depend on the atomic number of the element. Photon spectroscopy was carried out by means of an intrinsic germanium X-ray detector with a resolution of 180 eV (fwhm).

The sensitivity of the experimental arrangement is of the order of 10^{-9}–10^{-10} g for all elements with $Z \geq 14$. For elements such as V, Cr, Mn, Fe, Co, Ni, Cu, and Zn, the PIXE method can detect quantities of less than 1 ng on the target. The limit of detection for a given element depends on the X-ray emission (counting) rate of the neighboring elements on the spectrum.

RESULTS

The calculation of the concentration of chromium in a specimen is carried out by comparing the area of the characteristic chromium X-ray peak with that of an internal standard (e.g., nickel).

The spectra are analyzed on a graphic terminal connected to a CDC CYBER 750 computer. Background-corrected peak areas are calculated using a nonlinear, least-squares fitting procedure.

At the Cardiac Catheterization Laboratory, the coronary arteries are made visible in multiple roentgenogram projections by injecting a solution of 38 g iodine/100 mL Telebrix. X-ray films are exposed with a cine camera. The films are reviewed by specialized interpreters. When coronary artery disease is present, the degree of obstruction is assessed in terms of the percentage of coronary artery lumen that is occluded. The results showed that the patients fell into three groups: coronary artery

disease (CAD); heart disease, but relatively normal arteries (HD); and normal arteries with no heart disease (N).

The present results concern the analysis of 350 samples from 150 subjects, two-thirds of whom belonged to group CAD. The remaining third was divided approximately equally between groups HD and N. The results obtained are shown in Table 1. Measurements for nineteen subjects were not carried to completion for a variety of reasons; the first few samples analyzed showed a manganese peak (from $KMnO_4$) which partly masked that of chromium; samples with yields <40% were eliminated (these low yields resulted from sputtering losses during the final concentration to microliter volumes). For ten CAD subjects, the chromium peak was not distinguishable from the "chemical" background due to wet ashing and the oxidation of Cr(III) to Cr(VI). The plasma chromium level for these cases was probably less than 0.3 ng/mL, our limit of detection, and these values have not been included in the program for the calculation of the mean for group CAD; inclusion of these values would have slightly lowered the weighted mean shown in Table 2.

The observed chromium levels showed no significant dependence on the location from which the blood sample had been taken. The chromium level assigned to each patient is the mean of the two or four measurements (depending on whether a single catheterism, or both left and right catheterisms had been carried out). The spread between the two or four values for a single patient has been taken as representing the error of the measurement for that patient.

TABLE 1
Distributions of Plasma Chromium Levels in CAD, N, and HD Patients

Group	Chromium level, ng/mL	Fraction of group with chromium levels in given interval, %
CAD	<0.3	15
	0.3–1	30
(n = 67)	1–2	27
	2–4	15
	4–6	13
HD	0.3–2	32
	2–4	11
(n = 19)	4–6	37
	6–8	16
	> 8	4
N	4–6	20
	6–8	25
(n = 23)	8–10	35
	>10	20

TABLE 2
Statistical Analysis of Plasma Chromium Distribution

Group	Weighted mean ± standard deviation, ng/mL
CAD	1.05 ± 0.11
HD	1.72 ± 0.30
N	8.51 ± 0.47

Table 1 shows the distribution of chromium levels for each group of patients. These distributions (histograms) have been treated by the XFIT program (*13*) (giving a least-square fit for a single variable) in order to obtain a weighted mean and a standard deviation for each group (Table 2).

DISCUSSION

Our plasma chromium values are higher than those obtained by Versieck et al. (*14*), using neutron activation analysis (NAA) and by Vanderlinde et al. (*15*) using atomic absorption spectroscopy (AAS), but are in quite good agreement with those obtained in a number of other laboratories in recent years, using very different methods of analysis. Newman et al. (*16*) found a chromium level of 2.5 ± 3.3 ng/mL for a coronary population and 6 ± 3.3 for a noncoronary population, using NAA. A flameless AAS method developed by Thompson (*17*) gives plasma chromium values in the range 0.65–4 ng/mL. Gedik et al. (*18*) find normal plasma Cr levels from 4.4–6.1 ng/mL using a graphite furnace atomic absorption spectrophotometer. Liu and Abernathy (*19*) have measured serum Cr by radiochemical neutron activation in young subjects with normal glucose tolerance. The lyophilized serum was irradiated and ^{51}Cr was then extracted with a carrier as $BaCrO_4$. They found 1.46 ± 0.09 ng/mL for the pooled serum samples. Pekarek et al. (*20*) and Rabinowitz et al. (*21*) found values for plasma samples in the same range using a graphite furnace atomic absorption spectrophotometer. Serum chromium in normal subjects and in patients with recent and old myocardial infarction has been measured by AAS by Abraham et al. (*22*), who found 1.7 ng/mL. In 37 patients with acute myocardial infarction, the serum Cr levels rose to a mean of 6.4 ng/mL during the first 5 d after the infarct, then returned to normal during the next 5 d.

Over the last 20 yr, the measured levels of serum chromium have fallen from 100 to 0.1 ng/mL. The present results lie in a range starting below our limit of detection (0.3 ng/mL) and rising to an upper limit of about 10 ng/mL.

It seems improbable that our measurements suffer from systematic chromium pollution from the materials employed for blood collection, since no systematic dependence of chromium concentration on the location (method) of sampling was observed. As a further check, we measured serum chromium levels in samples from nine volunteers (doctors) with no heart disease. The samples were taken by venipuncture with a polypropylene-over-the-needle catheter (Intranule-Vygon). The serum chromium values obtained (8 ± 2 ng/mL) agreed with those of group N, who had been subjected to catheterization and cineangiography.

The acid digestion procedure, however, is a source of systematic pollution due to chromium contamination of the acids. We cannot obtain purified acids with less than 2 ng Cr/g, so that dry ashing would appear to be preferable. Some authors have shown (*14*) that there is no loss of chromium by volatilization in dry ashing. However our results (*11*) for dry ashing methods showed differences that were not reproducible. We found a loss of chromium from about 0–50% for an aliquot of the solution in HNO_3 after ashing at 500°C. Perhaps the dry ashing increases the retention of chromium in acid–insoluble residues, or gives rise to losses of a volatile Cr compound. We therefore chose a wet digestion method and subtracted the chromium due to contamination from acids and chemical reagents by the use of many blanks. These were found to have Cr contents of a few nanograms. In such conditions, with plasma volumes of 10–20 mL, we can measure chromium levels as low as 0.3 ng/mL.

With regard to the reliability of serum chromium measurements, it may be noted that those published in recent years are more frequently in the ng/mL range, rather than an order of magnitude lower, as found by Versieck et al. (*14*) and Vanderlinde et al. (*15*). There has been a tendency to consider the lower values to be the more reliable, invoking chromium contamination from the venipunction needle, for example, to account for the higher levels often observed.

Versieck et al. (*14*), obtained low values (0.16 ng/mL) by destructive neutron activation analysis (NAA). Samples were ashed in a hot oven for 24 h at temperatures up to 450°C before being irradiated. They did not observe volatilization of ^{51}Cr albumin or Cr loss from serum of humans receiving $^{51}CrCl_3$ intravenously or from serum of rats fed for one week with $^{51}CrCl_3$. It may be the case, however, that inorganic chromium is only slowly converted to a volatile (organic) form, the loss of which would not be detected by the foregoing, short-term tests (*23, 7*).

Whether the present results represent absolute or only relative plasma chromium levels, they clearly distinguish between two populations: group CAD + group HD on the one hand, and group N on the other. No subject in group N had a chromium level <4 ng/mL, whereas 87% of the CAD group showed Cr levels less than that value (Table 1).

It should be noted here that numbers of subjects in all three groups were under treatment with a variety of drugs (nitroglycerin, sedatives, beta-blockers, anticoagulants, analgesics, etc.), but there was no system-

atic difference in drug absorption between groups HD and CAD, on the one hand, and group N on the other. Only HD subjects, however, were under treatment with diuretics and digitalin derivatives. We are aware of no evidence suggesting that either of these drugs might provoke a systematic lowering of plasma chromium levels, but the possibility should not be ignored. We intend to investigate the possible effects of diuretics on plasma chromium levels in normal subjects. Possible effects of digitalin, on the other hand, could perhaps be examined in animal experiments, but these cannot be undertaken in our laboratory.

An earlier study by Newman et al. (*16*) on 32 subjects showed that those with cineangiographically-determined CAD had an average serum chromium level of 2.5 ng/mL, whereas those with no CAD showed an average value of 6.1 ng/mL. The distribution of chromium levels for the two groups, however, overlapped very widely, so that it was not possible to assign a particular subject to either group on the basis of his serum chromium level. Newman's non-CAD group (17 subjects) did contain nine subjects with no detectable organic heart disease, so that it could have been subdivided into groups HD and N (in the terminology of the present work). This was not done, however, so that the data of Newman et al. cannot be used to check our main conclusion, that fasting plasma chromium determinations may make it possible to distinguish many patients with normal arteries from those who may have CAD. The present results suggest that an upper limit may be established (6 ng/mL in this study) beyond which CAD may be considered to be extremely unlikely. Such an upper limit would make it possible to eliminate a proportion of cineangiographic examinations that presently serve only to demonstrate the absence of CAD.

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