Toenail cerium levels and risk of a first acute myocardial infarction: The EURAMIC and heavy metals study

Jorge Gómez-Aracena a,b,*, Rudolph A. Riemersma c,d, Mario Gutiérrez-Bedmar a, Peter Bode e, Jeremy D. Kark f, Antonio García-Rodríguez a, Lydia Gorgojo g, Pieter van’t Veer h, Joaquín Fernández-Crehuet a, Frans J. Kok h, José M. Martin-Moreno i for the Heavy Metals and Myocardial Infarction Study Group

a Department of Preventive Medicine, Universidad de Málaga, Spain
b The Nordic School of Public Health, Göteborg, Sweden
c Cardiovascular Research Unit, University of Edinburgh, Edinburgh, United Kingdom
d Department of Medical Physiology, University of Tromsø, Tromsø, Norway
e Interfaculty Reactor Institute, Delft University of Technology, Delft, The Netherlands
f Epidemiology Unit, Hadassah Medical Organization and Hebrew University—Hadassah School of Public Health and Community Medicine, Jerusalem, Israel
h Division of Human Nutrition and Epidemiology, University of Wageningen, Wageningen, The Netherlands
i Department of Preventive Medicine and Public Health, Universidad de Valencia, Spain

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Abstract

The association between cerium status and risk of first acute myocardial infarction (AMI) was examined in a case-control study in 10 centres from Europe and Israel. Cerium in toenails was assessed by neutron activation analysis in 684 cases and 724 controls aged 70 years or younger. Mean concentrations of cerium were 186 and 173 µg/kg in cases and controls, respectively. Cerium was positively associated with low socio-economic status, smoking, mercury, zinc and scandium (p < 0.001). Cases had significantly higher levels of cerium than controls after adjustment for age and centre (case-control ratio 1.074; 95% CI 1.002–1.151) and increased in further adjustment for other cardiovascular risk factors 1.085; 95% CI 1.025–1.149. The risk after adjustment for age and centre was higher with increasing cerium levels (p for trend = 0.02). After adjustment for BMI, history of hypertension, smoking, alcohol intake, diabetes, family history of CHD, β-carotene, lycopene, α-tocopherol, selenium, mercury and scandium, the OR for the highest quintile was 1.43 (95% CI 0.85–2.41; p-trend 0.08). When we applied this same model in non-smokers the odds ratios in the 4th and 5th quintiles of cerium as compared with the lowest were 2.09 (95% CI 1.05–4.16) and 2.81 (95% CI 1.21–6.52), respectively, p-trend 0.011. Our results suggest that toenail cerium levels may be associated with an increased risk of AMI, but more research is warranted to shed further light and fully understand the plausibility and public health implications of these findings.

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Keywords: Scandium; Antioxidant; Lycopene; Cardiovascular risk factor; EURAMIC

1. Introduction

Cerium is one element of the lanthanides series of rare earth metals and exists in trivalent and quadrivalent...
state (Haley, 1991). Among the lanthanides it is the 
most abundant in the earth crust, found primarily as 
monazite, bastnazeite, orthophosphate and fluorocarbon-
ate. It is incorporated in many industrial products (light-
ers, carbon arc lamps, glass additives, ceramics, etc.) 
(HEI, 2001). Cerium is also used as a diesel fuel catalyst. 
The general population may be exposed to cerium 
through oral and inhalatory routes from a variety of 
sources with different concentrations in the environment. 
It should be noted that orally administered cerium is 
generally poorly absorbed by the digestive tract (HEI, 2001); 
the NRCP (1978) reported that, in adult rodents, between 0.01% 
and 0.1% of administered radioactivity (in soluble 
forms of cerium) was absorbed by the gastrointestinal 
tract. Inhaled cerium compounds have been classified by 
NRCP into three categories according to the estimated 
rate of clearance: from the intermediate and slow clear-
ance groups, 20% would enter the blood and lymph nodes 
and 80% would clear to the gastrointestinal tract. Despite 
the paucity of data, there is concern about the health 
effects of cerium emissions, in the ambient atmosphere 
and as deposits on soil, vegetation and water close to 
motorways.

In fact, there are studies in which cerium has been asso-
ciated with human disease. High dietary intake of cerium 
compounds has been related to endomyocardial fibrosis 
(Valiathan and Kartha, 1990; Kutty et al., 1996). Intersti-
tial pneumoconiosis and lung fibrosis caused by cerium 
has also been reported (Sabbioni et al., 1982; McDonald 
et al., 1995; Porru et al., 2001). An excess mortality 
due to lung and bladder cancer in a French population 
living near an industrial site contaminated with cerium 
rich monazite has been identified (De Vathaire et al., 
1998).

Studies in animal models suggest that cerium in low 
doses stimulate a mitogenic response and intracellular 
generation of reactive oxygen species in cardiac fibro-
blasts (Nair et al., 2003). A very large animal study demon-
strated increased pulmonary carcinogenicity due to low 
doses of radioactivity and also non-radioactive cerium 
(Lundgren et al., 1996). Cerium particles also elicit a 
non-specific antigenic response due to their toxicity (HEI, 
2001).

The aim of this paper is to address the possible role of 
chronic exposure to cerium in coronary heart disease 
(CHD), an issue which has not been previously examined. 
We investigated the potential association of toenail cerium 
concentrations, with the risk of first myocardial infarction 
(MI) among participants in the EURAMIC (EUropean 
multicentre case-control study on Antioxidants, Myocar-
dial Infarction and Cancer of the breast) Study (Kardinaal 
et al., 1993) and the Heavy Metals Study (Gómez-Aracena 
et al., 2002; Guallar et al., 2002; Martin-Moreno et al., 
2003).

The possible confounding or interacting influence of 
other toenail trace metals (scandium and mercury) and of 
antioxidant status was also considered.

2. Methods

2.1. Design and subjects

The target population was men aged 70 years or younger, 
native residents of eight European countries and Israel: 
Finland (Helsinki), Germany (Berlin), Israel (Jerusalem), 
the Netherlands (Zeist), Norway (Sarpsborg), Russia 
(Moscow), UK (Edinburgh, Scotland), Switzerland (Zür-
ich), and Spain (Granada and Málaga). Subjects were 
excluded if they had a previous diagnosis of myocardial 
infarction, drug or alcohol abuse, major psychiatric disor-
ders, weight loss ≥5 kg or were institutionalised.

Cases were men diagnosed with a first acute MI (ICD 
9th Revision code 410), confirmed by characteristic ECG 
abnormalities and elevated enzyme levels who had been 
admitted within 24 h from the onset of symptoms. Cases 
were recruited from the coronary care units of participating 
hospitals.

The controls were subjects without a history of MI, 
recruited from the cases’ population catchment area and 
frequency-matched for age in 5-year intervals. In Finland, 
Israel, Germany, Scotland and Switzerland, random sam-
pling from local population registers was used for control 
selection. In Russia and in the two Spanish centres, popu-
lation registries could not be used because of incomplete 
coverage or legal restrictions, so controls were selected 
from patients admitted to hospital for disorders not known 
to be associated with dietary factors (renal colic, non-infec-
tious prostatism, acute appendicitis, non-infectious ear dis-
ease, hernia, volvulus, rectal or anal disease except cancer, 
aeorrhoids, or chronic infection). In the Netherlands, 
controls were selected from the catchment area of the 
patient’s general practitioner, and in Norway they were 
selected by inviting friends and relatives of the case (Kard-
inaal et al., 1993). In the Netherlands, Spain and Russia, 
methods of subject recruitment were combined. Cases 
and controls were recruited concurrently during 1991 and 
1992. The participation rates were 81% for cases and 64% 
for controls. Informed consent was obtained from study 
participants in accordance with the ethical standards of 
the responsible institutional review boards.

2.2. Data collection

Information on smoking habits, history of hypertension, 
angina pectoris, and diabetes was collected for all subjects 
by standard questionnaires. Socio-economic status, alcohol 
take and family history of cardiovascular diseases were 
assessed through locally developed questionnaires.

Toenail clippings from all 10 toes were collected within 
8 weeks of inclusion in the study and stored in small plastic 
bags at room temperature (Kardinaal et al., 1997). Nails 
were cleaned and washed before clipping and storing. Fur-
thermore, any attached debris was scraped off nail specimens 
in the reference centre prior to testing. The mean weight of 
the samples was 53.9 mg (SD 39.1). A subcutaneous
adipose tissue specimen was taken from the buttock by needle aspiration. In AMI patients, the adipose tissue sample was taken within seven days of hospital admission. A non-fasting venous blood sample was also obtained. In patients, blood samples were drawn within 24 h of hospital admission. Adipose tissue and serum samples were stored at −70 °C and transported on dry ice at −56 °C to the reference laboratories (Kardinaal et al., 1993).

2.3. Analysis of biological samples

The cerium concentration in toenails was measured by instrumental neutron activation analysis (INAA) at the Interfaculty Reactor Institute of Delft University of Technology in Delft, the Netherlands (Alfassi, 1994; Bode and de Goeij, 1998; Bode, 2000). Toenail clippings were irradiated for 4 h in a thermal flux of $5 \times 10^{12}$ cm$^{-2}$ s$^{-1}$. After a decay time of 21 days, the gamma-spectrum of the induced radioactivity was measured in a well-type germanium detector. The peak at 145.5 keV was used for the identification of $^{141}$Ce and the quantitative determination of the cerium content. The quantitative determination is based on the single comparator method (De Bruin and Korthoven, 1972) in which secondary cerium working standards of known purity and amount of mass have been used. For each centre samples from patients and controls were analysed together and randomly distributed across batches. Personnel at the Interfaculty Reactor Institute were blinded with respect to the case-control status of the samples. The neutron activation analysis of toenail selenium, mercury and scandium levels has been described previously (Kardinaal et al., 1997; Gómez-Aracena et al., 2002; Guallar et al., 2002).

The detection limit of cerium in toenails by the INAA under the experimental conditions given above amounts to approximately 5–20 ng, depending on the level of other constituents in the nail material. The quality control materials we used to inspect for analytical errors were NIST 1577b Bovine Liver (National Institute of Standards and Technology, Gaithersburg, MD, USA) and BCR-CRM 414 Trace elements in Plankton (Community Bureau of Reference, Commission of European Communities, Institute for Reference Materials and Methods, Geel, Belgium). The degree of accuracy of the cerium determinations had been established previously as part of the method validation by a large number of analyses of appropriate reference materials with a range of cerium levels. These analyses were performed independently, and indicated the reproducibility and accuracy of the method.

Analysis of adipose tissue was carried out at the TNO Nutrition and Food Research Institute in Zeist, the Netherlands. Beta-carotene, lycopene and alpha-tocopherol were determined by reverse-phase high performance liquid chromatography and spectrophotometric detection (Van Vliet et al., 1991). The sample was saponified and quantitatively split for vitamin and fatty acid determination. Vitamin concentrations were expressed in μg/g fatty acids.

2.4. Statistical methods

Since the distribution of cerium was right-skewed, log-transformations were used. Retransformed values are presented in the tables. The distribution of cerium in controls was used to compute cut-off points and medians for quintiles of exposure. The levels of cardiovascular risk factors across quintiles of cerium were compared among controls by ANOVA and $\chi^2$ tests. The centre-specific and adjusted overall mean case-control ratios of cerium and 95% confidence intervals (CI) were estimated by linear regression (Hosmer and Lemeshow, 2000).

The association of cerium with the risk of myocardial infarction was estimated through multiple logistic regression analysis. Odds ratios were calculated by comparing each of the upper 4 quintiles with the lowest quintile, based on the distribution among controls. Tests for trend were performed by assigning each subject the median value for the category and treating this value as a continuous variable in the logistic models. All reported $p$ values are two-tailed. Statistical analyses were performed with SPSS 10.0 (SPSS Inc., 1999 Chicago).

3. Results

We recruited 742 men with a first MI and 757 controls. Toenail clippings were not available for 58 cases and 33 controls. Therefore, we analysed data from 684 cases and 724 controls. In comparison with controls, cases had significantly higher BMI, lower HDL-cholesterol, and were more likely to have hypertension, diabetes, to smoke, to have family history of MI and to belong to a lower socio-economic group; there was also a slight significant difference in the mean age: 54.7 years in cases and 53.2 in controls (Table 1). Although total cholesterol was lower among cases compared to controls, we assumed that this probably reflected cholesterol-lowering effect due to the acute phase effect of the MI. Therefore, total cholesterol was not further considered in case-control comparisons.

Mean concentrations of toenail cerium were 186 μg/kg (95% CI 177–196) in cases and 173 (165–182) μg/kg in controls. Among the controls, participants from Helsinki or Berlin, had the lowest concentrations of cerium (136 and 141 μg/kg, respectively). The highest levels were found in Jerusalem, Granada and Moscow (291, 216 and 202 μg/kg, respectively)—a 2.1-fold range of variation.

The level of cerium in toenails was inversely associated with age ($p = 0.004$) and BMI ($p = 0.02$) (Table 3). Smoking either expressed as percentage of current smokers or as cigarettes per day (not shown) was also related to toenail cerium levels ($p = 0.001$ and 0.002, respectively). Positive association was also found between high toenail cerium concentrations with low socio-economic status ($p < 0.001$). The levels of cerium in toenails were related to those of mercury, zinc and scandium ($p = 0.001$, $p = 0.001$ and $p < 0.001$, respectively). The association between cerium
in toenails with lycopene in adipose tissue was of borderline significance ($p = 0.055$).

Pearson correlations in controls, adjusted for age and centre were significant between cerium and the other metals ($r = 0.10 - 0.16$, $p < 0.001$) and between cerium and lycopene ($r = 0.08$; $p = 0.03$). Interestingly, cerium levels in toenails were strongly related to those of scandium both in controls and in cases ($r = 0.62$ and $r = 0.48$, both $p < 0.001$, respectively). In cases, Pearson correlations for metals were also significant (not shown). These correlations between cerium and scandium varied among centres and were not always significant: for example, the lowest one was found in controls in Edinburgh ($r = 0.12$; $p = 0.571$). We have carefully analysed the potential interactions between cerium and each metal and antioxidant with respect to their association with the risk of myocardial infarction, but no significant estimates of joint effects were found ($p > 0.05$). Cerium levels were higher in cases compared to controls in 8 of the 10 centres studied; only Málaga and Edinburgh had ratios below 1 (Table 2). Overall, cases had significantly higher levels of cerium than controls after adjustment for age and centre (case-control ratio 1.074; 95% CI 1.002–1.151). The Ce case-control ratio did not really change after adjustment for other cardiovascular risk factors (1.085; 95% CI 1.025–1.149).

This association of increased cerium with higher risk of MI was further examined evaluating the odds ratios of disease by quintiles of cerium (Table 4). The risk of a first MI was higher with increasing cerium levels when we adjusted for age and centre ($p$ for trend = 0.02). After adjustments for classical risk factors for CHD (BMI, history of hypertension, smoking, alcohol intake, diabetes, family history of CHD), the odds ratio for MI in the highest quintile of cerium as compared with the lowest was 1.00 (95% CI 0.65–1.54) and the observed trend no longer significant ($p = 0.466$). This was entirely due to the adjustment for smoking, odds ratio 0.98, 95% CI 0.68–1.41, $p$-trend 0.271. The inclusion of β-carotene, lycopene, α-tocopherol, selenium and mercury generated a flat, non-significant trend across quintiles ($p = 0.578$). After adjustment for toenail Se the odds ratio for the highest quintile clearly increased (1.43, 95% CI 0.85–2.41; $p$-trend 0.088). Further adjustment

### Table 1
Cardiovascular risk factors in cases of myocardial infarction and controls

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cases (n = 684)</th>
<th>Controls (n = 724)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.7 (8.9)</td>
<td>53.2 (9.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 (3.9)</td>
<td>25.9 (3.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.46 (1.11)</td>
<td>5.56 (1.10)</td>
<td>0.11</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.98 (0.25)</td>
<td>1.09 (0.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypertension (%)</td>
<td>26.0</td>
<td>17.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (% current smokers)</td>
<td>61.3</td>
<td>37.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>8.4</td>
<td>3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>57.6</td>
<td>45.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low socio-economic status (%)</td>
<td>25.1</td>
<td>20.8</td>
<td>0.036</td>
</tr>
<tr>
<td>Alcohol use (g/day)</td>
<td>18.2 (27.2)</td>
<td>17.8 (24.0)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* a Expressed in means (SD) or percentages.
* b CHD: coronary heart disease.

### Table 2
Levels of cerium in toenails (μg/kg) in cases and controls

<table>
<thead>
<tr>
<th>Centre</th>
<th>Number of cases/controls</th>
<th>Mean in cases (CI 95%)</th>
<th>Mean in controls (CI 95%)</th>
<th>Case/control ratio (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zürich (Switzerland)</td>
<td>56/74</td>
<td>174 (155–195)</td>
<td>149 (135–165)</td>
<td>1.16 (1.00–1.36)</td>
</tr>
<tr>
<td>Málaga (Spain)</td>
<td>93/100</td>
<td>152 (133–173)</td>
<td>197 (174–223)</td>
<td>0.77 (0.64–0.92)</td>
</tr>
<tr>
<td>Moscow (Russia)</td>
<td>92/97</td>
<td>230 (202–263)</td>
<td>202 (178–230)</td>
<td>1.14 (0.94–1.38)</td>
</tr>
<tr>
<td>Granada (Spain)</td>
<td>55/52</td>
<td>236 (195–286)</td>
<td>216 (177–263)</td>
<td>1.09 (0.83–1.44)</td>
</tr>
<tr>
<td>Edinburgh (UK)</td>
<td>39/25</td>
<td>171 (148–196)</td>
<td>195 (164–232)</td>
<td>0.87 (0.70–1.09)</td>
</tr>
<tr>
<td>Helsinki (Finland)</td>
<td>56/62</td>
<td>178 (154–206)</td>
<td>136 (118–156)</td>
<td>1.31 (1.07–1.60)</td>
</tr>
<tr>
<td>Jerusalem (Israel)</td>
<td>57/58</td>
<td>314 (274–361)</td>
<td>291 (254–334)</td>
<td>1.08 (0.89–1.31)</td>
</tr>
<tr>
<td>Sarpsborg (Norway)</td>
<td>96/101</td>
<td>171 (146–201)</td>
<td>144 (123–168)</td>
<td>1.19 (0.95–1.49)</td>
</tr>
<tr>
<td>Berlin (Germany)</td>
<td>75/97</td>
<td>158 (138–181)</td>
<td>141 (126–159)</td>
<td>1.12 (0.93–1.35)</td>
</tr>
<tr>
<td>Zeist (The Netherlands)</td>
<td>64/57</td>
<td>159 (134–190)</td>
<td>146 (123–176)</td>
<td>1.09 (0.84–1.40)</td>
</tr>
<tr>
<td>Overall</td>
<td>683/723</td>
<td>186 (177–196)</td>
<td>173 (165–182)</td>
<td>1.074 (1.002–1.151)b</td>
</tr>
</tbody>
</table>

* a Geometric means adjusted for age.
* b Adjusted for age and centre.
* c Adjusted for age, centre, smoking, alcohol intake, BMI, diabetes, history of hypertension, family history of coronary heart disease, lycopene, selenium, mercury, zinc, scandium and toenail weight.
for SES did not really change these results. Adjustment for classical risk factors in non-smokers showed a p-trend of 0.137. When we included in the model, β-carotene, lycopene, α-tocopherol, selenium, mercury and scandium the odds ratios in the 4th and 5th quintiles of cerium as compared with the lowest were 2.09 (95% CI 1.05–4.16) and 2.81 (95% CI 1.21–6.52), respectively, p-trend 0.011 (Table 5).

4. Discussion

In this multicentre case-control study we observed an indication of an association between toenail cerium levels and risk of a first acute myocardial infarction after adjustment for confounding factors. This association was stronger among non-smokers.

This study was particularly designed to investigate the association between toenail trace metals and MI. Toenails and adipose tissue samples were collected shortly after the patients had suffered their first MI. Although a validation study has not been performed yet, using existing data, we assumed that cerium levels of toenail clippings provide a measure of relatively long-term exposure to this trace element, reflecting average exposure over the previous 3–12 months (Wilcosky, 1990; Hunter, 1998). Human

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.1</td>
<td>55.0</td>
<td>53.5</td>
<td>53.4</td>
<td>51.0</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0</td>
<td>26.2</td>
<td>26.1</td>
<td>26.0</td>
<td>25.2</td>
<td>0.020</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.39</td>
<td>5.59</td>
<td>5.62</td>
<td>5.57</td>
<td>5.65</td>
<td>0.177</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.07</td>
<td>1.05</td>
<td>1.12</td>
<td>1.13</td>
<td>1.09</td>
<td>0.513</td>
</tr>
<tr>
<td>History of hypertension (%)</td>
<td>10.8</td>
<td>19.2</td>
<td>10.0</td>
<td>13.5</td>
<td>10.3</td>
<td>0.376</td>
</tr>
<tr>
<td>Smoking (% current smokers)</td>
<td>34.8</td>
<td>36.5</td>
<td>40.2</td>
<td>25.1</td>
<td>54.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.488</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>55.8</td>
<td>49.2</td>
<td>48.6</td>
<td>53.4</td>
<td>49.9</td>
<td>0.472</td>
</tr>
<tr>
<td>Low socio-economic status (%)</td>
<td>14.8</td>
<td>12.6</td>
<td>14.1</td>
<td>14.6</td>
<td>25.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol (g/week)</td>
<td>135</td>
<td>103</td>
<td>134</td>
<td>113</td>
<td>138</td>
<td>0.460</td>
</tr>
<tr>
<td>β-Carotene (µg/g)</td>
<td>0.41</td>
<td>0.46</td>
<td>0.43</td>
<td>0.40</td>
<td>0.48</td>
<td>0.191</td>
</tr>
<tr>
<td>Lycopene (µg/g)</td>
<td>0.27</td>
<td>0.28</td>
<td>0.28</td>
<td>0.27</td>
<td>0.32</td>
<td>0.055</td>
</tr>
<tr>
<td>α-tocopherol (µg/g)</td>
<td>187.3</td>
<td>195.7</td>
<td>200.9</td>
<td>198.2</td>
<td>189.0</td>
<td>0.834</td>
</tr>
<tr>
<td>Selenium (mg/kg)</td>
<td>0.58</td>
<td>0.55</td>
<td>0.55</td>
<td>0.57</td>
<td>0.59</td>
<td>0.158</td>
</tr>
<tr>
<td>Mercury (mg/kg)</td>
<td>0.22</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
<td>0.29</td>
<td>0.001</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>101</td>
<td>103</td>
<td>103</td>
<td>113</td>
<td>118</td>
<td>0.001</td>
</tr>
<tr>
<td>Scandium (µg/kg)</td>
<td>4.38</td>
<td>5.36</td>
<td>4.38</td>
<td>8.91</td>
<td>20.82</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3

Age and centre adjusted levels of risk factors by quintiles (Q) of cerium among controls

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.1</td>
<td>55.0</td>
<td>53.5</td>
<td>53.4</td>
<td>51.0</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0</td>
<td>26.2</td>
<td>26.1</td>
<td>26.0</td>
<td>25.2</td>
<td>0.020</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.39</td>
<td>5.59</td>
<td>5.62</td>
<td>5.57</td>
<td>5.65</td>
<td>0.177</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.07</td>
<td>1.05</td>
<td>1.12</td>
<td>1.13</td>
<td>1.09</td>
<td>0.513</td>
</tr>
<tr>
<td>History of hypertension (%)</td>
<td>10.8</td>
<td>19.2</td>
<td>10.0</td>
<td>13.5</td>
<td>10.3</td>
<td>0.376</td>
</tr>
<tr>
<td>Smoking (% current smokers)</td>
<td>34.8</td>
<td>36.5</td>
<td>40.2</td>
<td>25.1</td>
<td>54.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.488</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>55.8</td>
<td>49.2</td>
<td>48.6</td>
<td>53.4</td>
<td>49.9</td>
<td>0.472</td>
</tr>
<tr>
<td>Low socio-economic status (%)</td>
<td>14.8</td>
<td>12.6</td>
<td>14.1</td>
<td>14.6</td>
<td>25.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol (g/week)</td>
<td>135</td>
<td>103</td>
<td>134</td>
<td>113</td>
<td>138</td>
<td>0.460</td>
</tr>
<tr>
<td>β-Carotene (µg/g)</td>
<td>0.41</td>
<td>0.46</td>
<td>0.43</td>
<td>0.40</td>
<td>0.48</td>
<td>0.191</td>
</tr>
<tr>
<td>Lycopene (µg/g)</td>
<td>0.27</td>
<td>0.28</td>
<td>0.28</td>
<td>0.27</td>
<td>0.32</td>
<td>0.055</td>
</tr>
<tr>
<td>α-tocopherol (µg/g)</td>
<td>187.3</td>
<td>195.7</td>
<td>200.9</td>
<td>198.2</td>
<td>189.0</td>
<td>0.834</td>
</tr>
<tr>
<td>Selenium (mg/kg)</td>
<td>0.58</td>
<td>0.55</td>
<td>0.55</td>
<td>0.57</td>
<td>0.59</td>
<td>0.158</td>
</tr>
<tr>
<td>Mercury (mg/kg)</td>
<td>0.22</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
<td>0.29</td>
<td>0.001</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>101</td>
<td>103</td>
<td>103</td>
<td>113</td>
<td>118</td>
<td>0.001</td>
</tr>
<tr>
<td>Scandium (µg/kg)</td>
<td>4.38</td>
<td>5.36</td>
<td>4.38</td>
<td>8.91</td>
<td>20.82</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4

Risk of first myocardial infarction by quintiles (Q) of cerium (relative risk ratios and 95% confidence intervals)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.1</td>
<td>55.0</td>
<td>53.5</td>
<td>53.4</td>
<td>51.0</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0</td>
<td>26.2</td>
<td>26.1</td>
<td>26.0</td>
<td>25.2</td>
<td>0.020</td>
</tr>
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For SES did not really change these results. Adjustment for classical risk factors in non-smokers showed a p-trend of 0.137. When we included in the model, β-carotene, lycopene, α-tocopherol, selenium, mercury and scandium the odds ratios in the 4th and 5th quintiles of cerium as compared with the lowest were 2.09 (95% CI 1.05–4.16) and 2.81 (95% CI 1.21–6.52), respectively, p-trend 0.011 (Table 5).
toenails grows at a rate of 1 cm every 9–12 months (Tosi and Piraccini, 1999). Garland et al. (1993) have studied the reproducibility of 16 trace elements in toenails and found correlations of 0.48 (selenium), 0.54 (arsenic) and 0.58 (zinc). Our group has studied metal exposure in selenium, mercury, zinc, scandium and recently also in chromium. There are other studies which have used toenails for investigating long-term inhaled exposure. For instance, Mortada et al. (2001) studied lead exposure from automobile exhaust. Agahian et al. (1990) found a high correlation \( r = 0.89 \) when they compared mean air arsenic concentrations exposure group with corresponding arsenic levels in fingernails. In the Nurses’ Health Study toenail nicotine levels was demonstrated to be a valid biomarker of tobacco exposure \( r = 0.8 \) (Wael et al., 2002). Therefore, it is unlikely that the measurements could have been affected by the disease process or a recent change of the diet.

Although we cannot completely exclude the possibility of nail contamination, these toenails are from men and it seems unlikely to have been contaminated by nail polish. Furthermore, as indicated by the study protocol, nails were collected, cleaned, stored and analysed in exactly the same way in cases and controls, thus allowing comparability between both groups. Nevertheless, the overall weight of nails was less in controls than in cases (49.9 vs. 57.8 mg, \( p < 0.001 \)).

Cerium is added to diesel fuel to improve its function. Indeed, cerium concentrations of 0.6 \( \mu \)g/m\(^3\) along a highway and of 1.25 \( \mu \)g/m\(^3\) in a street canyon have been reported. Concentrations due to deposition of cerium-containing particulate matter (PM) in soil are predicted to be 5–30 ppm near roads with heavy traffic by the year 2050 (HEI, 2001). Cerium and radiocerium may also be absorbed by vegetables or contaminant water (NRCP, 1978). We could not find good data on the cerium content of cigarettes. An early patent suggested adding cerium to tobacco to reduce the formation of carcinogens (TKT Tabak Forschungs—GmbH & Co., 1980). We do not know whether this patent has been or is applied. We found a significant association between cerium and smoking in controls. Furthermore, in our models it would appear that there is a strong association between smoking and cerium levels. Interestingly, this relationship between cerium and MI becomes stronger when the same model is applied to the data of the non-smokers.

As described before, main routes of exposure to cerium are inhalation and ingestion but cerium is poorly absorbed in the digestive tract converting inhalation in a world concern (HEI, 2001). The different concentrations of cerium in toenails among centres could be due to diverse exposure to inhalation. The highest concentrations of cerium found in toenails in Jerusalem may be consistent with the reports of air pollution in Israel. This is an increasing problem, mainly due to the rise of diesel engine vehicles (Karsenty and Leventhal, 2002). These authors underline the importance of fine PM, which are known as the most rapid way of cerium penetration in the blood stream through the lungs (HEI, 2001). Erel et al. (2002) have also reported high atmospheric concentrations of lead (23 ± 17 ng/m\(^3\)) transported to Jerusalem from Egypt, Turkey, and East Europe. Inhaled atmospheric soil dust might also explain the high levels of both scandium and cerium, highly correlated in this population. The relatively higher concentrations of cerium toenails in Moscow are also consistent with pollution. Ladonina et al. (1999), studying various heavy metals, in soils and vegetation of Southeastern Moscow reported a high degree of soil contamination by heavy metals which they attributed to industrial and railway pollution. Association between cerium and low socio-economic status may also be explained by pollution. Low income and minority populations are more likely to live near motor vehicles and main sources of many hazardous air in California (Gunier et al., 2003). However, we
appreciate that it will be difficult to disentangle the putative effects of cerium, cadmium, lead and other smoking or low socio-economic class related factors. Nevertheless, to our knowledge, this is the first study that suggests an association between cerium and risk of a first myocardial infarction.

As for the potential mechanism of action, the effect of cerium on experimental atherosclerosis appears not to have been fully understood yet. Animal studies have focused on the effect of radioactive cerium on carcinogenesis (Lundgren et al., 1996). Cerium (0.5 μM) stimulated a mitogenic response to cardiac fibroblasts due to intracellular generation of reactive oxygen species (Nair et al., 2003). Furthermore, cerium administration induces subendocardial fibrosis and increases the collagen content of the heart experimentally (Shivakumar et al., 1992; Kumar et al., 1996). CeCl3 also can cause, a rapid increase of triglycerides in liver of the rat (Salas et al., 1976) and lipid peroxidation in the chick liver (Basu et al., 1984). Cerium may reduce the oxygen binding capacity of haemoglobin in rats (Cheng et al., 2000). Cerium chloride at concentrations of between 5 and 100 μM induced apoptosis in rat alveolar macrophages in cultures (Lizon and Fritsch, 1999). Some of these may help to explain the high incidence of endomyocardial fibrosis in southern India probably due to the presence of cerium rich monazite in the soil (Valiathan and Kartha, 1990; Kuty et al., 1996).

We observed an inverse association between antioxidants status and risk of MI (Kardinaal et al., 1993, 1997; Gómez-Aracena et al., 1997). Toenail cerium was related to lycopene but apparently not to α-tocopherol, β-carotene or selenium. In any case, we decided to adjust for these antioxidants (and also for mercury) because of the role that these factors play in our case-control studies (Kardinaal et al., 1993; Guallar et al., 2002).

High toenail mercury is associated with an increased risk of MI in our population (Guallar et al., 2002). Mercury levels were related to those of cerium in the controls, but not in the patients with AMI. We did not find an interaction between cerium and mercury. There were also significant correlations between cerium and other metals, adjusted for age and centres both in cases and controls, although they tended to be less in cases than in controls.

The high correlation coefficient between cerium and scandium was particularly noteworthy. The scandium levels were especially high in quintile 5 of cerium; this might have influenced this high correlation. A similar correlation between these two trace elements has been described for plants (Markert, 1988). Rolling annual averages of cerium and of scandium in the atmosphere at four unrelated sites in the UK were remarkably related (Neath Port Talbot County Borough Council, 1998). Forty six elements, including scandium and cerium, were measured in 71 plants in Israel. Amongst several statistically significant correlation coefficients was the one between the levels of these two metals (Horowitz, 1999). The adjustment for scandium influenced the risk of MI considerably. It is important to point out that we do not understand how scandium can protect against the development of MI (Gómez-Aracena et al., 2002). We studied the possibility of collinearity between cerium and scandium. Using the model of simple linear regression the R square was 0.39 (variance inflation factor 1.64); thus we excluded collinearity (Kleinbaum et al., 1988). We examined this further and evaluated the odds ratios of MI by quintiles of cerium in tertiles of scandium, and after adjusting for age and centre we found a significant association between MI and cerium concentration in toenails in the lowest and middle tertiles (p < 0.05), but not in the highest level of scandium. More research about this potential interaction is warranted.

In conclusion, there is no clear plausible pathophysiological mechanism that could explain the increased risk of MI in those with higher cerium levels. Within a context of real concern about cerium pollution and limited available data, our results suggest that toenail cerium levels may be associated with an increased risk of AMI, but more research is warranted to shed further light and fully understand the plausibility and public health implications of these findings.

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Appendix

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References


