



## Levels of L-ascorbic acid and cadmium in the saphenous vein of patients with coronary artery disease are negatively correlated



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### ABSTRACT

**Background:** The aim of this study was the simultaneous determination of levels of cadmium and L-ascorbic Acid (AA) in human saphenous vein (SV) used in coronary artery bypass grafting (CABG) and check whether there is a relationship between these levels.

**Methods:** Human SV were collected from 40 patients (20 men and 20 women; age, 40–75 years) at the time of routine coronary artery surgical revascularization. The concentration of cadmium in the tissue was determined according to the GF AAS—atomic absorption method. The concentration of AA was assayed in supernatant by FIA method with spectrophotometric detection.

**Results:** AA concentration (mean  $\pm$  SD); men:  $98,7 \pm 13,18 \mu\text{g/g}$  tissue, women:  $96,06 \pm 11,98 \mu\text{g/g}$  tissue. Cadmium concentration (mean  $\pm$  SD); men:  $309 \pm 103,71 \text{ ng/g}$  tissue, women:  $348,5 \pm 255,71 \text{ ng/g}$  tissue. Correlations among concentrations of AA and cadmium were insignificant negative in the group of men (Pearson  $r = -0,1504$ ,  $p = 0,5269$ ) and in the group women (Pearson  $r = -0,339$ ,  $p = 0,144$ ).

**Conclusions:** Negative correlations among concentrations of AA and cadmium in human SV obtained in our study may indicate a protective effect of this vitamin in relation to toxic cadmium.

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## 1. Introduction

Coronary heart diseases (CHD) disease remains the important cause of death in most European countries. In 2009 CHD deaths (% of total) was greatest in Latvia and Slovakia (28%) for men and in Lithuania (40%) for women. The smallest mortality was in Portugal (7%) for men and in Luxembourg (6%) for women. In Poland for both sexes was 12% [33]. Coronary artery bypass graft (CABG) surgery increases survival in patients with severe coronary diseases and still remains one of the most common surgical procedures. Although arterial conduits are generally preferred, saphenous vein grafts (SVGs) continue to be used regularly. Patency of SVGs used for coronary artery surgery is a factor determining the survival of both the graft and the patient and is dependent on many factors [14]. One of the factors having a particularly harmful effect on human circulatory system is cadmium, a toxic metal present in certain foods, in tobacco smoke, and in ambient air. Systematic reviews and meta-analysis demonstrate the evidence in support of chronic cadmium

exposure as a risk factor for cardiovascular disease, peripheral arterial disease, stroke and heart failure [30,39].

AA is one of the substances which could protect vascular dysfunction caused by cadmium [6,28,27,8]. In our previous studies, we determined the levels of cadmium in human saphenous vein used in CABG [20] (article in Polish, abstract in English), the levels of cadmium [18,21] (articles in Polish, abstracts in English) and AA [19,23] (articles in Polish, abstracts in English) in blood of patients with stable coronary artery disease scheduled for CABG.

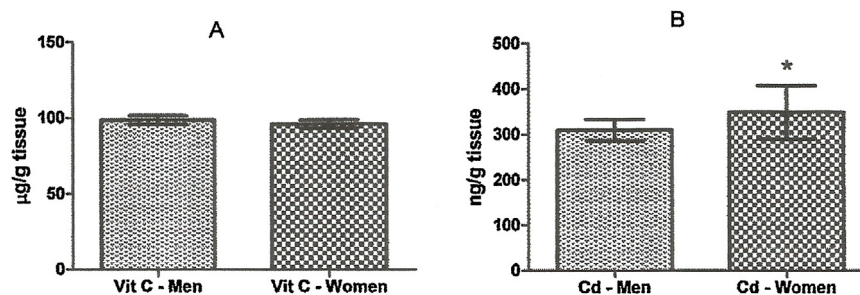
The aim of this study was the simultaneous determination of levels of cadmium and AA in human saphenous vein used in CABG and check whether there is a relationship between these levels.

## 2. Materials and methods

Human SV were collected from 40 patients (20 men and 20 women; age, 40–75 years) at the time of routine coronary artery surgical revascularization. Patients received typical preoperative cardiac treatment, such as  $\beta$ -blockers, calcium channel blockers, ACEI, nitrates and anticoagulants. This study was reviewed and approved by the local committee of the ethics on human research. Because the SV obtained during coronary artery bypass operation

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**Fig. 1.** Concentration of AA (A) and cadmium (B) in the human saphenous vein obtained from non-smoking patients with coronary artery disease, living in north-east region of Poland who were treated with coronary artery bypass grafting,  $n = 20$  for both, men and women group. Vertical lines represent  $\pm$  s.e. mean, \* $P < 0,05$  as compared between men and women.

was classified as a surgical specimen, its use was exempted by the committee from required patient consent. They declared their not smoking and lack of occupational exposure to cadmium.

SV fragments of approximately 5 cm were cut along the long axis of the vessel into two parts, each U-shaped. The first assayed for AA, the second for cadmium content.

The soft tissues were washed in physiological saline, with was subject to dry liberalization in an electric muffle furnaces at 105 °C for 2 h. The concentration of cadmium in the tissue mineralisates was determined according to the GF AAS—atomic absorption method (PHILIPS SCIENTIFIC PU 9100) equipped in an auto sampler and the cathode lamp of Cd operated at reference Cd line of 228.8 nm with electrothermal atomization in graphite couvette (PU 9390X; FS 90) as used. Mineralisates were obtained using mineralizer microwaves (ETHOSPLUS), 0.20 g of tissue was weighed and treated with 6,25 cm<sup>3</sup> of a mixture of HNO<sub>3</sub> 65% and H<sub>2</sub>O<sub>2</sub> 30% at the volumetric ratio 4:1 v:v. The time of mineralization was 20 min. For the first 10 min temperature was increasing up to 190 °C, and then it was kept at the level of 190 ± 5 °C.

All samples were analysed in fivefold. The mixture of palladium and magnesium as nitrates was used as a matrix modifier. The analytical quality of Cd measurements was checked by the use of Standard Reference Pig Kidney (No. 1688, BCR-186; Institute for Reference Materials and Measurements, European Union). Cd concentrations (mean  $\pm$  S.D.;  $n = 3$ ) determined by us in the reference material (2.64  $\pm$  0.11 µg/g) well agreed with the certified values (2.71  $\pm$  0.15 µg/g). The CV for Cd measurements was <5.0%. The limit of detection (was set at three times the standard deviation of the mean blank value) was 0047 µg/g dry wt.

The concentration of AA was assayed in supernatant by FIA method with spectrophotometric detection. The SV fragments after washing in physiological saline were weighed and homogenized in Teflon tube, then tissues were centrifugation centrifuged at 3000g for 20 min. All the procedures concerning preparation of reference solutions and experimental setup have been described previously [24,25].

### 2.1. Statistical analysis

Determining the level of cadmium were conducted five times for each sample, AA three times for each sample. The obtained results were developed statistically using the *t*-Student's test, variances analysis and Pearson's test. The differences were considered to be statistically significant at  $p < 0.05$ . The correlations between concentrations of AA and cadmium were evaluated by Pearson correlation. The mean, SD and Pearson coefficients values of levels of Cd and AA was performed for each samples.

### 3. Results

The results of the determination of AA and cadmium concentrations in the SV of the investigated group are presented in Table 1, and in Fig. 1.

Unpaired Student's *t*-test revealed statistically significant variances difference of cadmium concentrations between the two gender groups ( $p = 0,0002$ ). Analysis performed by Pearson's test showed no significant negative correlations among concentrations of AA and cadmium in the group of tissues taken from men (Pearson  $r = -0,1504$ ,  $p = 0,5269$ ), in the group of tissues taken from the women (Pearson  $r = -0339$ ,  $p = 0144$ ) and for both groups together (Pearson  $r = -0261$ ,  $p = 0104$ ).

### 4. Discussion

To our knowledge, levels of AA and cadmium in human SV have not been studied yet in the same tissue at the same time.

The levels of cadmium obtained in SV are similar to those obtained by us in our previous work [20]. Statistically significantly higher levels of cadmium were among women group. In this group there are several results significantly higher than the average value (760, 820, 950 ng/g tissue). This may be due to the fact that, contrary to the collected interview and declarations patients were occupationally exposed to cadmium or tobacco smoke. Levels obtained by us are slightly higher than the levels obtained by Jeng and co-authors in the internal spermatic vein (ISV) in the group of non-smokers, where the Cd content in the ISV was 59.84 ± 5.7 ng/g in the control group and 192.1 ± 24.2 ng/g in the varicocele group [12]. In turn Abu-Hayyeh and co-authors have received in the group of smokers the cadmium concentration of full-thickness aortas ranged from 10 to 6730 ng/g (mean 1460 ± 350 ng/g). In this work was no difference in the cadmium content of aortic media samples between normal diameters (2970 ± 590 ng/g) and aneurysmal diameters (3650 ± 100 ng/g; Mann-Whitney,  $P = 0.53$ ), and total cadmium content was associated with smoking, assessed as pack-years [1]. Levels of cadmium given by Abu-Hayyeh are much higher than those received by our and Jeng teams, and show a significant effect of smoking on the accumulation of cadmium in the blood vessels.

In the available literature we did not find results for determining the levels of AA in blood vessels. Our results show no differences in levels of AA depending on gender. Correlations between the levels of AA and cadmium are statistically insignificant, but for both sexes they are negative, stronger for women (Pearson  $r = -0339$ ,  $p = 0144$ ), than for men (Pearson  $r = -0,1504$ ,  $p = 0,5269$ ).

Several studies have shown a negative effect of cadmium on the human circulatory system. In studies involving several thousand people, Maria Tellez-Plaza & coauthors demonstrated that cadmium, at substantially low levels of exposure is an important determinant in cardiovascular disease mortality [41], cadmium

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