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ORIGINAL ARTICLE

Ischemia-Modified Albumin Expression: Is there a Difference between Male and Female Subjects?

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SUMMARY

Background: **Ischemia-modified albumin (IMA) derives from naive albumin, modified in the binding region of bivalent ions, as cobalt and iron. The cobalt, released from some types of hip prosthesis seems to be metabolized differently in males and females but the iron ion is more prevalent than cobalt and is detectable in the healthy population. Our aim was to verify if there are any gender- and age-related differences in IMA concentrations and if IMA correlates with cobalt and iron-related proteins.**
Methods: **IMA, albumin, iron, ferritin, transferrin, and cobalt were measured in 50 men and 50 women divided into two age/fertility-homogeneous groups.**
Results: **Men < 45-years-old showed a statistically significant lower IMA concentration than men ≥ 45 and fertile and menopausal women. Considering all the population studied, IMA does not seem to be correlated with age and is distributed differently by gender; also, Co distribution was different between males and females.**
Conclusions: **IMA did not correlate with cobalt, iron, ferritin, and transferrin in any group, except for fertile women where IMA presented a statistically significant correlation with serum iron values.**
Minor expression of IMA in young males together with the results obtained on serum iron in fertile females, could explain the higher accumulation of circulating Co in women compared to men and their different cobalt metabolism.
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INTRODUCTION

Albumin, the most abundant plasma protein, plays a fundamental role in the distribution of many metabolites, hormones, drugs and essential transition metal ions in the human body. Its action is achieved by the presence of several specific binding sites. In some pathological conditions, including cardiac ischemia, an albumin variant called Ischemia-Modified Albumin (IMA) has been identified. It has the same sequence as the original protein with a post-transcriptional modification of a few amino acids at the N-terminal, which repre-

sents the binding site of some bivalent metals [1-3]. It has been suggested that these changes are related to the production of reactive oxygen species during ischemia and/or hypoxia, acidosis, and reperfusion [4]. However, increased IMA concentrations do not seem to depend purely on myocardial involvement as it is also increased in other diseases associated with oxidative stress such as obesity, type 2 diabetes mellitus, hypercholesterolemia, renal disease [5-7], as well as in patients with gastric, prostate, neuroblastoma, and soft tissue cancers [8-11].

Evidence of differences in gender specificity are incomplete [12].

The modification is found in 90% of ischemia cases; therefore, the IMA assay has been validated, and later approved by the FDA (US Food and Drug Administration), as a clinical marker of ongoing myocardial infarction [13]. The quantification of IMA in circulating blood is usually performed with a colorimetric test (ACB® albumin-cobalt binding test) based on a side-effect of the post-transcriptional modification: the modification of IMA reduces its binding efficiency to transition metals [2]. The test was developed by Bar-Or et al. to detect structural changes in the albumin N-terminus [1] and later improved by Lee [2].

In this method, IMA concentration can be determined by adding a known amount of Co(II) to a serum specimen and measuring the unbound Co(II) by colorimetric assay using dithiothreitol (DTT). There is an inverse relationship between the amount of albumin-bound cobalt and the intensity of the color formation.

Based on the knowledge of the principle that the dosage of IMA is determined by the addition of cobalt ion, our group, which has had a long-term interest and studied the toxic effects produced *in vivo* by the cobalt ion, was interested in further study. Cobalt can be released in abnormal quantities by a particular type of hip prosthesis, in which the joint sliding surfaces are made of a cobalt-chromium alloy (Metal-on-Metal, MoM). In the presence of some risk factors, circulating ion levels may reach acute lethal or sublethal toxicity concentrations, up to over 600 micrograms/liter, when the reference value for non-exposed subjects is below 1.

It is also known that females are exposed to a greater risk of failure when implanted with MoM implants [14]. In addition, a greater accumulation of cobalt in the female population has been observed after exogenous exposure [15-17], compared to values in non-exposed individuals ranging from 0.1 and 0.6 μg/L, respectively. Starting from these observations, we decided to verify if IMA could represent one of the elements responsible for different transport/clearance of cobalt ion in men and women.

The primary aim of the study is to verify the distribution of IMA concentration in healthy subjects, stratified by gender, age, and by fertility status in female subjects, as this topic has not been treated up to now.

Secondary aim was to verify if IMA was correlated with circulating cobalt and with the two main proteins involved in iron metabolism, transferrin and ferritin.

MATERIALS AND METHODS

Patients enrollment

One hundred thirty-eight patients were screened, 38 were excluded due to either the presence of metallic implants (24) or diabetes (11) or both conditions (3). This observational study enrolled 100 subjects in the waiting list for elective orthopedic surgery (primary hip or knee prosthesis, ligament reconstruction, arthroscopy). Written, informed consent was obtained from all patients (according to Ethical Committee approval no. 8567 9/3/2015). Exclusion criteria were the presence of metallic implants, recent fractures, pregnancy, current or previous oncological pathologies, hemochromatosis, current or previous hepatic disease, thalassemia, severe anemia (Hb < 9), obesity and cholesterol status, renal disease, and diabetes. Enrolled patients were uniformly distributed for gender and age: there were 50 males and 50 females, in male group 25 were below 45 years old and 25 over; in female group 25 were fertile and 25 menopausal (Table 1).

Fertility status was ascertained for each woman; menopause was considered as amenorrhea for at least 12 months in the fifth decade of age. All female subjects with eumenorrhea were considered fertile. In case of uncertainty, patients were not enrolled.

Samples collection and blood analysis

Peripheral blood samples were harvested using disposable intravenous cannulas, withdrawn, and transferred into trace elements BD vacutainer tube (Becton Dickinson, Plymouth, PL6 7BP, UK).

Blood samples were centrifuged at 800 rcf (relative centrifuge force) for 7 minutes to collect serum; total albumin, ferritin, transferrin, and serum iron were measured immediately following routine colorimetric test methods (Cobas analyzer, Roche Diagnostics, USA), while the remaining samples were frozen and stored at -80°C until IMA ELISA assays. (Cusabio Biotech Co., LTD. Wuhan, Hubei Province 430206, P. R. China) and cobalt assay. ELISA IMA assay employs the quantitative sandwich enzyme immunoassay technique. The ELISA kit had a detection range up to 200 IU/mL, with an intra-assay precision < 8% (coefficient of variability, CV%) and inter-assay precision of < 10% (CV%). The normal value of IMA assessed by this kit is < 10 IU/mL, as declared by the manufacturer and previously validated [18].

ICP-MS (ELAN DRC II, Perkin Elmer, Waltham, MA, USA) equipped with dynamic cell reaction (DRC) was used for the cobalt assay. A reaction system with ammonia gas was used for the elimination of spectral interferences. The samples of serum were diluted (1:20) with Triton 0.05% for inorganic trace analysis (Merck KgaA). The curve and sample solutions were pumped into the spray chamber using a peristaltic pump. The

blank samples were used to correct for any contamination in each batch. The concentration of metal ions was expressed as ppb.
The calibration standards were prepared by standard solutions of single elements ranging from 0.5 to 1,000 ppb: cobalt in HNO3 2% mono elemental standard solution (Carlo Erba Reagenti, Milano, Italy) and chromium in HCl atomic absorption standard solution (Sigma-Aldrich, Milwaukee, WI, USA).
The accuracy of the method was determined according to the mean values obtained on certified reference materials (Environmental and Occupational G-EQUAS for blood and serum, Erlangen, Germany). The coefficient of variation was 6.1% and the limits of detection, calculated as three standard deviations of the background signal obtained on 10 white samples, were 0.05 ppb in all the matrices.
The method accuracy was assessed by the external quality assessment program and it was certified for the above-mentioned metallic elements by G-EQUAS of the German Society of Occupational and Environmental Medicine.

Statistical analysis
GPower was used to perform calculations on sample size, effect size, and statistical power. The minimal significance (α) and statistical power (1-β) were set at 0.05 and 0.90, respectively. Calculations were performed for multiple groups (one-way ANOVA; *F*-distribution) [19].
The normality of the data distribution was verified with the use of the Shapiro-Wilk test. Results are presented as the median (range) for non-normal data.
A non-parametric statistical test (Kruskal-Wallis) was used in evaluations of the difference in distribution in the four groups for IMA and normalized IMA (IMA/albumin).
Pearson's correlation test was used for the correlation between IMA or normalized IMA with age, iron, transferrin, ferritin, and cobalt. The Mann-Whitney test was used to analyze the difference of expression levels of Co and IMA between women and men. Statistical significance was set at $p < 0.05$.
The statistical analysis was performed using SPSS v. 14.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows a summary of the results for IMA, total albumin, serum iron, transferrin, ferritin, and cobalt expressed as median, maximum, and minimum.
IMA median values observed in the 4 groups were well within the normality range. Only 5 patients had IMA values above 10 U/mL (two menopausal women, two men over 45 years old and one younger man); for none of these 5 patients was it possible to verify the presence of any cardiac pathology shortly after blood sampling, which would justify the higher IMA value. However, in 4 of these cases, IMA values were very close to the normality range and only one menopausal woman had a significantly high value of 39 U/mL.
The result of this analysis was that IMA concentration in young men was significantly lower than in the other 3 groups both considering IMA and IMA normalized (Kruskal-Wallis test, $p = 0.001$ for both) That is why the results of IMA in women are higher than in men, regardless age.
These results were represented by the boxplots in the Figure 1, where only IMA results were graphically represented.
We repeated the analysis considering women divided in two age groups ($19 < 45$ and $31 \geq 45$): this also showed no difference (data not shown).
A correlation was verified between IMA (normalized and not) and age (Pearson's correlation, $p = 0.021$ and $p = 0.041$, respectively) but not between IMA and iron, transferrin, ferritin or cobalt.
Both IMA and cobalt values are distributed differently according to gender (Mann-Whitney, $p = 0.004$ and $p = 0.001$, respectively) even if the difference has no clinical value, such as always falling inside reference value range.

DISCUSSION

Results for IMA dosage demonstrate that women have higher circulating levels than men, and this is particularly true when women of any age or fertility status are compared to younger men.
We measured IMA levels by immunoenzymatic assay (ELISA) that specifically recognizes the epitope responsible of Co binding, instead of using the colorimetric assay, despite the latest being widely used and standardized. The main reason for this choice resides in the fact that the colorimetric assay is based on the binding ability of IMA to Co and this would not be applicable to patients that have high levels of circulating Co due to professional exposure or, more likely, to the presence of a cobalt releasing implant. Moreover, the albumin binding site for cobalt could be hampered by free fatty acids [20].
Patients wearing a metal-on-metal hip prosthesis sometimes have high levels of circulating cobalt, particularly in women. Females, despite the smaller dimension of the prosthesis, have significantly higher levels of circulating cobalt (unpublished data).
Therefore, the present study investigated if there are gender and age differences in IMA expression in unexposed subjects, determination of IMA with the immunoenzymatic assay, and also doing a correlation analysis for IMA and other Co and iron related parameters. Many papers that validated the role of IMA as a marker of several pathologies, cardiopathies in particular, have never considered any gender effect, or these have not clearly emerged from the presented data [12]. Also, the reference values used in the clinics do not take gender

Table 1. Concentrations (expressed as median, maximum, and minimum of IMA, albumin, iron, transferrin, ferritin and cobalt in all groups: 25 fertile women (18 < 45 years and 7 ≥ 45 years), 25 menopausal women (1 < 45 years and 24 ≥ 45 years), 25 men < 45 years and 25 men ≥ 45 years.

Reference values		Age (years)	IMA (< 10 IU/mL)	Iron (37 - 145 mg/100 mL)	Transferrin (200 - 360 mg/100 mL)	Ferritin M: 30 - 400 F: 15 - 150 (ng/mL)	Albumin (3.5 - 5.2 g/100 mL)	Cobalt (0.1 - 0.6 µg/L)
Fertile women	n	25.0	25.0	25.0	25.0	25.0	25.0	25.0
	median	40.0	6.4	73.0	272.0	38.0	4.7	0.4
	minimum	19.0	0.0	24.0	178.0	10.0	4.4	0.3
	maximum	53.0	9.8	165.0	351.0	179.0	5.2	1.1
Menopausal women	n	25.0	25.0	24.0	25.0	25.0	25.0	25.0
	median	62.0	3.4	72.0	259.0	82.0	4.6	0.3
	minimum	43.0	0.0	46.0	162.0	23.0	4.1	0.3
	maximum	82.0	39.6	136.0	336.0	1,623.0	5.3	0.8
Men ≥ 45 years	n	25.0	25.0	24.0	25.0	25.0	25.0	25.0
	median	62.0	3.3	94.0	262.0	177.0	4.8	0.4
	minimum	45.0	0.0	51.0	182.0	52.0	3.6	0.0
	maximum	87.0	16.7	148.0	326.0	682.0	5.1	1.3
Men < 45 years	n	25.0	25.0	25.0	25.0	25.0	25.0	25.0
	median	35.0	0.0	91.0	251.0	139.0	4.9	0.4
	minimum	19.0	0.0	52.0	222.0	36.0	4.4	0.3
	maximum	43.0	12.5	158.0	309.0	303.0	5.3	1.1
Total	n	100.0	100.0	98.0	100.0	100.0	100.0	100.0
	median	47.0	2.6	84.5	261.5	89.0	4.7	0.4
	minimum	19.0	0.0	24.0	162.0	10.0	3.6	0.0
	maximum	87.0	39.6	165.0	351.0	1,623.0	5.3	1.3

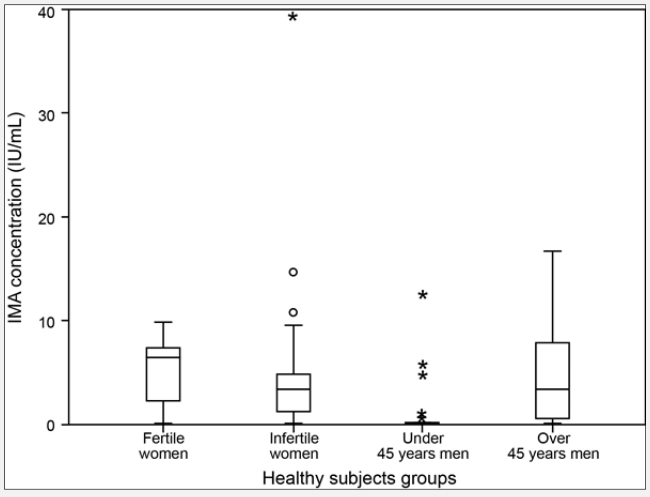

Figure 1. IMA concentration in all groups.

Boxes are limited by values of the 25th and 75th percentile. The horizontal line crossing the box represents the median value. The vertical lines are extended from min to max value. Outliers are indicated by * and °, depending on their distance from 75th percentile (* for values greater than 3 interquartile differences and ° for values greater than 1.5 interquartile differences, respectively).

into account and mainly assayed using the colorimetric method. Conversely, our results demonstrated the presence of a gender and age difference. Our study showed that male and young subjects had significantly lower levels of IMA compared to the other studied categories. From literature data, two different explanations for these findings can be found.

Niki showed that an increased Total Antioxidant Capacity (TAC) reduces IMA expression [21]; this is because the formation of free radicals may alter the ability of three amino acids (aspartate, alanine, and histidine) at the albumin N-terminus to bind free metal ions, including cobalt. Some studies have reported higher TAC levels in the saliva of younger subjects [22], and significantly lower salivary TAC in women than in men [23]. Moreover, age and gender differences were observed in oxidative stress markers in plasma [24], and this could explain the reason why younger male subjects have a lower level of IMA compared to women of any age and compared to older men.

In addition, it is known that there is a different response to oral administration of Co ion in women compared to men. In fact, some toxicological studies have demonstrated that by administering oral Co 1.0 mg/day to 10 healthy volunteers, 5 males and 5 females, for a period of 31 or 89 days, Co concentration in serum or whole blood was 2 times higher in female compared to male subjects [17,25]. This is probably due to differences in absorption and excretion of Co in women, who generally absorb more and excrete less Co than men [26]. This gender and age difference could be a co-cause that, theoretically, could explain the different metabolism and excretion of Co in male and female patients with MoM prosthesis.

The secondary objective of this study was to verify if there was a correlation between IMA and cobalt, iron and some iron-correlated proteins, which have different concentrations in men and women and mostly in fertile and menopausal women. In fact, it is known that cobalt and iron share a common intestinal uptake mechanism of absorption [27].

IMA was not correlated with blood concentration of cobalt, iron, ferritin, and transferrin in any of the studied groups with the exception of fertile women where IMA was positively correlated with iron concentration. As clinically expected, a statistically significant difference in transferrin, albumin, and ferritin values has also been observed between fertile and menopausal women. This evidence of gender difference in iron metabolism and IMA involvement supports, at least indirectly, our theory of a gender influence in the metabolism of bivalent ions, including Co, which is an important factor in orthopedic settings; in fact, cobalt has a different distribution between man and women subjects.

The strength of the present study is related to the multiple determinations performed on patients, aiming at clarifying a possible relationship between IMA and other metabolites.

Among the limitations of the present study is the lack of data on the oxidative stress defense level presented by the enrolled patients, which could in turn influence their IMA level [28]. In addition, no nvestigation on diet and smoking habits was performed. Conversely, the subjects enrolled in our study belong to a population for which all major risk factors responsible for the alteration of IMA expression have been excluded.

CONCLUSION

The different expression of IMA by gender and, in particular, the lower expression of IMA in young males together with the results on cobalt, iron, and iron related proteins can explain the higher accumulation of circulating Co by exogenous or endogenous intake in the female compared to the male population.

This study gives important information on the expression of IMA in a population not exposed to metals and therefore it represents the basis for further studies on patients with MoM prosthesis.

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Declaration of Interest:

The authors declare that there are no conflicts of interest.

References:

1. Bar-Or D, Lau E, Winkler JV: A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia - a preliminary report. J Emerg Med 2000;19(4):311-315 (PMID: 11074321).

2. Lee E, Eom JE, Jeon KH, et al. Evaluation of albumin structural modifications through cobalt-albumin binding (CAB) assay. J Pharm Biomed Anal 2014;91:17-23 (PMID: 24434278).

3. Paustenbach DJ, Tvermoes B., Unice KM, et al. A review of the health hazards posed by cobalt. Crit Rev Toxicol 2013;43:316-362 (PMID: 2365655).

4. Bar-Or D, Rael L, Bar-Or R, et al. The cobalt-albumin binding assay: insights into its mode of action. Clin Chim Acta 2008;387: 120-127 (PMID: 17964561).

5. Piva SJ, Duarte MM, Da Cruz IB, et al. Ischemia-modified albumin as an oxidative stress biomarker in obesity. Clin Biochem 2011;44:345-347 (PMID: 21159315).

6. Caglar GS, Oztas E, Karadag D, et al. Ischemia-modified albumin and cardiovascular risk markers in polycystic ovary syndrome with or without insulin resistance. Fertil Steril 2011;95: 310-313 (PMID: 20701906).

7. Duarte MM, Rocha JB, Moresco RN, et al. Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. Clin Biochem 2009:42: 666-671 (PMID: 19318029).

8. Mastella AK, Moresco RN, da Silva DB, et al. Evaluation of ischemia-modified albumin in myocardial infarction and prostatic diseases. Biomed Pharmacother 2009;63:762-766 (PMID: 19375 269).

9. Stachowicz-Stencel T, Synakiewicz A, Owczarzak A, et al. Ischemia-Modified Albumin as a biochemical marker in children with neuroblastoma and soft tissue sarcomas. Clin Lab Anal 2011;25: 255-258 (PMID: 21786329).

10. Fidan E, Mentese A, Kavgaci H, et al. Increased ischemia-modified albumin levels in patients with gastric cancer. Neoplasma 2012;59:393-397 (PMID: 22489694).

11. Chan MY, Pronovost PJ: Clinical utility of biomarkers in myocardial injury. Curr Opin Anaesthesiol 2004;17(1):49-55 (PMID: 17021528).

12. Grovender R, De Greef J, Delport R et al. Biological variation of ischaemia-modified albumin in healthy subjects. Cardiovasc J Afr 2008;19:141-144 (PMID: 18568173).

13. Keating L, Benger JR, Beetham R, et al. The PRIMA study: presentation of ischemia modified albumin in the emergency department. Emerg Med J 2006;23:764-768 (PMID: 16988302).

14. AOANJRR. Australian Orthopaedic Association National Joint Replacement Registry. Annual Report 2017. https://aoanjrr.sahmri.com/documents [accessed 18.07.18]

15. Tower SS. Arthroprosthetic cobaltism: neurological and cardiac manifestations in two patients with metal-on-metal arthroplasty: a case report. J Bone Joint Surg Am 2010;92:2847-2851 (PMID: 21 037026).

16. Bradberry SM, Wilkinson JM, Ferner RE. Systemic toxicity related to metal hip prostheses. Clin Toxicol (Phila) 2014;52:837-847 (PMID: 25132471).

17. Finley BL, Unice KM, Kerger BD, et al. 31-day study of cobalt (II) chloride ingestion in humans: Pharmacokinetics and clinical effects. J Toxicol Environ Health A. 2013;76:1210-1224 (PMID: 24283372).

18. Facchin F, Catalani S, Bianconi E, et al. Albumin as marker for susceptibility to metal ions in metal-on-metal hip prosthesis patients. Hum Exp Toxicol 2016;36(4):319-327 (PMID: 27206702).

19. Faul F, Erdfelder E, Lang A-G, et al. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences.Behavior Research Methods 2007:39, 175-191 (PMID: 17695343).

20. Coverdale JPC, Katundu KGH, Sobczak AIS et al. Ischemia-modified albumin: Crosstalk between fatty acid and cobalt binding. Prostaglandins Leukot Essent Fatty Acids 2018:135:147-157 (PMID: 30103926).

21. Niki E. Assessment of antioxidant capacity *in vitro* and *in vivo*. Free Radical Biology and Medicine 2010:49(4):503-515 (PMID: 20416370).

22. Krawczyk D, Sikorska-Jaroszyńska MHJ, Mielnik-Błaszczak M, et al. Dental caries and total antioxidant status of unstimulated mixed whole saliva in patients aged 16-23 years. Advances in Medical Sciences 2012;57(1):163-168 (PMID: 22472470).

23. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clinical Science 2003;105(2):167-172 (PMID: 12650638).

24. Veglia F, Cighetti G, De Franceschi M, et al. Age- and gender-related oxidative status determined in healthy subjects by means of OXY-SCORE, a potential new comprehensive index. Biomarkers 2006;11(6):562-573 (PMID: 17056475).

25. 26. Tvermoes BE, Unice KM, Paustenbach DJ, et al. Effects and blood concentrations of cobalt after ingestion of 1 mg/d by human volunteers for 90 d. Am J Clin Nutr 2014;99:623-646 (PMID: 24500148).

26. Unice KM, Kerger BD, Paustenbach DJ, et al. Refined biokinetic model for humans exposed to cobalt dietary supplements and other sources of systemic cobalt exposure. Chem Biol Interact 2014; 216:53-74 (PMID: 24726710).

27. Barceloux D, Cobalt. Clin Toxicol 1999;37:201-206 (PMID: 103 82556).

28. Lainiala OS, Moilanen TP, Hart AJ, et al. Higher Blood Cobalt and Chromium Levels in Patients With Unilateral Metal-on-Metal Total Hip Arthroplasties Compared to Hip Resurfacings. J Arthroplasty 2016;31(6),1261-1266 (PMID: 26775067).