Impact of Aging on Urinary Excretion of Iron and Zinc
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ABSTRACT
PROJECT: Data about the influence of aging on urinary excretion of iron and zinc are scarce. The objective of the present study was to compare the concentration of zinc and iron in the urine of healthy elderly subjects and younger adults.
PROCEDURE: Seven healthy elderly subjects and seven younger adults were selected and submitted to biochemical, clinical, and nutritional tests. After a fasting period, 12-hour urine was collected for the determination of iron and zinc concentrations by graphite furnace atomic absorption spectrophotometry.
RESULTS: Urinary zinc and iron concentrations of the elderly subjects were not significantly different from that of younger adults. However, the total zinc and iron urinary clearance in 24 hours for the elderly was significantly higher compared with that of younger adults.
CONCLUSION: There is an increase in urinary iron and zinc clearance with aging. The values reported in this manuscript may be used as references in future studies.
KEYWORDS: micronutrients, iron, zinc, physiology of aging, atomic absorption spectrophotometry

Introduction
Minerals, iron and zinc in particular, are inorganic elements widely distributed in nature that perform various metabolic functions such as hormone and enzyme production and macronutrient metabolism, among others. Iron deficiency is one of the more severe nutritional deficiencies, affecting 30% of the world population, and zinc deficiency is also one of the more severe deficiencies.1 Deficiency of both iron and zinc is prevalent among the elderly.

The elderly population is increasing, and this age range (65 years and more) is more susceptible to deficiencies of different nutrients. Despite the importance of minerals, few studies have reported urinary values for the elderly. Urinary values of minerals are usually correlated in different clinical conditions.2–4 However, data about urinary excretion in elderly subjects considered to be healthy are scarce, although knowledge about this topic is fundamental for the understanding of the kinetics and the age-related changes in the metabolism of these minerals. Thus, the objective of the present investigation was to study the urinary excretion and urinary clearance of iron and zinc in healthy elderly subjects and younger adults.

Material and Methods
Subjects. Seven healthy persons aged 60–70 years (five women, mean age 65 years and two men, mean age 66 years) were studied. Volunteers were selected by interview from 132 patients followed at the ophthalmology and geriatrics outpatient clinics in University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (HCFMRP-USP). Basic activities of daily living were assessed by the Barthel...
scale, and instrumental activities of daily living by the Lawton scale. A group of seven younger adults (four women, mean age 28 years) was also studied in outpatient clinics to check up in University Hospital, HCFMRP-USP.

**Ethics.** All volunteers gave written informed consent to participate, and the study was approved by local Human Research Ethics Committee.

All subjects in the study were submitted to careful clinical and biochemical examination, and exclusion criteria were body mass index below 18.5 and above 24.5 kg m⁻²; self-reported weight loss during the last six months; cigarette smoking; alcoholism; anemia; diabetes; cardiovascular, neuromuscular, hepatic, and renal diseases; and the intake of medications that could change absorption and excretion of minerals (ie diuretics, beta-blockers).

All volunteers were admitted to the Metabolism Research Unit of HCFMRP at 7:00 am after a 12-hour fast. Room temperature was kept constant at 23°C, during the 12 hours of the experiment. A standardized diet was offered every two hours, with a total of 125 kcal/kg/day (Table 1). Iron and zinc contents of the food were determined using a food contents table. Liquid intake was standardized at 10–20 mL/kg to maintain a constant urinary flow and avoid dehydration. A zinc- and iron-free container was used for delivery water. The protocols of diet and urine collection are described elsewhere—15 N glycine protein metabolism study.

Urine was collected during the 12 hours of study. Urine volume was measured in a graduated cylinder, and urine was preserved in 1 N HCl. All materials employed were left in 30% nitric acid in Milli Q® water for 24 h. A fasting (time zero) sample was collected for the determination of the fasted urinary zinc and iron concentrations.

For zinc determination, urine was diluted in ultrapure Milli Q® water, with the addition of 3% suprapure nitric acid. For iron determination, 5 mL of urine was diluted in Milli Q® water with the addition of 3% suprapure nitric acid up to 50 mL.

Urine samples were analyzed with an atomic absorption spectrophotometer (model 6200®, Shimadzu, Kyoto, Japan) having a deuterium lamp background, in the Laboratory of Mass Spectrometry of School of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, Brazil.

For zinc determination, the equipment was used in the flame mode. Gases employed for combustion were acetylene (99.7% purity) and synthetic air (20% O₂ and N₂) at a flow of 2.2 and 15 L/minute, respectively. Iron was determined with a graphite furnace (GFA-EX7®) coupled to the atomic mass spectrometer. Argon was employed for analysis during the atomization period.

Correlation coefficients for iron and zinc standards were 0.993 and 0.999, respectively. The automatic micropipette of the equipment was cleaned with ultrapure water containing 0.3% of ultrapure nitric acid.

Blood samples were collected in time zero and biochemical blood tests (complete hemolytic cell count, plasma glucose, creatinine, and urea levels) were analyzed in the laboratory of the Clinics Hospital of the Ribeirão Preto Medical School, University of São Paulo. Creatinine clearance was calculated according to Cockcroft and Gault.

**Statistical analysis.** Descriptive data are reported as means ± SD. The Kolmogorov–Smirnov Z test showed that the data had normal distribution, and the Student’s t-test was used to analyze group means for all variables. Data were analyzed statistically using the SPSS 16.0 software, with the level of significance set at P < 0.05.

**Results.** Regarding general aspects, the elderly volunteers had a high score for the basic and intermediate daily life activities. Regarding physical activity, all volunteers stated that they were sedentary. Mean educational level was incomplete high school for the elderly subjects and complete high school for the younger adults. Mean age was 28 ± 2.7 years for younger adults and 65 ± 2.8 years for the older ones.

Laboratory tests were normal for both groups, as shown in Table 2. Serum glycemia and urinary creatinine differed between groups although both groups had adequate reference values, with the absence of diabetes or renal changes. Zinc consumption was 13 ± 2 mg, and iron consumption was 10 ± 3 mg. Urinary zinc concentration (mean ± SD) was 85 ± 36 µg/L for the elderly subjects and 61 ± 36 µg/L for the younger adults (P = 0.467), and urinary iron concentration was 101 ± 45 µg/L for the elderly subjects and 59 ± 46 µg/L for the younger adults (P = 0.105). Table 3 and Figure 1 present the urinary excretion values for both groups. There were significant differences between younger adults and elderly subjects regarding iron and zinc concentrations corrected for urinary creatinine.

**Discussion**

The laboratory tests of all volunteers were within the normal range regarding nutritional assessment, metabolism, and renal function. Urinary creatinine and creatinine clearance were reduced in the elderly subjects compared to the younger adults, probably because of their lower muscle mass and the effects of aging on renal function. It should be pointed out that none of the volunteers were taking supplements at the time of collection and that all of them were sedentary. De Leo and Di Francesco reported that urinary creatinine excretion was 0.47 g/day in elderly subjects older than 90 years. In contrast,

**Table 1.** Mean dietary intake of the volunteers during the in the experiment.

| EACH MEAL   | CALORIES (kcal) | IRON (mg) | ZINC (mg) |
|-------------|-----------------|-----------|-----------|
| Rice        | 82              | 0.54      | 0.34      |
| Meat        | 72              | 1.41      | 3.32      |
| Soya oil    | 18              | 0.00      | 0.00      |
| Lemon juice with sugar | 101 | 0.12      | 0.24      |
| **Total**   | **5 kcal/kg weight** | **2.07**  | **0.97**  |
among adults aged 20–45 years, urinary creatinine excretion may vary from 1.80 to 1.17 g/day. These values are close to those observed in the present study.

In the present study, mean urinary iron excretion was close to literature values in healthy adults (58 ± 3 µg/L, age up to 30 years). The values found for iron loss are well above the ones described in other researches for younger volunteers, where it ranged from 51.8 to 88 µg day⁻¹ (Refs. 15–17) and close to the amounts found in patients with hypertension, arthritis, and obesity, where it ranged from 470 to 550 µg day⁻¹. Regarding zinc loss in urine, the values found in this study are within the range described by Schroeder and Nason, in 1971, and above the output found by Helwig et al. in 1966. However, a recent study in 2011 reported lower urinary iron excretion than the present values for all age ranges. It should be pointed out that the technique used in that study (inductively coupled plasma mass spectrometry (ICP-MS)) is more sensitive for plasma than for urine.

Regarding urinary zinc, mean excretion was lower than reported data (439 ± 32 µg/L). The values found in the present study were within the reference values for the same method of determination. However, comparison of the present urinary zinc values to the values obtained in a study on postmenopausal women showed greater excretion in the young adults of this study and lower excretion in the elderly subjects. Schroeder and Nason reported dietary zinc consumption similar to that observed in the present study, but a greater zinc excretion. A literature review about zinc excretion showed that the zinc excretion values observed in the present study were within the range for healthy persons.

When we corrected the iron and zinc values for urinary creatinine per 24 hours, these values were lower for the elderly, suggesting the occurrence of a reduction in urinary filtration rate with age. The use of urinary creatinine as a parameter allows correction for age, sex, and nutritional and hydration states. Some authors do recommend this correction although there is no significant association with age.

In conclusion, in this study the absolute urinary zinc and iron values did not differ between healthy elderly subjects and among adults aged 20–45 years, urinary creatinine excretion may vary from 1.80 to 1.17 g/day. These values are close to those observed in the present study.

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In conclusion, in this study the absolute urinary zinc and iron values did not differ between healthy elderly subjects and
younger adults, even though the elderly subjects had a lower creatinine clearance. Therefore, we suggest that an increase in urinary iron and zinc clearance occurs with aging. In addition, these data can be used for comparison in future studies on elderly subjects.

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Author Contributions
Conceived and designed the experiments: KP, JSM, EF. Analyzed the data: KP, RFM, GJP. Wrote the first draft of the manuscript: KP. Contributed to the writing of the manuscript: RFM, JSM, JCM. Agree with manuscript results and conclusions: KP, RFM, JSM, EF. Jointly developed the structure and arguments for the paper: KP, EF. Made critical revisions and approved final version: KP, EF. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with ethical and legal guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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