

Thiamin, Selenium, and Copper Levels in Patients with Idiopathic Dilated Cardiomyopathy Taking Diuretics

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Objective - To analyze the association of thiamin, selenium, and copper serum levels with cardiac function in patients with idiopathic dilated cardiomyopathy using diuretics, and also to compare them with levels in control patients with no evidence of disease.

Methods - The study comprised 30 patients with heart disease and 30 healthy control individuals. Thiamin was analyzed by measuring the activity of erythrocytic transketolase and the effect of thiamin pyrophosphate. Selenium and copper serum levels were measured by hydride generation and flame atomic absorption spectrophotometry, respectively.

Results - Thiamin deficiency was observed in 10% of the control individuals and in 33% of the patients with heart disease ($p=0.02$). The mean selenium and copper serum levels in control individuals and patients with heart disease were, respectively, $73.2 \pm 9.9 \mu\text{g/L}$ (56.5 to 94.5 $\mu\text{g/L}$) and $72.3 \pm 14.3 \mu\text{g/L}$ (35.5 to 94 $\mu\text{g/L}$) ($p=0.77$); $1.1 \pm 0.4 \text{mg/L}$ (0.6 to 1.8 mg/L) and $1.2 \pm 0.4 \text{mg/L}$ (0.6 to 2.2 mg/L) ($p=0.27$). No association between the levels of these nutrients and cardiac function was observed.

Conclusion - Thiamin deficiency was significantly more frequent in patients with heart disease. No significant difference was observed between the mean selenium and copper serum levels in control individuals and in patients with heart disease. The results suggest possible benefits with thiamin replacement in patients taking diuretics.

Keywords: thiamin, selenium, copper, diuretics, cardiomyopathy

In addition to the well-known adverse effects of the prolonged use of diuretics, thiamin deficiency induced by the use of diuretics has been reported since the late 1970s^{1,2}. An Israeli study³ assessed the hypothesis that prolonged treatment with furosemide in patients with heart failure was associated with a clinically significant deficiency of thiamin due to an increase in the urinary excretion of that nutrient. This deficiency was observed in 21 of the 23 patients with heart failure using furosemide and in only 2 of the 16 control individuals ($p<0.001$). Thiamin replacement also caused a mean 13% increase in ejection fraction in 4 of the 5 patients who underwent echocardiography. This study used a technique reported by Brin⁴ to measure the effect of thiamin pyrophosphate on the erythrocytic transketolase activity. The mechanism by which diuretics increase the urinary excretion of thiamin is the increase in the urinary flow, common to all diuretics^{5,6}. A randomized double-blind study⁷ assessed the effect of thiamin replacement on its organic level, on functional capacity, and on left ventricular ejection fraction in patients with heart failure using furosemide at the dosage $\geq 80 \text{mg/day}$. The effect of thiamin pyrophosphate was also assessed, and correction of the deficiency, increased diuresis, and an improvement in ejection fraction were observed. More recently, the thiamin status in rats receiving intraperitoneal doses of furosemide was assessed, and only the greater doses of furosemide induced thiamin deficiency, which could be reversed with replacement of that nutrient⁸.

The importance of selenium in human nutrition was emphasized in 1979, when Chinese scientists reported that its supplementation prevented the development of a cardiac disease known as Keshan disease in children living in areas with a selenium-poor soil⁹. The recommended selenium dietary allowances (RDA) in the United States of America are 55 $\mu\text{g/day}$ for women and 70 $\mu\text{g/day}$ for men. The selenium level in agricultural products is proportional to that nutrient's content in the soil where the agricultural products are cultivated. The geochemical composition of certain regions, such as Finland, New Zealand, the eastern coast of the USA, and China, reveals selenium deficiency in the soil¹⁰. This is the reason why studies carried out in a number of

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countries about the normal selenium serum level reported values ranging from 0.53 $\mu\text{mol/L}$ in Finland to 2.01 $\mu\text{mol/L}$ in Canada ¹¹ (tab. I). As far as we know, no similar study has been conducted in the Brazilian population, and, in our country, the geographical regions are as different as the composition of their soils.

The results of a number of studies about cardiac disease induced by selenium deficiency, such as Keshan disease that has been controlled with oral replacement of sodium selenite, have been published ¹²⁻¹⁴. Most of the studies reported patients who had undergone long-term parenteral nutrition without selenium replacement, or patients with acquired immunodeficiency syndrome and severe undernourishment ¹⁵⁻¹⁷. However, the association between low selenium levels and cardiovascular diseases remains controversial.

A low copper-content diet also results in cardiomyopathy in rats ¹⁸. The National Research Council in the USA established the safe content of daily copper intake between 1.5 and 3.0 mg/day. The exact mechanisms of cellular injury in copper deficiency have not yet been clarified. However, because the superoxide dismutase enzyme activity is reduced with copper deficiency, we can assume that the lesions found in the myocardial basement membrane result from the loss of protection against the free oxygen radicals produced ¹⁸. The treatment with antioxidants of rats with cardiac disease induced by copper deficiency resulted in a reduction in cardiac hypertrophy and milder mitochondrial injury ¹⁹. In 15-day-old pigs receiving a copper-deficient diet for 8 weeks, an increase in the heart weight/body weight ratio and electrocardiographic alterations were observed, confirming the findings in rats ²⁰. In patients undergoing myocardial revascularization, a lower concentration of microelements was observed, with the occurrence of heart failure in the early postoperative period, more frequent in patients with lower copper serum levels ²¹.

The present study aimed at assessing thiamin, selenium, and copper serum levels in patients with idiopathic dilated cardiomyopathy using diuretics, and their comparison with those of an equal number of healthy individuals. The nutritional status of these patients with cardiac disease was assessed, as were the possible associations between undernourishment and the deficiency of the micronutrients

studied and between their serum levels and cardiac function, which was evaluated on echocardiography.

Methods

From 1999 to 2000, we carried out an observational study comprising 30 patients with idiopathic dilated cardiomyopathy and an equal number of healthy controls. All participants were informed about the nature of the study and its objectives and provided written informed consent. The research project was submitted to and approved by the committee on ethics and research at the Hospital Universitário Pedro Ernesto - UERJ. The groups were paired by age, sex, and skin color (white and nonwhite). The patients had been diagnosed with idiopathic dilated cardiomyopathy ²² and were taking diuretics on a daily basis for a period ≥ 15 days. Patients with renal and hepatic dysfunction and alcoholic individuals were excluded from the study ²³⁻²⁵.

The patients underwent anamnesis, physical examination, laboratory tests, electrocardiography, single- and 2-dimensional echocardiography with Doppler, and nutritional assessment. The blood samples were withdrawn between 8 and 9 AM after a 12-hour fasting period. Routine biochemical measurements, including all electrolytes, hepatic function test, and lipid profile were performed.

Thiamin assessment was performed by measuring the erythrocytic transketolase activity and the effect of thiamin pyrophosphate, as reported by Myron Brin ⁴. The following values were considered as normal limits: erythrocytic transketolase activity $\geq 800 \mu\text{g hexose} \cdot \text{mL}^{-1} \cdot \text{h}^{-1}$; effect of thiamin pyrophosphate $< 16\%$. Thiamin deficiency was defined as the simultaneous presence of low erythrocytic transketolase activity and the high effect of thiamin pyrophosphate ²⁶.

The sera in which selenium and copper were measured were stored in a refrigerator. The serum levels of the micronutrients were obtained by hydride generation and flame atomic absorption spectrophotometry, respectively according to the methodologies of Navarro et al ²⁷ and Terrés-Martos et al ²⁸. The accuracy and precision of the method were tested with 2 sera with internationally recognized standardized selenium and copper concentrations as follows: 1) Contox trace metal serum control - Kaulson Laboratory, Inc., NJ, USA; 2) Seronorm trace elements serum, produced by SERO AS, Billingstad, Norway.

Nutritional evaluation was performed through nutritional history, anthropometric assessment, measurement of serum albumin, lymphocyte count, and global subjective assessment ^{29,30}.

The means were compared with the Student *t* test for independent samples. We adopted $p \leq 0.05$ as the significance level. The chi-square test was used to compare frequencies. To assess the association between numerical data, the correlation (*r*) and determination (*r*²) coefficients were calculated.

Results

The composition of the groups studied is shown in table II.

Table I - Mean selenium serum or plasma values in $\mu\text{mol/L}$ and $\mu\text{g/L}$ in adults of different countries ⁶

Region	$\mu\text{mol/L}$	$\mu\text{g/L}$
Finland (Helsinki)	0.53	41.73
New Zealand (Dunedin)	0.60	47.24
Germany (Mainz)	1.03	81.10
Sweden (Lund)	1.08	85.03
Italy (Rome)	1.14	89.76
Japan (Hiroshima)	1.24	97.63
USA (Mort. Gr.)	1.40	110.23
England (Southampton)	1.47	115.74
Canada (Toronto)	2.01	158.26

1 $\mu\text{g} = 0.0127 \mu\text{mol}$.

Table II - Characteristics of the groups studied

Group	Mean age (years)	Sex		Color	
		M	F	white	nonwhite
Cardiac patients	43.2 *	23	7	22	8
Controls	39.6 *	23	7	22	8

M - male; F - female; * p>0.10. The groups were paired by age, sex, and skin color.

Furosemide dosages ranged from 20 to 160mg/day. Only 1 patient was not taking furosemide but was taking thiazide and spironolactone, and it appeared in the prescription of 10 (33%) patients at the dosages of 25 (6 patients - 20%) or 100 mg/day (4 patients - 13%). Six (20%) patients took thiazides at the dosages of 12.5 (3%), 25 (7%), and 50mg/day (10%). In 5 (16%) of these patients, furosemide was added, and, in 2 (6%) of these patients, spironolactone was added. One (3%) patient took a combination of the 3 diuretics. Forty-six percent of the patients with cardiac disease took furosemide doses \geq 80mg/day.

The following medications were also used in the patients with cardiac disease: digoxin (97%), angiotensin-converting enzyme inhibitors (86%), amiodarone (13%), coumarin anticoagulants (13%), carvedilol (23%), and acetylsalicylic acid (6%). Eighty percent of the patients used the combination of digitalis, diuretics, and angiotensin-converting enzyme inhibitors.

The functional classes of the patients with cardiac disease (NYHA) were as follows: I, 17%; II, 53%; III, 23%; and IV, 7% (tab. III).

Table III - Results (means and percentages) of the groups studied

	Controls	Cardiac patients	p value
Electrolytes:			
Sodium (mEq/L)	141	140	NS
Potassium (mEq/L)	4.2	4.5	NS
Calcium (mg/dL)	9.3	9.3	NS
Phosphorus (mg/dL)	3	3.4	NS
Magnesium (mg/dL)	2	1.9	NS
Chloride (mEq/L)	102	99	NS
Hepatic tests:			
AST (UI)	21	36	0.001
ALT (UI)	24	40	0.02
TB (mg/dL)	0.7	1.2	0.01
Glucose (mg/dL)	93	98	NS
Creatinine (mg/dL)	0.8	0.9	NS
TC (mg/dL)	210	213	NS
FC (% of patients):			
I	100	17	
II	-	53	
III	-	23	
IV	-	7	
EF (%)	65	31	
ETA (μ g hexose.ml ⁻¹ .h ⁻¹)	1002 \pm 155	863 \pm 293	0.02
ETP (%)	13.8 \pm 7.2	17.7 \pm 10.8	NS
Thiamin deficiency (%)	10	33.3	0.02
Selenium (μ g/L)	73.2 \pm 9.9	72.3 \pm 14.3	NS
Copper (mg/L)	1.1 \pm 0.4	1.2 \pm 0.4	NS

ns- nonsignificant; AST- aspartate aminotransferase; ALT- alanine aminotransferase; TB- total bilirubin; TC- total cholesterol; CF- functional class (New York Heart Association); EF- ejection fraction; ETA- erythrocytic transketolase activity; ETP- effect of thiamin pyrophosphate.

The groups had similar nutritional status according to both the objective and global subjective assessments, with cases of overweight and obesity.

No significant alterations were observed in the electrolytes or in the hepatic and renal functional tests in either group. Dyslipidemia was highly prevalent both in patients with cardiac disease and in the controls (83.3% and 76.6%, respectively), table III.

Left ventricular ejection fraction (LVEF) in patients with cardiac disease ranged from 12 to 57% (mean = 31%). The subjective assessment of left ventricular function showed severe systolic dysfunction in 83.3% of the patients, moderate systolic dysfunction in 10%, and mild systolic dysfunction in 6.7% of the patients. Left bundle-branch block was observed in 60% of the patients with cardiac disease. Coronary angiography was performed in 50% of the patients with cardiac disease and was normal in all of them.

The means of the erythrocytic transketolase activity in the controls and in patients with cardiac disease were 1002 \pm 155 and 863 \pm 293 μ g glucose.mL⁻¹.h⁻¹ (ranges: 706 to 1347 and 145 to 1344 μ g glucose.mL⁻¹.h⁻¹), respectively (p=0.02). The mean effect of thiamin pyrophosphate was 13.8 \pm 7.2% (1.4 to 31.5%) in the controls, and 17.7 \pm 10.8% (1.3 to 46.2%) in the patients with cardiac disease (p=0.12). Thiamin deficiency was observed in 3 controls and in 10 patients with cardiac disease (p=0.02), table III. No significant difference in the frequency of thiamin deficiency was observed between patients with cardiac disease taking furosemide doses < 80mg/day and those taking furosemide doses greater than that value (p=0.07). The use of spironolactone did not decrease thiamin deficiency frequency in the patients studied with cardiac disease (p=0.28). Thiamin deficiency was not more frequent in the patients taking the furosemide/thiazide combination (p=0.17). No association was observed between thiamin serum level and left ventricular ejection fraction in patients with cardiac disease in the assessment performed either through erythrocytic transketolase activity (r = - 0.05) or through the effect of thiamin pyrophosphate (r=0.07). No significant difference was observed in the mean left ventricular ejection fraction of patients with cardiac disease who had thiamin deficiency (LVEF = 32.6%) and those who did not have thiamin deficiency (LVEF = 30.7%), p=0.68. None of the 10 patients with thiamin deficiency was undernourished.

In regard to selenium serum level, the accuracy (exactness) and precision (reproducibility) of the method are shown in table IV. With the reference material of Seronorm, 102.4% accuracy and 5.3% precision were obtained.

The mean selenium serum levels in controls and patients with cardiac disease were, respectively, 73.2 \pm 9.9 μ g/L (56.5 to 94.5 μ g/L) and 72.3 \pm 14.3 μ g/L (35.5 to 94 μ g/L) with p=0.77. The results are visually compared in figure 1. No association was observed between selenium serum levels and left ventricular ejection fraction (r = - 0.10; r² = 0.01).

In regard to copper serum level, the method's accuracy and precision observed with the use of the reference mate-

Table IV - Accuracy and precision of selenium and copper measurements					
	Material	Concentration		Accuracy (%)	Precision RSD (%)
		Certified	Measure		
Selenium (mg/L)	Seronorm NIST-RM 3149	80 ± 13	81.9 ± 4.4	102.4	5.3
Copper (mg/L)	Contox RM-0148	0.8 ± 12	0.8 ± 0.03	98.7	3.8

RSD- relative standard deviation; NIST- National Institute of Standards; RM- reference material.

rial Contox are shown in table IV. Accuracy of 98.7% and precision of 3.8% were obtained.

The mean copper serum levels in controls and patients with cardiac disease were, respectively, 1.1±0.4 mg/L (0.6 to 1.8mg/L) and 1.2±0.4mg/L (0.6 to 2.2mg/L), p=0.27. Visual comparison of the data is shown in figure 2. No association was observed between copper serum levels and left ventricular ejection fraction in patients with cardiac disease (r = -0.25; r²=0.06).

Discussion

Due to the lack of Brazilian studies reporting the normal range in our population for erythrocytic transketolase activity, for the effect of thiamin pyrophosphate, and for selenium and copper serum levels, the formation of a control group with individuals with no evidence of disease or use of any medication paired by age, sex, skin color, and nutritional status was required.

From the clinical point of view, most patients with cardiac disease were compensated in NYHA functional classes I or II, even though they had severe myocardial disease according to echocardiographic data (83.3% had severe systolic dysfunction).

Both groups had similar nutritional profiles as follows: mean BMI of controls and patients with cardiac disease, respectively, 26.2 and 26kg/m²; mean body fat percentage, respectively, 24.3 and 25%; mean CMB, respectively, 26 and 24.4cm; and no significant alteration in serum albumin. Overweight and obesity were present in both groups.

Three patients with cardiac disease had an increase in hepatic aminotransferases (AST and ALT) and total bilirubin greater than twice the normal limit. On echocardiography, these three patients had severe systolic dysfunction, 2 of whom had left ventricular ejection fraction < 20%. On physical examination, they had tender hepatomegaly and jugular venous distension, suggesting hepatic congestion as the cause of the enzymatic alteration, which is the reason why we did not find it necessary to measure hepatitis viral markers.

Assessment of thiamin levels should include erythrocytic transketolase activity and the effect of thiamin pyrophosphate on erythrocytic transketolase activity, which reflects the percentage of increase in this activity after the

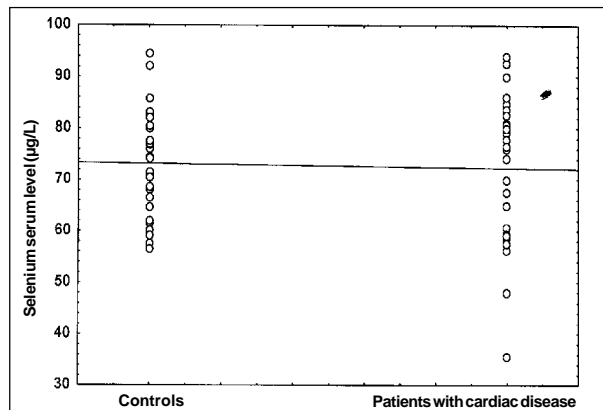


Fig. 1 - Selenium serum level. Two patients with cardiac disease had levels below 50 mg/L.

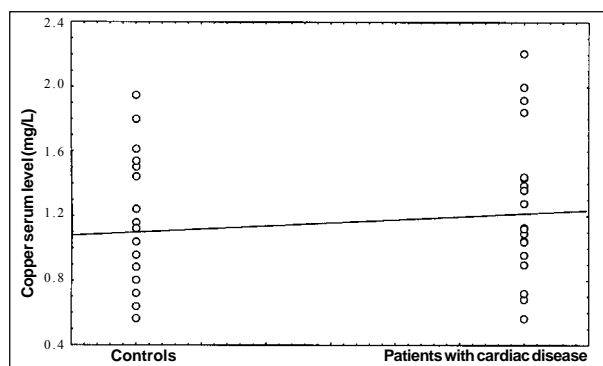


Fig. 2 - Copper serum level. The groups had similar copper serum levels.

addition of thiamin pyrophosphate to the hemolysate. However, until the present time, no consensus exists in regard to the normal levels of erythrocytic transketolase activity due to the use of different methods of measurement by several laboratories³⁰. Two aspects should be considered in the analysis of the erythrocytic transketolase activity³¹: a) the method involves the activity of other erythrocytic enzymes, such as ribose isomerase and xylulose epimerase, which do not depend on thiamin, and, in case of their deficiency, this may influence the result of the test, because no increase in erythrocytic transketolase activity will be detected (assessed by the formation of hexoses in the hemolysate), and occasional cases of thiamin deficiency will not be identified; b) thiamin deficiency may also cause a reduction in apotransketolase and a weak response of that to thiamin pyrophosphate, with the same consequence. These aspects show the importance of considering the simultaneous existence of low erythrocytic transketolase activity and the high effect of thiamin pyrophosphate as a criterion for the diagnosis of thiamin deficiency.

Dietary standardization was not adopted in this study because the objective of the study was to assess the status of the micronutrients in the nutritional reality of its participants. By limiting thiamin intake to 1.5mg/day, Seligmann et al³ assessed the role played by diuretics in thiamin depletion, but they imposed a regimen of that micronutrient ingestion

on study participants, which may not have matched the dietary reality of the patients. The usual ingestion of a higher thiamin content, both due to the presence of food richer in that nutrient or due to its conservation and preparation avoiding the action of thiaminases, could overcome the losses imposed by the diuretics, reducing the clinical importance of the increased urinary excretion of thiamin in that population.

Daily ingestion of the nutrients was not estimated in the study because of the difficulty in precisely establishing the nutrient's content in the food ingested, as well as their amount, in the absence of a standardized diet. The influences of the processes of food conservation and preparation in the bioavailability of thiamin also contributed making this assessment difficult³⁰, as did ignorance about the selenium and copper contents in food cultivated in Brazil.

In a previous study³², considering only the elevated effect of thiamin pyrophosphate as a criterion for thiamin deficiency, a possible effect of spironolactone as a saver of thiamin spoliation induced by furosemide was observed. In the present study, a significant difference between the means of the effect of thiamin pyrophosphate in takers and nontakers of spironolactone was observed; however, adopting the new criterion here presented (low erythrocytic transketolase activity and elevated effect of thiamin pyrophosphate), no significant difference in the prevalence of thiamin deficiency between the groups was observed ($p=0.28$). Even though greater thiamin spoliation should be expected in patients taking the combination furosemide/thiazide due to the increased urinary flow, it was not observed in the present study, maybe because of the small number (5) of patients taking that combination.

The mean selenium serum level in controls ($73.2 \pm 9.9 \mu\text{g/L}$) was similar to that reported by Navarro et al²⁷ in Spain ($74.90 \mu\text{g/L}$); when compared with those shown in table I, it is among the lowest levels, above only those reported in Finland and New Zealand, regions with selenium-poor soils. Despite the report by Inoko et al³³ of a case of cardiomyopathy related to the selenium serum level of $62 \mu\text{g/L}$, the most frequently reported levels in the literature are below $50 \mu\text{g/L}$ ^{11,13,15}. In the Chinese cardiomyopathy cases, the selenium serum levels reported were below $30 \mu\text{g/L}$ ¹¹. However, we do not know the role played by intermediate serum levels in worsening the already present cardiomyopathy or cardiomyopathy with other etiologies. No association was observed between the selenium and copper levels in patients with cardiac disease and their cardiac function assessed by left ventricular ejection fraction. We believe

that this was due to the existence of another etiological mechanism for the myocardial disease of the patients studied, in addition to the record of only 2 cases with low selenium serum levels, which did not allow a better evaluation of the possible influence of this deficiency in worsening the underlying disease.

The mean copper serum level in the control group ($1.09 \pm 0.39 \text{mg/L}$) is similar to that reported by Terrés-Martos et al²⁸ (1.10mg/L), and to others observed in other countries, such as the USA ($0.9 \pm 0.03 \text{mg/L}$)³⁴ and Japan ($0.98 \pm 0.11 \text{mg/L}$)³⁵. No significant difference was observed between the mean copper serum level in controls and that in patients with cardiac disease ($p=0.27$), suggesting a good content of that element in the food ingested by the patients studied. The preferentially hepatic excretion of copper minimizes the influence of the diuretics in copper spoliation.

In this study, thiamin deficiency was significantly more frequent in patients with cardiac disease, occurring in 1/3 of the patients, raising the possibility that the systematic replacement of that micronutrient might contribute to better myocardial performance in patients taking diuretics for prolonged periods. However, it is worth emphasizing the nonexistence in the Brazilian market of a commercial product with a thiamin unit dose adequate for that purpose. The only product available for oral administration has 300mg/tablet , which seems excessive, even though the minimum dose necessary to compensate for the losses induced by diuretics is yet to be defined. As thiamin deficiency has not been associated with protein-caloric undernourishment, we should consider the possibility of that vitamin deficiency even in the absence of the classical signs of undernourishment.

No significant difference in the mean selenium and copper serum levels was observed between controls and patients with cardiac disease. However, 2 patients with cardiac disease (6.6%) had selenium $< 50 \mu\text{g/L}$, which we consider significant, because such levels have already been reported as sufficient to induce myocardial dysfunction^{6,8,10}. This finding raises the question of the possible benefit of selenium replacement in patients with idiopathic dilated cardiomyopathy refractory to the conventional therapeutic schemes. Even though serum measurement should precede replacement of the microelement, we should consider the difficulty of this procedure in Brazil, especially in public health units. However, the results here presented seem not to justify the systematic replacement of selenium and copper in idiopathic dilated cardiomyopathy.

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