SELENIUM STATUS AND ITS RELATION TO MARKERS OF OXIDATIVE STRESS IN OBESE WOMEN

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Abstract

Objective: The present study evaluated the relationship between selenium status and markers of oxidative stress in obese women.

Methods: This cross-sectional study included 89 women, divided into two groups, case (n = 44) and control (n = 45). Selenium intake was assessed by a 3-day food record. Selenium concentrations in plasma, erythrocytes, and urine were determined by inductively coupled plasma optical emission spectrometry. Lipid peroxidation was determined by measuring the plasma levels of thiobarbituric acid reactive substances (TBARS). Glutathione peroxidase (GPx) activity was assayed using an automatic biochemical analyzer.

Results and Discussion: The mean concentrations of selenium in plasma and erythrocytes were lower in obese women than in the control group ($p < 0.05$). The mean values for TBARS concentrations and GPx activity were higher in obese women than in the control group ($p < 0.05$). A significant positive correlation was obtained between erythrocyte selenium concentration and GPx activity. However, no significant correlation was obtained between the selenium biomarkers and plasma concentrations of TBARS ($p > 0.05$).

Conclusion: Selenium concentrations in plasma and erythrocytes are reduced in obese women. Moreover, GPx activity was increased and had a positive correlation with selenium in erythrocytes, which suggest the influence of this mineral on oxidative stress in the obese women evaluated.

Keywords:
Obesity; Selenium; Oxidative Stress; Glutathione Peroxidase.

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Resumo

Objetivo: O presente estudo avaliou a relação entre os níveis de selênio e marcadores de estresse oxidativo.

Métodos: Estudo transversal envolvendo 89 mulheres, separadas em dois grupos, casos (n= 44) e controle (n=45). A ingestão de selênio foi avaliada por meio de um registro alimentar de três días. As concentrações de selênio no plasma, eritrocitos e urina foram determinadas por espectrometría dee emissão óptica. A peroxidação lipídica foi determinada por meio da avaliação das substâncias reativas ao ácido tiobarbitúrico (TBARS). A atividade da glutationa peroxidase (GPx) foi realizada usando analizador bioquímico automático.

Resultados e Discussão: As concentrações médias de selênio no plasma e os eritrocitos foram menores nas mulheres obesas que no grupo controle ($p <0,05$). Os valores médios das concentrações de TBARS e da atividade GPx foram maiores nas mulheres obesas que no grupo controle ($p <0,05$). Foi obtida uma correlação positiva significativa entre a concentração de eritrócitos selênio e atividade da GPx. No entanto, não foi observada correlação significativa entre os biomarcadores de selênio e as concentrações plasmáticas de TBARS ($p> 0,05$).

Conclusão: As concentrações de selênio no plasma e os eritrócitos estão reduzidas nas mulheres obesas. A atividade de GPx estava aumentada e teve uma correlação positiva com o selênio nos eritrócitos, que sugere a influência desse mineral no estresse oxidativo presente nas mulheres avaliadas.

Palavras-chave:
Obesidade; Selênio; Estresse Oxidativo; Glutationa Peroxidase.

INTRODUCTION

Obesity is a chronic disease characterized by the excessive accumulation of body fat. The etiology of obesity is complex, involving several environmental, metabolic, and molecular factors. It is considered one of the main risk factors for various comorbidities, such as type 2 diabetes mellitus, cardiovascular diseases including heart disease and stroke, and some types of cancers[1-3].

Adipose tissue dysfunction in obesity contributes to the manifestation of several metabolic complications, such as low-grade chronic inflammation, endothelial dysfunction, changes in glucose and lipid metabolism. These associated complications have important implications since they lead to excessive production of reactive oxygen species, resulting in oxidative stress in obesity[4-7].

Several biochemical and nutritional disorders have been investigated in obese individuals in order to understand the mechanisms involved in the pathogenesis of this disease. Many studies have attempted to identify the contribution of mineral to oxidative stress. Selenium, in particular, is important because it plays an essential role in controlling the excessive production of reactive oxygen species[8,9].

However, it is essential to point out that selenium performs the antioxidant function by means of selenoproteins, proteins in which contain selenium in their catalytic site. Some selenoproteins with known antioxidant functions include glutathione peroxidase (GPx), thioredoxin reductase (TR), and selenoprotein P (SelP), which catalyze the reduction of hydrogen peroxide (H_2O_2), free organic hydroperoxide, phospholipid hydroperoxide, and peroxynitrite (ONOO-), inhibits the oxidation of proteins and lipids and participates in the regulation of redox signaling[8-11].

The selenoproteins GPx1 GPx3, GPx4, and SelP are hyper-expressed in white adipose tissues and are involved in the intracellular redox balancing during differentiation of pre-adipocytes to mature adipocytes, demonstrating importance of action of these selenoproteins in control oxidative stress in obesity[12].

Recent studies have demonstrated that changes in selenium homeostasis and activity of selenoproteins in obese individuals seem to limit the antioxidant defense in the population. However, the existing data is insufficient for elucidation of the mechanism of selenium involvement in oxidative stress in obese population[13-15].

Therefore, considering the metabolic repercussions of obesity, such as oxidative stress, as well as the importance of selenium in the process, determination of the selenium status and elucidation of its relation to oxidative stress markers would contribute to a better understanding of the role of this nutrient in mechanisms involved in metabolic disorders associated with obesity. The aim of this study was to evaluate the relationship between selenium status and different markers of oxidative stress in obese women.

METHODS

Study design

This was a cross-sectional study that included 89 women (obese, 44; control, 45), between the ages of 18 and 50 years, who were recruited at the Ambulatory of Getúlio Vargas Hospital (Teresina, Piauí). Participants were selected according to the following criteria: body mass index (BMI) between 18.5 and 24.9 kg/m² (control group) or between 30 and 39.9 kg/m² (obese group); nonsmokers; not pregnant or lactating; not drink alcohol chronically, present conditions inflammatory intestinal or recent infections, absence of endocrine disease, diabetes mellitus, cardiovascular disease, hypertension, cancer, chronic renal failure, and liver disease (self-reported data); not being in treatment for weight loss; not taking vitamin and mineral supplements and/or medicines that could affect selenium status.

Written informed consent was obtained from all the individual participants included in the study. This study was approved by the ethics committees of the Getúlio Vargas Hospital and of the Federal University of Piauí. The purposes and procedures of the study were explained to the participants prior to the data collection.

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Anthropometric measurements

For anthropometric evaluation, body weight, height and waist circumference were assessed with participants in an upright position and without shoes. The height and weight were measured with an anthropometer and a digital balance, respectively. The measurement of waist circumference was performed using a flexible, inelastic tape surrounding the natural waistline; the narrowest area between the chest and the hips was used as a reference value[16]. For classification of nutritional status, we calculated BMI, dividing the participant's body weight by the square of the height[17].

Dietary intake assessment

Energy, macronutrient, and selenium intake was estimated from three-day diet records. The software NutWin (version 1.5)[18] was used to calculate the dietary energy and nutrient content using food composition data derived primarily from the Brazilian Table of Food Composition[19]. For selenium content, we considered — preferentially, in this order — data of the analysis of Brazilian foods performed by Ferreira et al.[20], the Brazilian Institute of Geography and Statistics' Table of Food Composition[21], and the US Department of Agriculture's National Nutrient Database for Standard Reference[22]. The estimated average requirement (EAR) for selenium (45 μg/day) was used as a reference value for adequate intake[23]. The dietary selenium intake was adjusted for energy and corrected for intra- and interpersonal variance.

Sample collection and preparation

Blood collection was collected by a nurse technician in the morning, between 7 and 9 a.m., after the participants had fasted for about 12 hours. Venous blood (11 mL) was collected and distributed in separate tubes as follows: (1) vacuum tube containing EDTA anticoagulant for analysis of selenium (4 mL) and (2) vacuum tube containing EDTA anticoagulant for the analysis of glutathione peroxidase activity and thiobarbituric acid reactive substances – TBARS (4 mL).

Plasma was separated from whole blood by centrifugation at 1764 × g for 15 min at 4°C (CIENTEC® CT-5000R, Brazil). Subsequently, plasma was extracted with an automatic pipette, placed in the previously demineralized polypropylene microtubes, and stored at −35°C. To isolate the erythrocytes for the measurement of selenium concentration, the methods proposed by Whitehouse et al.[24] were used. The red cell mass was washed with 3.5 mL of isotonic saline solution (0.9% NaCl) then slowly homogenized by inversion, and was centrifuged at 2401 × g for 10 min. Finally the supernatant was aspirated and discarded. This procedure was performed three times to remove any contaminants from erythrocytes (i.e., platelets and leukocytes).

The 24-h urine was collected in a demineralized bottle that was weighed before and after collection in a semi-analytical balance to determine the urinary volume from density. After homogenization of the total content, 8 mL of urine were removed and stored in demineralized microtubes at −35°C for later analysis[25].

Assessment of Biochemical Parameters of Selenium

Blood and urinary selenium concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP 720/OES, Varian Inc., United States), according to the methodologies proposed by Blazewicz et al.[26], Harrington et al.[27], and Niedzielski and Siepak[28]. The device used was a hydride generator system ($NaBH_4$) mounted on a V-Groove platform with the Babington-type nebulizer, with 70 mm coupled gas orifice. The device was set to the following experimental conditions: power: 1.4 kW; plasma flow (gas): 15 L/min; auxiliary gas flow rate: 1.5 L/min; spray chamber type: cyclonic; and nebulizer flow: 0.7 L/min.

Plasma samples were diluted at 1:20 (v/v) as follows: 3.0% (w/v) 1-butanol, 0.1% (v/v) TAMA (high purity surfactant) and 0.05% (v/v) HNO_3. Samples of erythrocytes/packed red blood cells were diluted at 1:60 (v/v) as follows: 3.0% (w/v) 1-butanol, 0.2% (v/v) TAMA (high surfactant purity) and 0.1% (v/v) HNO_3. For urine samples, we used a microwave-assisted Mars 6 system (CEM®, USA) with acid digestion of 65% HNO_3 (1 mL HNO_3: 9 mL of deionized water). After digestion, the solutions were diluted at 1:40 v/v in ultra-pure water and placed in polytetrafluoroethylene volumetric flasks.

Se(VI) was reduced to Se(IV) in 4M HCl by heating the diluted solution at 90°C for 30 min. The choice of spectral lines analysis was based on both its sensitivity and the spectral interference, being optimized for selenium readings at a wavelength of 196.026 nm and 203.985 nm. Samples were measured in triplicate.

A stock solution of 1,000 μg/mL of selenium (Spex®CertiPrep, USA) was used to prepare calibration curves and optimize of analytical conditions. All standards were prepared in the same way as the samples. Calibration curves were prepared at the following concentrations: 1, 5, 10, 20, 50, and 100 μg/L. The detection limits were determined from the equation: 3 × standard deviation of 10 measurements of blank, divided by the slope of the calibration curve. Samples of certified reference material (Seronorm®Oligoelement Serum, Norway) were determined to validate the analytical measurements in ICP-OES.

To evaluate the selenium status, the following cutoff values were adopted: plasma selenium: 80–95 μg/L (for maximization of plasma glutathione peroxidase)[29]; erythrocyte selenium: 0.18–0.55 μg/g Hb[30]. Urinary selenium was evaluated according to the urinary excretion of this mineral over 24 h and by calculating clearance.

Calculation of selenium clearance

Selenium clearance was calculated based on the plasma mineral concentrations and their amounts excreted in urine over 24 h using the following formula[31]:

Se selenium, *VU* rate of production urinary.

Determination of hemoglobin

The concentration of hemoglobin in the red cell mass was determined using cyanmethemoglobin method in order to express the erythrocyte selenium concentrations and of the glutathione peroxidase activity in erythrocytes[32]. The absorbance was read at the wavelength of 540 nm by visible ultraviolet spectrophotometer (Bel Photonics®, SP1102, Brazil).

Determination of 24-h urinary creatinine

Urinary creatinine was assessed with an automatic biochemical analyzer (ChemWell T, Awareness Technology Inc., USA) using a K Creatinine Kit (Labtest®, Brazil), based on the Jaffé reaction. The creatinine values were used to express the urinary concentrations of selenium and as a marker of completeness of urine over 24 h[33].

Determination of the Glutathione Peroxidase Activity in Erythrocytes

Determination of glutathione peroxidase activity in erythrocytes was determined using an automatic biochemical analyzer, LabMax 240 (Labtest®, Brazil) and Ransel 505 kit (Randox Laboratories®, UK), according to the protocol described earlier Paglia and Valentine[34]. The decrease in absorbance of the samples was measured at a wavelength of 340 nm. The results were expressed as enzyme units per gram of hemoglobin (U/gHb). The reference values of glutathione peroxidase were adopted as proposed by RANDOX (27.5 to 73.6 U/gHb).

Determination of Thiobarbituric Acid Reactive Substances

Thiobarbituric acid reactive substances (TBARS) in plasma were determined using the method suggested by Ohkawa et al.[35]. Prior to sample processing, analytical calibration curves were prepared at concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 nmol/mL, using 1,1,3,3-tetraethoxypropane as standard. Absorbance was readusing a UV/Vis Bel Photonicsspectrophotometer, SP 1102 model (Osasco, SP, Brazil), at a wave length of 532 nm.

Statistical Analysis

Data were analysed using the software SPSS for Windows® version 22.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to verify the homocedasticity of the data. To compare outcome measures between the two subject groups, the Student's *t* test and the Mann-Whitney *U* test were used for parametric and nonparametric data, respectively. Additionally, Pearson's coefficient test was used to identify any potential cor-

relations between data sets. The difference was considered statistically significant when the p value was <0.05, admitting an error of 5%.

RESULTS

The mean values of age and anthropometric parameters, along with the respective standard deviations, used to assess the nutritional status of obese patients and the control group are shown in Table 1. There was a statistically significant difference in the weight parameters, body mass index, and waist circumference ($p < 0.05$).

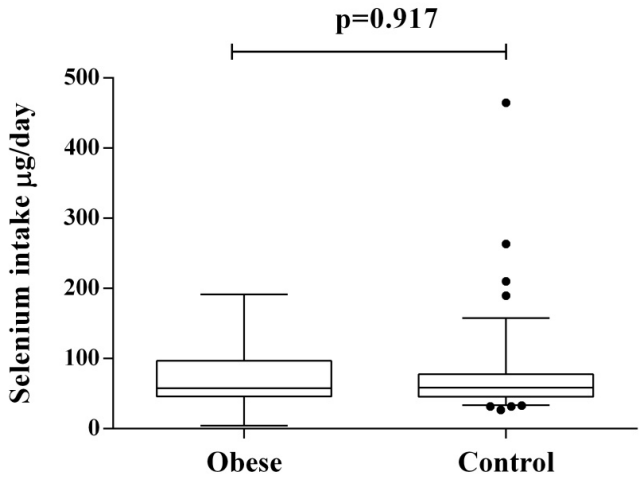
Table 1 — Mean values and standard deviations of age, body weight, height, body mass index and waist circumference for obese patients and control group

Parameters	Obese Women (n=44) Mean ± SD	Control Group (n=45) Mean ± SD	p
Age (years)	38,30 ± 6,681	36,96 ± 7,33	0,371
Body Weight (kg)	83,30 ± 9,52*	53,57 ± 5,02	< 0,001
Height (m)	1,53 ± ,068	1,55 ± ,055	0,290
BMI (kg/m2)	35,07 ± 2,43*	22,10 ± 1,55	< 0,001
WC (cm)	100,94 ± 8,46*	72,58 ± 4,83	< 0,001

* Values significantly different between the obese and control groups using Student's t test ($p < 0.05$). BMI = Body Mass Index; WC = Waist Circumference; SD = standard deviation; p = p value.

Figure 1 shows the dietary selenium intake in obese patients and the control group. The difference in mineral consumption was not statistically significant between the groups ($p = 0.917$), and selenium intake was adequate in both groups in relation to EAR. Furthermore, 80% of obese women and 79.1% of the controls showed selenium intake above the EAR value.

Figure 1 — Selenium intake in the obese patients and control group



Mann-Whitney *U* test ($p = 0.917$).Reference values for selenium intake: EAR = 45 mg/day[23].

The mean concentration of the selenium status biomarkers in obese and control subjects is shown in Table 2. The differences in plasma and erythrocyte selenium concentrations, and clearance of selenium was statistically significant between the two groups ($p<0.05$).

Table 2 — Mean values and standard deviations of biomarkers of nutritional status of selenium obese women and control group

Parameters	Obese Women (n=44) Mean ± SD	Control Group (n=45) Mean ± SD	p
Plasma selenium (μg/L)	62,12 ± 6,83*	86,57 ± 10,31	<0,001
Erythrocyte selenium (μg/gHb)	0,27 ± 0,06*	0,33 ± 0,06	<0,001
Urinary selenium[a] (μgSe/g creatinine)	45,04 ± 19,62	45,33± 22,86	0,960
Selenium *clearance* (mL/min)	0,60 ± 0,21*	0,40 ± 0,19	<0,001

[a] Urinary selenium: obese (n = 30) andcontrol (n = 28)

* Values significantly different between obese and control group. Student's t test (p <0.05). Values: selenium plasmatic = 80-95 μg /L[29]; selenium erythrocyte = 0.18 to 0.55 μg / gHb.[30]

GPx activity of erythrocytes and concentration TBARS in the two groups is shown in Table 3. The activity of GPx in erythrocytes and TBARS values were significantly higher in obese women than in the control group (p < 0.05).

Table 3 — Mean values and standard deviations of biomarkers of nutritional status of selenium obese women and control group

Parameters	Obese Women (n=44) Mean ± SD	Control Group (n=45) Mean ± SD	p
GPx (U/gHb)	41,82 ± 12,27*	33,95 ± 8,68	0,001
TBARS (nmol/ml)	2,04 ± 0,41*	1,75 ± 0,37	0,001

* Values significantly different between obese and control group, Student's t test (p <0.05). GPx = Glutathione peroxidase. TBARS = thiobarbituric acid reactive substances.

The linear correlation analysis between the selenium status biomarkers and activity of GPx in erythrocytes in obese patients and the control group is shown in Table 4. A positive correlation was found between the erythrocyte selenium levels and the GPx activity in erythrocytes (p < 0.05).

Table 4 — Simple linear correlation analysis between the biomarkers of nutritional status of selenium and glutathione peroxidase activity in erythrocyte in obese and control group

Parameters	GPx (U/gHb)			
	Obese Women		Control Group	
	r	p	r	p
Plasma selenium (μg/L)	-0,255	0,095	-0,258	*0,087*
Erythrocyte selenium (μg/gHb)	0,386*	0,010	0,476*	*0,001*
Urinary selenium[a] (μgSe/g creatinine)	-0,297	0,110	-0,232	*0,236*

[a] Urinary selenium: obese (n = 30) andcontrol (n = 28)

* Pearson's linear correlation (p < 0.05).GPx = Glutathione peroxidase.

The linear correlation analysis between the selenium status biomarkers and plasma TBARS in obese patients and the control group is presented in Table 5. No significant correlation was found between the observed parameters.

Table 5 — Simple linear correlation analysis between the biomarkers of nutritional status of selenium with thiobarbituric acid reactive substances in obese women and control group

Parameters	TBARS (nmol/ml)			
	Obese Women		Control Group	
	r	p	r	p
Selenium dietary (μg/dia)[a]	0,026	0,877	-0,063	0,705
Plasma selenium (μg/L)	0,017	0,912	-0,016	0,915
Erythrocyte selenium (μg/gHb)	-0,128	0,408	0,197	0,194
Urinary selenium[b] (μgSe/g creatinine)	0,145	0,445	-0,161	0,413

[a] Dietary selenium: obese (n = 37) and control (n = 39).

[b] Urinary Selenium: obese (n = 30) and control (n = 28). Pearson's linear correlation ($p < 0.05$). TBARS = thiobarbituric acid reactive substances.

DISCUSSION

This study evaluated the relationship between selenium status, GPx activity, and plasma concentrations of thiobarbituric acid reactive substances in obese and normal weight women. The correlation between these variables was investigated in both the groups. The results obtained indicated that selenium intake showed no statistically significant difference between the two groups, and that consumption was in adequate amounts in accordance with the recommended values[23].

The food survey data from this study showed that adequate amounts of selenium in the diets consumed by obese and normal weight women can be attributed to intake of the nutrient from food sources such as meat, fish, and chicken[20,36]. Moreover, it is important to note that the reported selenium contents in food composition tables is based most on studies conducted in other countries, which may lead to overestimation of the intake of the mineral due to variation in selenium concentrations in soil, which is the main determinant of its concentration in food[37].

Plasma selenium analysis revealed that obese women had significantly lower concentrations of the mineral than the control group. Thus, the values of this nutrient in plasma does not seems to be influenced by the amount of selenium found in the diet consumed by these women. However, other factors, may justify this result, such as oxidative stress in obesity, which requires a constant supply of nutrients and antioxidant enzymes to prevent against excessive reactive oxygen species, and may have contributed to reducing selenium levels in plasma of obese women in this study. We highlight that plasma concentrations of TBARS were significantly elevated in obese women.

In this sense, stands out that elevated values of plasma TBARS in obese women may be due the increased production of pro-inflammatory adipokines in adipose tissue, which, in turn, activate NADPH oxidase, a key enzyme for the production of compounds that induce lipid peroxidation[2,38,39]. Similar observations were reported by Chielle et al.[40] and Morais et al.[41].

Others factors also have been proposed to influence the plasma concentrations of selenium, such chronic low-grade inflammation, one of the main characteristics of obesity, since this metabolic disorder reduces the synthesis of SelP in hepatocytes, selenoprotein responsible for transporting up to 70% of selenium in plasma. The suppression of SelP synthesis in liver, which is one of the main route for selenium release in the organ, increases its availability for the synthesis of urinary metabolites and, consequently, increases the selenium clear-

ance, as was observed in obese women in this study. Thus, reduced selenium concentrations in plasma can be attributed to the contribution of relevant factors associated with obesity.

Similarly, analysis of selenium levels in erythrocytes revealed that obese women had significantly lower concentrations of selenium than the control group. In this context, it has been proposed that oxidative stress in obese women increased the demand of selenium for synthesis of GPx and other selenoproteins associated with antioxidant activities in specific tissues, which, in turn, may have led to reduction in selenium content of erythrocytes due to their mobilization from this compartment.

GPx activity in erythrocytes was found to be significantly higher in obese women than in the control group. Similar results were reported by Codoñer-Franch et al.[42], who found high levels of enzyme activity in erythrocytes of obese children compared to that in the control group. The existence of a compensatory homeostatic mechanism that prioritizes the synthesis of this enzyme in response to increased oxidative stress in obese women may explain such an observation[43].

Moreover, the result of a positive correlation between GPx activity and selenium in erythrocytes strengthening the role of selenium as an antioxidant mineral. A similar pattern was observed in the study by Cominetti et al.[44], who reported a positive correlation between selenium content and GPx activity in erythrocytes.

To better understand the role of selenium in controlling oxidative stress in obese women, a correlation analysis between different markers of nutritional status of the mineral and plasma concentrations of TBARS was conducted. The analysis, however, did not yield significant results in the present study. Nevertheless, it is worth emphasizing that selenium plays an important role in the stages of initiation and propagation of lipid peroxidation by inhibiting the formation of hydroxyl radicals and reduction of lipid hydroperoxides, thereby preventing increased reactivity and formation of toxic products[45,46].

Some factors may have contributed to the absence of correlation between selenium and TBARS, such as lack of specificity of the marker evaluated in the study and the possible participation of other antioxidant nutrients that control lipid peroxidation, such as α-tocopherol, vitamin C, coenzyme Q10, and β-carotene[5,38,45,46].

Thus, given the complexity of the role of selenium as an antioxidant nutrient, further studies on the topic are needed to obtain a better understanding of the behavior of this nutrient in the metabolic complications associated with obesity, particularly oxidative stress.

CONCLUSION

The results obtained in this study demonstrated that selenium concentrations in plasma and erythrocytes are reduced in obese women, indicating that nutritional status of selenium is altered. Moreover, GPx activity was increased and had a positive correlation with selenium in erythrocytes, which suggest the influence of this mineral on oxidative stress in the obese women evaluated. Taken together, these results demonstrate the importance of selenium as a nutrient able to control oxidation processes in human body.

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