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Lack of Relationships Between Serum Prolactin Concentrations and Classical Cardiovascular Risk Factors in Eastern Croatian Older Adults

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BEF 1 **Sanja Bekić**
BDF 1 **Šefket Šabanović**
C 2 **Nataša Šarlija**
EF 1 **Zvonimir Bosnić**
EF 3 **Nikola Volarić**
ABCDEFG 1,3 **Ljiljana Majnarić Trtica**

1 Department of Internal Medicine, Family Medicine and the History of Medicine, Faculty of Medicine, University Josip Juraj Strossmayer, Osijek, Croatia
2 Department of Statistics, Faculty of Economics, University Josip Juraj Strossmayer, Osijek, Croatia
3 Department of Physiology and Immunology, Faculty of Dental Medicine and Health, University Josip Juraj Strossmayer, Osijek, Croatia

Corresponding Author: Ljiljana Majnarić Trtica, e-mail: ljiljana.majnarić@gmail.com

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Background: Relationships between serum prolactin concentrations and various CV risk factors in older adults have rarely been assessed. The aim of this study was to examine the relationships between serum prolactin concentrations and CV risk factors in older patients with multiple CV risk factors.





Material/Methods: This case-control study included 92 patients, 50–89 years old (median, 69 years), with multiple CV risk factors. We used data from general practice electronic health records and biochemical laboratory tests. Patients were divided according to categories of CV risk factors.

Results: Serum prolactin concentrations were significantly higher in elderly people (≤ 65 vs. >65) and in men (70.65 ± 58.02 vs. 150.82 ± 114.05 mIU/L), as well as in patients with lower renal function (156.70 ± 127.23 vs. 72.53 ± 37.25 mIU/L, the bottom vs. top quartile of creatinine clearance), higher serum homocysteine and TSH concentrations, and in those who used NSAID and statins. Parameters indicating chronic inflammation (CRP) and renal function decline (creatinine clearance) were significantly and independently correlated with increased serum prolactin concentrations in multiple regression analysis.

Conclusions: When assessing the relationships between prolactin and CV risk factors in older people with multiple CV risk factors, the effect of renal function decline and chronic inflammation should receive attention.

MeSH Keywords: **Aging • Cardiovascular Diseases • Prolactin**

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Background

Emerging evidence suggests the role of the pituitary hormone prolactin (PRL), known for its physiological role in lactation, in atherosclerotic processes and development of cardiovascular (CV) disease [1–4]. In patients with prolactinomas, associations have been found between increased serum PRL concentrations (hyperprolactinemia) and the range of metabolic and vascular disorders, including increased body weight, impaired endothelium-dependent vasodilation, increased inflammation, insulin resistance, and abnormal serum lipids (dyslipidemias) [5,6].

Studies on the relationship between serum PRL concentrations and CV risk factors, performed in humans without prolactinomas, have not provided consistent results, probably reflecting differences in study designs and patient characteristics [7,8]. Even large, prospective, community-based studies have not been able to draw more definitive conclusions on this issue. In the Framingham Heart Study, increase in serum PRL concentrations was found to increase the probability of having low-HDL cholesterol in women and incident hypertension and diabetes in men [9]. Results of the Study of Health in Pomerania (SHIP) did not support the role of PRL as a risk factor for incident metabolic syndrome (MS) and diabetes mellitus type 2 (DM2) [10]. In the subsequent study of the same cohort, no changes were found in serum PRL concentrations across the ranges of BMI values, while there were associations with measures of abdominal obesity, but only in women, not in men [11]. These assessments can be compromised with the complex network known to regulate PRL secretion and signalling and involvement of this hormone in many homeostatic processes [12]. In these terms, a range of biological and psychosocial factors can influence serum PRL concentrations, including: age, sex, BMI, sex steroid hormones, basal temperature, nutrition, levels of stress and exercise, some medications, and renal function decline [12,13].

A recent prospective study suggested increased serum PRL concentrations as a predictor of all-cause and CV mortality [14]. Another study, however, did not support PRL as a biomarker for CV risk prediction [15].

Relationships between serum PRL concentrations and CV risk factors have not been specifically addressed in older population, despite of the fact that CV disease is its top cause of death [16]. Several factors can compromise results of such studies. For example, a variety of influences which are concomitant to aging, such as comorbidities, were found to change the levels and function of multiple hormonal systems [17]. The cumulative prognostic benefit of classical CV risk factors was found to decline [18]. Features such as comorbidities, frailty, cognitive and functional declines, were found to modify composition and impact of CV risk factors on CV prediction [19].

One of the major disorders proposed to link increased serum PRL concentrations with CV disease, in older population, is renal function decline, due to the large prevalence of this disorder and the strong impact on both, CV risk factors generation and elevation of serum PRL concentrations [20].

Experimental studies indicate age-dependent changes in the pituitary mechanisms of regulation of PRL secretion [21]. The main changes include reduction of the inhibitory dopamine system and dampening of the circadian rhythms. Specifically in women, impact of the sex hormone estrogen, a positive regulator of PRL secretion, is decreased. Only a few studies assessed changes in serum PRL concentrations with age. They were inconsistent with respect to the patterns found in women, while in men, a slight increase with age has been observed [22–24]. These studies did not include assessment of comorbidities and CV risk factors. In the present study, we wanted to shed more light on this complex issue. We used a large data set consisting of a number of clinical and biochemical parameters indicating CV risk factors in older people who were examined in general practice and assessed relationships between these parameters and serum PRL concentrations.

Material and Methods

Participants

The study included 92 respondents, 34 M/58 F, 50–89 years old (median, 69 years). They were recruited from several general medicine practices located in the town of Osijek (approximately 80 000 inhabitants) in eastern Croatia, which is a region that has a high burden of CV disease, exceeding the average of Croatia. The study was approved by the local ethics committee.

Only respondents at least 50 years old who gave their signed informed consent were included in the study. Respondents using antidepressants or antipsychotic drugs were excluded due to the known effect of these medications of increasing serum PRL concentrations [13].

Data description

Some parameters selected in the data set were used from patient health records, including: age, diagnoses of hypertension, DM2 and chronic cardiomyopathy, and information on continuous use of non-steroidal anti-inflammatory drugs (NSAID) and hypolipidemic drugs statins, both known to influence serum PRL concentrations. Continuous use of these medications was defined either as monthly prescription in the case of NSAID, or as 3 and more months of continuous use in the case of statins.

A measure of obesity, BMI (kg/m^2), was obtained from respondents. They were also screened for cognitive impairment using the Mini-Mental State Examination (MMSE), which is the most widely used screening test for the assessment of cognitive functioning, validated in many populations, including an older Croatian population [25]. During the examination, a health professional asks patients a range of questions designed to test everyday mental skills. The score of 24 or less (out of the maximum 30) indicates cognitive impairment when screening is performed in a general population. The test allows quantification of cognitive impairment, given that it is relatively sensitive in diagnosing overt dementia, but is less accurate in distinguishing cognitively healthy individuals from those with mild cognitive impairment. It cannot discriminate Alzheimer's from other types of dementia. Therefore, individuals screened as positive on cognitive impairment (MMSE <25) must undergo additional diagnostic evaluation and a prolonged follow-up for definitive diagnosis.

Laboratory tests were chosen to provide information on metabolic CV risk factors, including: total cholesterol, HDL cholesterol, triglycerides, fasting blood glucose, and glycosylated hemoglobin A1c (HbA1c) (a measure of the average glucose value in the past 3 months). Insulin measurement in fasting state was performed to approximate the level of insulin resistance [26]. Creatinine clearance and serum homocysteine were used as measures of renal function decline [27]. Increased serum homocysteine concentrations (hyperhomocysteinemia) has been reported to be a CV risk factor. The level of inflammation was indicated with C-reactive protein (CRP) and total leukocytes count. Thyroid-stimulating hormone (TSH) was measured, in addition to PRL, to determine the possible effect of hypothyroidism, a frequent disorder in the older population, on elevation of serum PRL concentrations [28].

Blood samples were analyzed according to the standardized procedures in the Department of Clinical Laboratory Diagnostics of the Clinical Hospital Osijek, Croatia.

PRL was quantitatively determined in serum using the CMIA method (chemiluminescence microparticle immunoassay) on an Abbott Architect i1000 analyzer. An original manufacturer reagent, the Architect prolactin reagent kit (7K76), was used for this procedure. Serum TSH and homocysteine concentrations were determined on Beckman Coulter (TSH) and Abbott Architect i1000 (homocysteine) analyzers using the CMIA method. Serum insulin concentrations were determined on a Siemens Immulite 2000 using the CMIA method.

Statistical analysis

Data were analyzed using the statistical software package Statistica 12. Numerical data are presented as means and standard deviations (SD) and categorical data are presented

as absolute frequencies. Sex-dependent distributions of categories of BMI are presented as relative frequencies.

Differences in serum PRL concentrations were determined according to stages of severity of particular CV risk factors. These stages were defined as quartiles for numerical parameters indicating CV risk factors, and as categories for categorical parameters. To reveal the possible confounding effect of age on the expression of particular CV risk factors, these defined respondent subgroups were additionally assessed according to differences in age.

When serum PRL concentrations were presented with 2 categories and the sample was of the appropriate size, the *t* test was used, otherwise, we used the Mann-Whitney U test. When serum PRL concentrations were presented with more than 2 categories and the sample was of the appropriate size, ANOVA was used, otherwise, we used Kruskal-Wallis ANOVA. The same tests were used when assessing differences in age. The level of significance of $p < 0.05$ was considered statistically significant.

Some results considered to be of particular importance for the study's objectives, are also presented graphically as box-whisker plots.

A multiple regression analysis was performed to explain relationships between serum PRL concentrations and CV risk factors, especially according to their possible age-dependent variations. Forward and backward selection procedures were used to find the model with the best description performances.

Results

Patient characteristics, including sex-dependent distributions of age and BMI and proportions of subjects diagnosed with hypertension, DM2, and chronic myocardopathy are provided in Table 1.

As related to parameters indicating CV risk factors, significant differences in serum PRL concentrations were found in subjects with lower renal function (expressed as quartiles of creatinine clearance) and higher serum concentrations of homocysteine and TSH, as well as in those who used NSAIDs and statins, compared to those who did not (Tables 2, 3).

Serum PRL concentration was significantly elevated in elderly subjects compared to those younger than that age (>65 vs. ≤ 65), and this was more prominent in men than in women (Table 3).

For quartiles of creatinine clearance and homocysteine, significant differences were found for serum PRL concentrations and patient age (Table 2).

Table 1. Patient characteristics.

Age (years)	Mean ± standard deviation	Minimum–Maximum
Male	69.97±7.79	52–89
Female	66.29±7.87	50–89
BMI (kg/m ²)	Male (%)	Female (%)
<25	20.59	13.79
25–30	41.18	36.21
>30	38.23	50.00
DM 2		
Yes	34	
No	58	
Hypertension		
Yes	76	
No	16	
Chronic cardiomyopathy		
Yes	24	
No	68	

BMI – body mass index; DM2 – diabetes mellitus type 2.

For quartiles of CRP and categories of MMSE, significant differences were found for patient age but not for serum PRL concentrations (Tables 2, 3).

Graphical presentation of serum PRL concentrations according to MMSE and patient age showed significantly higher values for elderly patients (>65 years) compared to their younger counterparts (Mann-Whitney, $p=0.00$) (Figure 1).

Graphical presentation of serum PRL concentrations according to quartiles of creatinine clearance (a measure of renal function decline) showed higher values in patients with lower renal function (indicated with quartiles of creatinine clearance <1.4 and 1.4–1.63 ml/s/1.73 m²) (Figure 2). The bottom to the top quartiles comparison (quartiles <1.4 and ≥2.02 ml/s/1.73 m²) showed a statistically significant difference (Kruskal-Wallis ANOVA, $p=0.0003$).

The LR model selected most of the examined parameters, indicating good choice of the parameters used in the study with respect to their benefit for prediction of increased serum prolactin concentrations (Table 4). These selected parameters indicate either metabolic disorders or disorders associated with chronic renal impairment. Only 2 of these selected parameters – CRP (a marker of chronic inflammation) and creatinine clearance (a measure of renal function decline)

– showed significant and age-independent associations with serum PRL concentrations.

Discussion

Our results showed a tendency of serum PRL concentrations to slightly increase in elderly subjects (+65 years old), compared to those younger than that age, this feature being more prominent in men than in women (Table 3). These results are in line with other studies in which PRL secretion in women was found to decrease after menopause and to slightly increase again after the age of 80, in contrast to men, in whom a continuous slight increase with age has been observed [22,23]. Chronic medical conditions, concomitant with aging, and cumulative psychological stress were found to modify serum PRL concentrations along with the PRL feedback mechanisms regulating secretion [17,20,21,28–30]. Recent evidence indicates an even more complex network influencing serum PRL concentrations in older people, including increased inflammation, impaired immune response, and maladaptive remodelling of the neuroendocrine system, on the background of structural changes of the brain and the hypothalamus [31,32]. PRL was found to play a modulatory role in immune and inflammatory processes [33]. Results of the present study, in general, support this view.

Our results do not support an association between increased BMI (indicating increased body weight) and serum PRL concentrations, either for women or for men (Table 3). Also, large community-based studies, even those performed in a longitudinal manner, do not provide consistent results in this regard, despite the fact that experimental findings clearly show involvement of PRL in mechanisms associated with increased body mass, such as insulin resistance, impaired endothelium dependent vasodilation, and leptin secretion and signalling [9–11,34–36]. Reasons for these inconsistencies may be different patient selection criteria, which can hinder comparison of study results, and the possibility that PRL synthesized locally in adipose and other peripheral tissues has the dominant role in fine-tuning the changes in body composition and shape [1,37–39]. In older people, especially those with multiple CV risk factors, another reason should also be taken into account: the tendency of their BMI values to diverge, either towards obesity or frailty (indicated by low BMI), a feature which can cause different effects on the hormonal regulatory loops [33,40]. In our sample, over 20% of males and 13% of females showed low BMI values (BMI <25) (Table 1).

Similar to BMI, serum PRL concentrations showed no associations with parameters indicating features of MS, including: fasting blood glucose, HbA1c, HDL cholesterol, triglycerides, diagnoses of hypertension and DM2, and fasting blood insulin (Tables

Table 2. Differences in serum PRL concentrations and age according to quartiles of numerical parameters indicating CV risk factors.

CV risk factor Quartiles	N	Serum PRL conc. (mIU/L) Mean ±SD	P-value	Age (years) Mean ±SD	P-value	
Total cholesterol (mmol/L)						
<5.25	22	98.57±48.69	0.75	66.95±9.21	0.81	
5.25 <6.20	27	140.56±118.27		68.81±8.01		
6.20 <7.15	20	106.72±62.93		67.70±8.52		
≥7.15	23	115.16±117.78		66.91±6.52		
CRP (mg/L)						
<3.8	13	118.03±83.21	0.52	68.07±9.78	0.04*	
3.8 <4.4	31	100.21±51.50		65.64±6.18		
4.4 <5.05	25	127.48±83.37		71.04±8.57		
>5.05	23	126.91±147.00		66.43±7.75		
Creatinine clearance (ml/s/1.73 m²)						
<1.4	22	156.70±127.23	0.003	72.86±8.19	0.0003	
1.4 <1.63	22	144.00±110.98		67.17±7.89		
1.63 <2.02	24	97.77±60.58		67.47±6.51		
≥2.02	23	72.53±37.25		63.08±6.53		
HDL-cholesterol (mmol/L)						
Male <1.14	16	145.15±142.55	0.58	70.00±8.90	0.65	
	1.14 <1.40	13		131.37±72.56		71.23±6.18
	1.40 <1.70	3		75.78±37.17		69.00±6.08
	≥1.70	2		114.52±56.64		63.00±12.72
Female <1.14	6	75.23±38.61	0.79	62.83±10.34	0.61	
	1.14 <1.40	10		137.52±142.80		64.10±7.15
	1.40 <1.70	20		100.82±63.58		68.15±8.10
	≥1.70	22		109.87±79.90		66.54±7.24
Fasting glucose (mmol/L)						
<5.3	22	169.84±152.62	0.19	66.63±9.66	0.98	
5.3 <5.65	24	95.24±52.94		67.75±8.46		
5.65 <6.7	21	90.89±50.055		68.71±8.64		
≥6.7	25	112.64±72.83		67.56±5.33		
HbA1c (mmol/mol)						
<3.82	22	113.87±101.41	0.98	68.22±9.10	0.73	
3.82 <4.14	23	133.51±132.06		66.78±9.25		
4.14 <4.56	24	107.48±62.21		66.75±5.83		
≥4.56	23	112.67±74.12		68.91±7.81		
Triglycerides (mmol/L)						
<1.10	20	136.29±120.57	0.85	64.85±9.02	0.48	
1.10 <1.50	21	115.16±78.46		68.76±7.62		
1.50 <2.20	26	99.21±53.45		68.88±7.97		
≥2.20	24	120.97±120.09		67.70±7.54		
Insulin (mIU/L)						
<14.90	23	110.82±53.28	0.48	67.81±11.41	0.87	
14.90 <19.00	24	123.67±86.11		68.58±5.46		
19.00 <26.65	23	108.36±110.35		66.95±7.04		
≥26.65	22	122.23±122.38		67.14±7.80		
Leukocytes (×10⁹ L)						
<5.59	23	110.86±63.61	0.51	69.04±7.24	0.43	
5.59 <6.80	22	110.90±57.29		69.50±9.19		
6.80 <7.62	24	133.57±126.17		66.58±6.50		
≥7.62	23	110.94±113.89		65.60±8.76		

Table 2 continued. Differences in serum PRL concentrations and age according to quartiles of numerical parameters indicating CV risk factors.

CV risk factor Quartiles	N	Serum PRL conc. (mIU/L) Mean ±SD	P-value	Age (years) Mean ±SD	P-value
Homocysteine (umol/L)					
<9.58	23	97.82±98.77	0.07	63.26±7.55	0.0008
9.58 <11.75	23	105.11±75.43		68.60±6.58	
11.75 <14.50	23	109.70±56.35		66.08±7.83	
≥14.50	23	154.62±128.53		72.65±7.32	
TSH (mIU/L)					
<0.95	23	93.16±105.02	0.046	65.78±8.84	0.58
0.95 <1.47	24	118.47±56.62		67.54±7.90	
1.47 <2.21	21	137.85±119.13		67.47±8.65	
≥2.21	23	119.22±94.38		69.39±6.53	

CRP – C-reactive protein; CV – cardiovascular; PRL – prolactin, TSH – thyroid stimulating hormone. * Bolded significant or borderline significant p-values.

Table 3. Differences in serum PRL concentrations and age according to categories of categorical parameters indicating CV risk factors.

CV risk factor	N	Serum PRL conc. (mIU/L) Mean ±SD	P-value	Age (years) Mean ±SD	P-value
Age (years)					
Male ≤65	8	70.65±58.02	0.011*		
>65	26	150.82±114.05			
Female ≤65	26	98.07±96.40			
>65	32	115.95±76.45			
BMI (kg/m ²)					
Male <25	7	128.32±37.93	0.48	71.14±10.74	0.76
25–30	14	203.90±225.95		70.64±5.59	
>30	13	105.25±79.82		68.61±8.49	
Female <25	8	109.98±44.71	0.51	69.25±8.05	0.51
25–30	21	108.12±82.99		66.81±8.46	
>30	29	107.24±97.53		65.10±7.40	
Hypertension					
No	16	136.41±153.98	0.56	68.18±8.09	0.59
Yes	76	122.11±113.26			
DM 2					
No	58	119.60±108.85	0.67	67.28±7.85	0.53
Yes	34	112.06±65.60		68.91±8.02	
Chronic cardiomyopathy					
No	68	111.15±85.90	0.39	67.13±7.75	0.24
Yes	24	132.86±117.19		69.12±8.66	
NSAID					
No	70	125.52±101.55	0.05	68.00±7.43	0.72
Yes	22	89.11±63.55		66.60±9.50	
Statins					
No	74	123.21±103.37	0.03	68.24±8.21	0.11
Yes	18	90.54±36.89		65.22±6.44	
MMSE					
≥25	56	108.71±79.79	0.34	64.94±7.64	0.00
<25	36	129.42±114.52		71.56±6.63	

BMI – body mass index; CV – cardiovascular; MMSE – mini-mental state examination; NSAID – non-steroidal anti-inflammatory drug; PRL – prolactin. * Significant p-values are bolded.

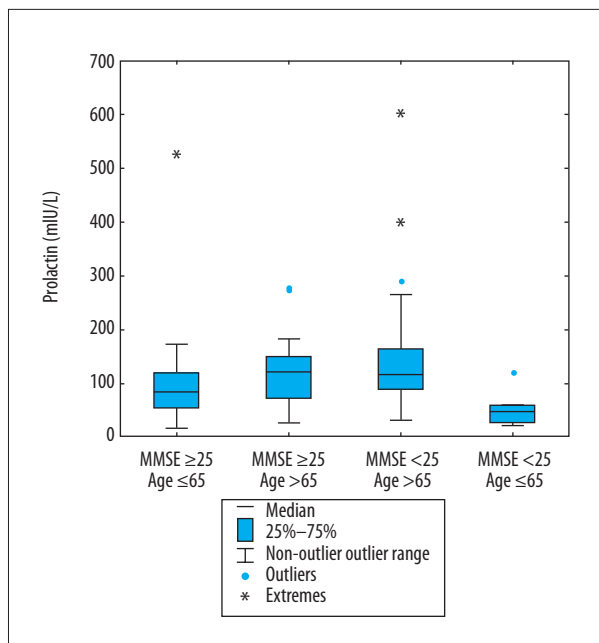


Figure 1. Age (≤ 65 , >65)- and MMSE score (<25 , ≥ 25)-dependent serum prolactin concentrations.

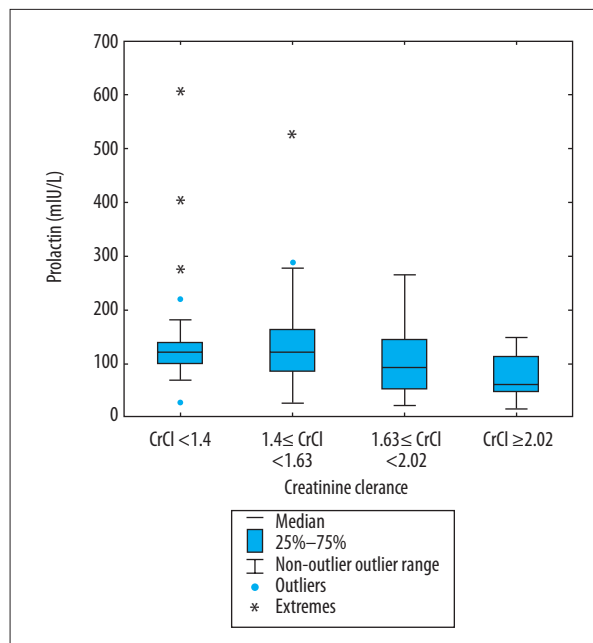


Figure 2. Serum prolactin concentrations according to quartiles of creatinine clearance.

Table 4. Multivariate regression model.

N=90	t (77)	p-Value
Intercept	1.94990	0.054829
Total cholesterol	0.38751	0.699445
Triglycerides	-0.64122	0.523283
HDL-cholesterol	-0.88962	0.376443
BMI	-1.41904	0.159924
Insulin	1.10623	0.272070
Leukocytes	-0.45831	0.648017
CRP	2.16635	0.033378*
Creatinine clearance	-2.65478	0.009639
Homocysteine	-0.10297	0.918257
TSH	-0.84085	0.403037
Age	-0.75558	0.452204

BMI – body mass index; CRP – C-reactive protein; TSH – thyroid stimulating hormone. * Significant p-values are bolded.

2, 3). MS is defined as a cluster of CV risk factors, including abdominal obesity, hypertension, impaired fasting glucose or DM2, and dyslipidemia characterized by increased triglycerides and decreased HDL cholesterol [41]. Increased body weight (indicated by BMI ≥ 25) is not always associated with MS, although in very obese people (BMI >30), features of MS are usually observed [42]. Our sample contained many very obese people (50%

for F and 38.2% for M) (Table 3). A possible reason for the lack of associations between serum PRL concentrations and these metabolic factors may be bias due to the small sample size and the relatively large number of parameters used for analysis. However, much larger studies did not provide clear answers to this question, despite the fact that experimental findings strongly support the role of PRL in insulin resistance, the key mechanism underlying these disorders [10,11,34,35]. Even in the large prospective Framingham Heart Study (n=3232), in a well-adjusted predictive model, PRL was not associated with a comprehensive panel of incident CV disease risk factors [9]. Substantial differences that exist between that study and our study compromise the comparison of the results. Participants in the Framingham Heart Study were on average younger and had a wider age range than in our study, and elderly people were not included at all. In the baseline measurement, CV risk factors presented much less burden than in our study. Results of different studies are difficult to compare, and future studies should pay more attention to justification of patient characteristics. The panel of the target CV risk factors should be adjusted accordingly. This study, although it had a limited sample size, provides some hints for future studies, which should be prepared on a larger scale. For example, our results indicate that in older people, some other factors, apart from the classical CV risk factors, can drive elevation of serum PRL concentrations, as some recent evidence also shows [19].

Parameters significantly associated with serum PRL concentrations, when explained in light of the current data, have enabled a reconciliation of the possible scenario of the pathologic

aging processes, in which increased serum PRL concentrations play a part. Specifically, the importance of chronic inflammation, indicated with the parameter CRP, has been emphasized (Tables 3, 4). That the level of inflammation, although independently associated with serum PRL concentrations in the LR model, is an age-dependent phenomenon, is indicated by the result showing that variations in age, but not in serum PRL concentrations, are associated with graded values of the parameter CRP (Tables 3, 4). Increased systemic inflammation is reflective of the cumulative effect of age-related comorbidities [43,44]. With respect to PRL, including both that of pituitary origin and that synthesized locally, in tissues and organs, evidence suggests their modulating role in immune responses and inflammation [33,38,45,46]. When these pieces of evidence are put together with our results, this all suggests the involvement of PRL in aging processes, which also include inflammation. In these terms, PRL can be considered as a part of a wider network in which the neuroendocrine and the immune systems interact with each other, operating through shared ligands and receptors [47].

Our findings support that increased serum PRL concentrations in older patients with multiple CV risk factors can indicate increased systemic inflammation, showing significant associations between the continuous use of medications with anti-inflammatory properties, statins and NSAID, and lowered serum PRL concentrations (Table 3). Both medications were found to decrease serum PRL concentrations [48,49].

When the fact that statins are prescribed to people at high risk of atherosclerotic CV disease and the fact that atherosclerosis is an inflammatory disease are put together, then the association between statins and serum PRL concentrations found in this study can also indicate the involvement of PRL in atherosclerotic vascular changes [50]. Evidence suggests that PRL augments many mechanisms involved in atherosclerosis development [1–3,35]. When an apparently contradictory result – the lack of association between serum PRL concentrations and total serum cholesterol, a major atherosclerosis risk factor – is added to this scenario, this suggests the mediating role of PRL in inflammatory processes of the vascular wall, rather than its active participation in metabolic derangements (Table 2).

Results showing significant associations between increased serum concentrations of PRL and another pituitary hormone, TSH (Table 2), indicating hypothyroidism, and low MMSE score (<25), indicating cognitive decline, but specifically for elderly participants (old 65 years and more) (Table 3, Figure 1), further enlarge the scope of this study by detailing aging pathologic conditions, which include increased serum PRL concentrations. Mechanisms of action of these hormones – PRL and TSH – on the development of cognitive decline in elderly people are not fully understood. Hypothyroidism, a frequent disorder in the

elderly, usually expressed in a subclinical form (elevation of TSH and normal levels of free thyroid hormones), is a known risk factor for age-related cognitive decline [51]. The compensatory elevation of TRH, the hypothalamic TSH-releasing factor, also causes increased PRL pituitary secretion [28]. By acting through the mechanism of inflammation amplification, these 2 hormones can exert their effect on the development of cognitive decline in 2 ways: either by accelerating atherosclerosis and vascular brain damage, or by promoting inflammatory brain changes [28,52,53].

Our study, by proposing the role of subclinical hypothyroidism and increased serum PRL concentrations in mechanisms of age-related cognitive decline, adds to the evidence supporting a causal link between inflammation and cognitive decline, which is an emerging area of research. A major limitation of this study, the small sample size, may compromise the validity of these results. In addition, the MMSE score, used for measuring cognitive function, cannot make a distinction between atherosclerotic vascular and inflammatory changes of the brain that underlie cognitive decline [25]. A confirmation of our results comes from other, larger, community-based studies, in which subclinical hypothyroidism has been identified as the predictive factor of cognitive decline and dementia, including both atherosclerotic vascular and inflammation-based types [54,55].

Our results allow an even a more complex view of these age-related pathologic conditions in which increased serum PRL concentrations and systemic inflammation play a role, by placing these disorders into the common framework of age-related renal function decline. This is indicated by significant associations found between increased serum PRL concentrations and low creatinine clearance (<1.4 ml/s/1.73 m²), increased serum homocysteine concentrations (≥14.50 umol/L), and age over 72 years (Table 2) and confirmed with the multivariate model in which creatinine clearance was selected as an independent determinant of increased serum PRL concentrations (Table 4). As indicated by our results, PRL in serum starts to rise when creatinine clearance decreases below 1.63 ml/s/1.73 m² (Table 2). Evidence suggest that both PRL clearance and production are altered in chronic renal impairment [56]. Apart from the pro-inflammatory action of this hormone, retention of oxidative and toxic metabolites, including homocysteine (also indicated with our results), relative to stages of renal function decline, can increase the level of the systemic inflammation and in other ways promote structural brain changes, which are known to stay in the background of age-related cognitive decline [57,58].

Accelerated atherosclerosis, usually marked in older subjects with multiple CV risk factors may in turn promote vascular kidney changes and renal function decline [59,60]. Along with this process, atherosclerotic vascular brain changes can cause changes in cognitive functions. A hypothesis which arises from

this evidence is that the metabolic CV risk factors may be only confounding the increased serum PRL concentrations, providing a possible explanation for the lack of their associations found in this and some other studies. A limitation of these results is the MMSE, a psychometric tool used in this study as a measure of global cognitive decline, which therefore cannot make distinctions between particular cognitive functions, although these functions are known to be affected differently by age [61].

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Conclusions

Results of this study indicate that interpretation of serum PRL concentrations in older populations with multiple CV risk factors should include the impact of renal function decline and chronic inflammation. The major strength of this study is its potential to provide better understanding of the complex aging pathophysiology mechanisms that involve increased serum prolactin concentrations. Results suggest the need for creating new laboratory reference ranges of serum prolactin concentrations, specifically for use in older populations. This study provides directions for future studies, which should be prepared prospectively and on a larger scale.

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