

1 Article

2 Mediation and moderation analysis of the 3 relationship between total protein concentration and 4 the risk of depressive disorders in older adults with 5 function dependence in home care

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13

14 **Abstract:** Due to its devastating consequences, late life depression is an important public health
15 problem. The aim of the study was the analysis of variables which may potentially influence risk of
16 depression (GDS-SF). Furthermore, the aim was to study possible mediating effect of given
17 variables on the relationship between the total protein concentration and risk of depression in
18 older-adults with chronic diseases, and physical function impairment. The research sample
19 included a total of 132 older adults with chronic conditions and physical function impairments,
20 remaining under a long-term care in residential environment. Negative linear correlation was
21 observed between patients' physical functionality, total protein concentration, concentration of
22 HDL cholesterol, arm circumference, and the risk of depression. Considerably stronger relationship
23 was observed between total protein concentration, and GDS-SF, in elderly suffering from sensory
24 dysfunction ($b = -6.42$, 95% CI = -11.27; -1.58). The effect of the mediation between depression risk
25 is correlated to total protein concentration in blood serum, and the mediators are probably low
26 function impairment and low levels of 25 (OH)D vitamin. Cohort control research is suggested to
27 confirm the hypothesis.

28 **Keywords:** depression; total protein; elder people; physical function; long-term care

29

30 1. Introduction

31 Functional status, understood as the ability to perform basic and instrumental activities of the
32 daily living (ADL), is of key importance when it comes to assessing the quality of life (QOF) in older
33 adults, as well as in younger people, in case of health deficits which cause deterioration of the
34 functional status. Research of mortality predictors, prove that functional status of the elderly is an
35 independent predictive factor for mortality [1-3].

36 Physical function impairment in patients is a particularly problematic issue in long-term home
37 care, and may lead to numerous health problems, as well as resulting in difficulties when providing
38 care in the home environment. Nursing homecare is becoming a significant sector in the healthcare
39 system for older adults. A long-term nursing care is of great importance for this healthcare system.
40 In Poland, the long-term nursing care is a relatively new and developing discipline. In 1999,
41 Residential long-term care facilities, i.e. nursing homes and care homes for the chronically ill, and
42 incapacitated, were established. In 2004, medical services provided at homes, under the name of
43 long-term nursing care [4], began to be contracted. Long-term nursing care can be provided only to

44 patients scoring 0-40 points in the Barthel index, along with a referral from a doctor working within
45 a health insurance scheme [5]. Other than the above mentioned, the elderly with physical function
46 impairments, who are staying in home environment, are under the care of informal carers (usually
47 family members), who support patients with slight or moderate dependence in the Barthel index.

48 The results of SHARE research, conducted in 2010-2011, show that the proportion of older
49 adults with functional limitations, being a basis concerning the demand for certain assistance forms
50 in daily life, is high in Poland.

51 Nearly 20% of people aged 65-79, and over 40% of those aged over 80, declare to have problems
52 with performing activities of daily living - (ADL). Higher percentage of the elderly (23% of those
53 aged 65-79, and 54% of those over 80) declare problems with performing instrumental activities of
54 daily living - (IADL) [6].

55 Due to the important role of the long-term care, healthcare sector in patient's living
56 environment, the demand is extremely high. However, the supply of long-term care in Poland is
57 mainly informal, and is to be organised by the family- the care is provided primarily by the closest
58 family members in the patient's home.

59 Several various and independent estimations consistently indicate that the most of elderly are
60 under an informal care, between 80% [7] and 93% [8]. The reasons for high supply of informal care
61 results from traditional family relations. Frequently, older adults reside with their children (high
62 cohabitation index).

63 In Polish review literature, there are sparse estimates concerning quantity and the functional
64 status of those in need of long-term care. When patients with physical functional impairment in
65 home care were examined, using the Barthel index, the group n = 296 included 21.3% of the examined
66 scoring 0 points, and 78.7% of the examined scoring 5-40 [4,9]. In other study, concerning the
67 assessment of functional status of patients, with long-term home nursing care, using the Barthel
68 index (n = 190), almost two-thirds of the examined (64.21%) were patients with total dependence
69 (Barthel index 0-20), i.e. demanding considerable help in performing the activities of daily life or
70 unable to self-care. Remaining patients, 35.79%, were characterized by moderate dependence,
71 scoring 21-40 in Barthel index. In cumulative assessment of patients with long-term nursing care (n =
72 575), between 2004 - 2008 in north-eastern Poland, 45% (n = 259) of people scored 0 points in Barthel
73 index. The highest value of points qualifying for long-term nursing care, in Barthel scale - 40 points,
74 was found in 4.9% (n = 28) of the examined. The rest, 50.1% (n = 288), scored between 5-35 points,
75 and between 1-5 in the Barthel index, in the assessment of patients' self-care [11].

76 Physical functional impairment and resulting mobility limitations, are the reasons for
77 impairments of physiological and biochemical reactions of human organism. The functions of
78 cardiovascular, respiratory, nervous, and muscular-bone systems, along with water-electrolyte
79 balance, mainly calcium metabolism, are distorted, affecting the blood clotting. The tempo of energy
80 conversion has also changed, and the organism homeostasis is distorted [12]. Hypokinesis changes
81 body composition, protein metabolism, hormonal profile and muscle function [13-15]. Additionally,
82 hypokinesis results in the decrease in production of certain neurotransmitters and neuromodulators
83 in the brain, eg. noradrenaline and endorphins. There might occur despondence and lowering in
84 adaptation capacities of the central nervous system (concentration difficulties, sleep disorders,
85 anxiety, aggression, as well as depression) [15].

86 The research concerning factors affecting the recovery, or maintaining the condition of patients
87 with chronic diseases, and physical functional impairment, who are under the care of an informal
88 carer and long-term care, should constitute one of the priority research areas in home care.

89 The aim of the study was the analysis of variables (medical, biochemical, and anthropometric)
90 which may potentially influence risk of depression. Furthermore, the aim was to study possible
91 mediating effect of given variables on the relationship between the total protein concentration and
92 risk of depression (GDS-SF) in older-adults with chronic diseases, and physical function
93 impairment.

95 **2. Materials and Methods**96 *2.1. Study design*

97 149 patients with long-term home care in 5 facilities (4 in urban, and 1 in rural area) took part in
98 the study. The study was conducted between September 2016, and February 2017. Information was
99 collected through a direct interview, by nurses, working in long-term home care, who were
100 professionally assisting the families of the patients, during the home visits. The nurses (n = 18) of
101 long-term home care were acquainted with the study project, and prepared for data collection,
102 taking measurements, and materials for laboratory tests, in accordance with established study
103 procedure. On the first day, the nurse filled in the questionnaire with the patient, took
104 measurements, and informed about the preparation for the laboratory tests to be taken on the
105 following day (blood collection in the morning, in the fasted state, after no food intake for 8 - 12
106 hours). On the second day, patient's venous blood was collected in order to mark biochemical
107 parameters. Blood was transported in special containers to the laboratory, in the time, no longer than
108 30 - 60 minutes from the moment of collection.

109 Inclusion criteria were as follows: physical function impairment in the Barthel index: 0 - 85
110 points, medical diagnosis of systemic atherosclerosis (ICD10 - I70), lack of coexisting carcinoma or
111 renal insufficiency, and lack of cognitive function impairments measured by MMSE \geq 7 (the
112 Mini-Mental State Examination), as well as the patient being cared for in home conditions. 149 people
113 qualified for the study. However, after conducting detailed analysis of the collected materials in the
114 questionnaire (due to its incompleteness), finally 132 people, whose information was complete, were
115 included in the analysis.

116 A written consent for the study was granted by the managers and directors of the health
117 facilities, where the nurses performing research were employed. Each patient was orally informed
118 by the nurses regarding the objective of the study, and the scope of the questionnaires,
119 measurements, and then a written consent was taken from the patients. The study was voluntary
120 and anonymous. Nurses completed the questionnaires during a patient interview, and provided the
121 patient with necessary information and explanation. During each stage of the data collection,
122 patients could resign or refuse further participation in the study.

123 The implemented study procedure was approved by Bioethics Committee of Medical
124 University in Lublin (No. KE-0254/13./2016), and was consistent with the Declaration of Helsinki.
125 The study was realized in the frame of own research, financed by Medical University of Lublin.

126 *2.2. Measurements*

127 Research tools consisted of two questionnaires: Geriatric Depression Scale - Short Form
128 (GDS-SF) and the Barthel index. Questionnaires were supplemented by a sheet collecting
129 sociodemographic information, and medical interview data. There were also anthropometric
130 measures taken, along with the collecting of venous blood for laboratory tests.

131 **Geriatric Depression Scale Short Form (GDS-SF)** in the Polish version [17] was used to
132 measure the degree concerning the risk of incidence of depression symptoms. GDS-SF scale was
133 created as a screening tool to diagnose intensity of depression symptoms. Scale in the shortened
134 version consists of 15 statements describing basic depression symptoms, the examined person
135 confirms or denies its presence (yes/no) during the past two weeks.

136 Interpretation of the shortened version is based on the number of scored points: 0-5 no risk of
137 depression, 6-15 there exists risk of depression symptoms. Reliability of the scale was measured with
138 the alfa Cronbach indicator and its value was 0.86.

139 **The Barthel index.** The assessment concerning activities of the daily living was conducted
140 using the Barthel index. The index takes into consideration: meal consumption, mobility,
141 maintenance of personal hygiene, using the lavatories, taking full body baths, mobility on flat
142 surfaces, walking the stairs, dressing, bladder and bowel control. Depending on the range of the
143 patient's self-care, the patient may score between 0-100 points [18]. The amount of scored points
144 shows the degree of disability, and describes the person's demand for care. Functional independence

145 of patients is differentiated depending on which category their score is qualified: 86-100 points -
146 patient deals well with activities of the daily living; 21-85 - patient partly deals well with the
147 activities of the daily living; 0-20 points - patient cannot deal with the activities of daily living alone
148 [19]. The assessment of the applicability of the Barthel index in Polish condition, as a reliable tool
149 (indicator α -Cronbacha = 0.78 ÷ 0.89; correlation co-indicator test-retest R = 0.93 ÷ 0.95) for measuring
150 the range of self-reliance when performing activities of the daily living in older adults, was proven
151 by research [20].

152 **Demographic and clinical variables.** Collected data, describing the examined group on
153 sociodemographic grounds, were: gender, age, marital status ("single" or "in a relationship"), and
154 living arrangements ("alone" or "with family"). Variables, taken into consideration when assessing
155 the medical state, were concerned with comorbidities, i.e. systemic atherosclerosis, diseases (or lack
156 thereof) of: cardiovascular, respiratory, endocrine, neurological, sensory, mental, rheumatic, and
157 other systems, as well as medicinal substances intake and its amount. At the end of this part of the
158 questionnaire, the interviewer calculated number of years the patient has been under long-term care.

159 2.3. Anthropometric measurements.

160 Because it was not possible to measure patients' height and weight in home conditions
161 (bed-ridden patients), to perform anthropometric assessment, following criteria were noted: calf,
162 arm, foot-knee length, as well as skinfold under the shoulder and skinfold of arm triceps muscle.

163 *Circumference of calf* was measured with accuracy up to 0.1 cm in the supine position using
164 inelastic measuring tape. The tape was placed around a calf without compressing the subcutaneous
165 tissue and was moved along the calf length to get the maximal circumference. The measures of both
166 calves were registered as an average, resulting from the measurements of two trials for each limb,
167 which were combined to establish an average for both legs.

168 *The length of shin bones* was measured between the distal end of the medial condyle of the tibia,
169 and the apex of medial malleolus. The location of anthropometer was parallel to long axis of tibia
170 [21].

171 *Skin fold thickness* (SFT) under the shoulder was measured using the Harpenden calliper
172 (Harpenden Calliper) in precisely chosen points. Prior to using, the calliper was thoroughly
173 calibrated to check the strains (so they are not loose) using an object with known dimensions.

174 *Triceps Skinfold* (TSF) was measured in a middle point of the non-dominant arm, with the upper
175 limb freely stretched along the body. Next, the skinfold was pinched between fingers, and the
176 calliper was used. The measurement was performed twice, and the average was used for further
177 analysis.

178 2.4. Blood collection and biochemical analysis.

179 Blood samples were collected in fasted state, after all night rest and moderate-size supper the
180 previous day. Blood was collected to a tube containing a clotting activator and a separation
181 substance (granulate). Plasma was separated using centrifugation of 3000 spins per minute, for 10
182 minutes. Centrifuged serum was used to mark the levels of biochemical parameters. Total protein
183 concentration, total cholesterol, triglyceride, and HDL cholesterol were marked using Advia1800
184 Siemens apparatus, with original reagents. LDL cholesterol was measured with Friedewald formula.
185 D3 25-OH Vitamin was marked on Cobas E411 Roche apparatus, with using of original set of
186 reagents and electrochemiluminescence method. B12 vitamin and folic acid levels were marked
187 using Advia Centaur Siemens apparatus, by chemiluminescence method.

188 2.3. Statistical analysis

189 Categorical variables were reported as number and percentages. The distribution of
190 quantitative variables were described by the mean value (M) and the standard deviation (SD) or
191 median (Me), and lower (Q1) and upper quartile (Q3). The Shapiro-Wilk test was used to assess
192 conformity with a normal distribution.

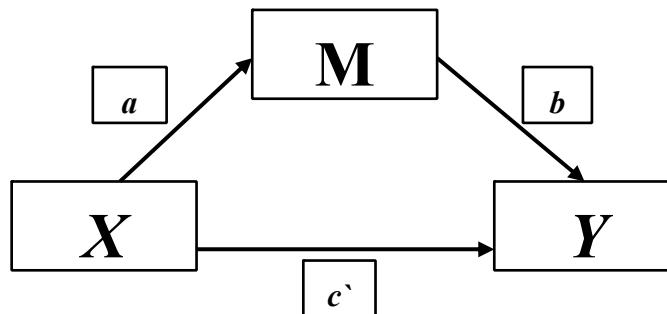
193 Linear regression was used to assess the relation between the values of the scale of depression
 194 risk (GDS-SF) and considered variables (age, gender, results of anthropometric measurements,
 195 laboratory test and comorbidities).

196 Furthermore, in order to study whether age, gender, or examined comorbidities change
 197 direction or magnitude relationship between total protein concentration, and the risk of depression;
 198 models with interaction term between the total protein concentration and the consider variable
 199 (moderator) were performed. The significance of the given moderator was assessed by the change of
 200 proportion concerning the explained variance (ΔR^2).

201 In order to investigate the mechanism of the relationship between total protein concentration,
 202 and the risk of depression, mediation analysis was used, by taking into consideration the third
 203 variable - mediator. The scheme of applied mediation analysis is shown on Figure 1. „The PROCESS
 204 macro” procedure was used [22]. Several models (steps) of linear regression were performed. The
 205 results of mediation analysis were presented by giving three factors (effects) from models:

- 206 1- Providing the coefficient for a given mediator (after controlling for total protein concentration)
 207 with 95% confidence interval (95% CI);
- 208 2- Direct effect, (c') coefficient of regression model for the total protein concentration after
 209 controlling for mediating variable (mediator) with 95% CI;
- 210 3- Indirect effect with 95% CI, calculated with taking into consideration „biascorrected” and
 211 „accelerated” correction. Effect is the product ($a \cdot b$, on the attached scheme) of the coefficients (in
 212 regression model) between total protein concentration and the studied mediating variable, and
 213 between the coefficient (in regression model) between the mediating variable and depression.

214 To establish the significance of the mediation effect, bootstaping method was used (made for
 215 5000 drawings). This procedure estimates the indirect effect using the bootstaping technique
 216 (generating empirical representation of the sample distribution, treated as a population
 217 representation). All analyses were made with IBM SPPS v.22 software, as a statistical significance for
 218 bilateral tests $\alpha = 0.05$ was applied.



227 **Figure 1.** Model presenting the examined mediation effect. X: independent variable (protein
 228 concentration); M: mediator; Y: dependent variable (GDS-SF); a: association between X and M; b:
 229 association between M and Y after controlling for X; c' : indirect effects.

230 3. Results

231 3.1. Characteristics of the studied groups according to sociodemographic and medical data

232 Table 1 presents characteristics of the studied group. Analysis included 132 patients under
 233 long-term home care. The average age in the studied group was 78.75 – 8.75 years. Majority of the
 234 group were women (n = 103, 78%) and unmarried (n = 83, 62.9%). As many as 118 people (79.2%)
 235 lived with their families. The group of the examined patients included only those with slight (0-40
 236 points), and moderate (41-85 points) dependency in the Barthel index. Average assessment of
 237 physical function was 43.20 (SD = 27.06). The largest group of 60 people (45.5%) consisted of patients
 238 scoring 21 - 40 points in the Barthel index; 44 people (33.3%) scoring 0-20 points, and 28 people
 239 (21.2%) scoring 41 - 85. All patients were under home care, which they have used on average for 3.59
 240 years (SD = 2.68). Average amount of medications prescribed by the doctors taken by the patients
 241 was 7.9 (SD = 2.8). Patients were characterized by comorbidities, with domineering conditions being

242 rheumatic (77.9%), sensory (65.1%), psychiatric (51.7%), endocrine (45%), and those of the nervous
243 system (26.5%).

244 In the assessment concerning the risk of depression symptoms occurrences, according to
245 GDS-SF, the average was 7.34 points (SD = 3.10). Lack of depression risk (0-5 points) occurred in 25%
246 (n = 33), and in other 75% (n = 99) there was a risk of depressive disorders.

247 38 people (28.8%) had total protein concentration within the 5.56 -6.61 mg/dl frame; 66 of the
248 examined (50%) 6.62 - 7.37 mg/dl, and 28 people had total protein concentration ranging in 7.38 - 8.14
249 mg/dl.

250 **Table 1.** Characteristics of the examined group.

Variable	n = 132
Medical interview	
Under long-term care (years)	3.91 ± 2.61*
Result in the Barthel index	43.20 ± 27.06*
Result in GDS scale	7.34 ± 3.10*
Amount of medicinal substances taken	7.9 ± 2.8*
Comorbidities	
Respiratory system (n, %)	19 (14.4)**
Endocrine system (n, %)	54 (40.9)**
Nervous system (n, %)	35 (26.5)**
Sensory (n, %)	12 (9.1)**
Psychiatric (n, %)	55 (41.7)**
Rheumatic (n, %)	77 (58.3)**
Laboratory test results	
Total protein (g/dl)	7.09, 6.61 - 7.37***
25 (OH)D vitamin (ng/ml)	14.41, 8.64-26.63***
B12 vitamin (pg/ml)	390.76 ± 143.35*
Folic acid (ng/ml)	5.4, 3.74-9.92***
Total cholesterol (mg/dl)	183, 158-218***
HDL Cholesterol (mg/dl)	53.55, 42.62-64.22***
LDL Cholesterol (mg/dl)	106.5, 86-130.75***
Triglycerides (mg/dl)	115, 88.25-158.75***
Anthropometric variables	
Calf circumference (cm)	36.05 ± 5.42*
Arm circumference (cm)	30.79 ± 7.90*
Tibial bones length (cm)	48.02 ± 5.44*
Thickness of the skinfold under the shoulder (cm)	2.6, 2.1-4***
Skinfold of arm muscle triceps (cm)	2.3, 2-3.8***

251 Data are presented as: *mean ± SD; **n (%); ***median and Q1-Q3.

252 *3.2. Relation between GDS-SF and studied criteria*

253 Table 2 shows results of simple linear regression. Negative linear association was observed
254 between the results of the Modified Barthel Index, total protein concentration, concentration of HDL
255 cholesterol, arm circumference, and value of the GDS-SF scale. Along with the increase of the point

256 value in the GDS-SF scale, the values of Barthel index assessment, total protein concentration, HDL
 257 cholesterol, and arm circumference decreased.

258 **Table 2.** Multi-variable analysis of the linear regression of the depression risk (GDS-SF) determinant
 259 in the examined group.

Variable	b	95%CI
Gender		
Male	0.40	(-0.89; 1.70)
Age	0.02	(-0.04; 0.09)
Marital status		
In a relationship	-0.86	(-1.97; 0.24)
Cohabitant		
Alone	0.75	(-0.54; 2.03)
Medical interview		
Under long-term healthcare (years)	-0.04	(-0.26; 0.17)
Results in the Barthel index	-0.02	(-0.04; -0.004)
Amount of medicinal substances taken	0.004	(-0.36; 0.36)
Morbidities:		
Respiratory system	-1.45	(-2.95; 0.06)
Endocrine system	-0.33	(-1.42; 0.76)
Nervous system	0.23	(-0.98; 1.44)
Sensory	-0.36	(-2.22; 1.50)
Psychiatric	-0.35	(-1.42; 0.72)
Rheumatic	0.51	(-0.58; 1.60)
Laboratory tests results		
Total protein	-1.10	(-2.08; -0.13)
25 (OH)D Vitamin	-0.008	(-0.02; 0.007)
B12 Vitamin	-0.001	(-0.004; 0.003)
Folic acid	0.001	(-0.05; 0.06)
Total cholesterol	-0.004	(-0.01; 0.007)
HDL Cholesterol	-0.03	(-0.07; -0.002)
LDL Cholesterol	0.001	(-0.01; 0.01)
Triglycerides	-0.001	(-0.01; 0.009)
Anthropometric variables		
Calf circumference	-0.08	(-0.18; 0.02)
Arm circumference	-0.079	(-0.15; -0.01)
Tibia bone length from foot base to the knee	-0.003	(-0.10; 0.10)
Thickness of under the shoulder skinfold	0.05	(-0.007; 0.12)
Skinfold of arm triceps muscle	0.06	(-0.008; 0.12)

260 b - coefficient from linear regression

261 *3.3. Moderation Model*

262 Table 3 presents the results concerning testing moderators of relationship between total protein
 263 concentration, and the value of the GDS-SF scale, which is equal to occurrence of interactions

264 between total protein, and the examined moderator. For this, estimates of the linear regression
 265 models were presented (separately for each moderator): model 1 involving total protein, and the
 266 examined variable and model 2 includes interaction between total protein concentration, and the
 267 examined variable. Moreover, determination coefficients (R^2) and its change(ΔR^2), when the
 268 interaction term was incorporated, were given.

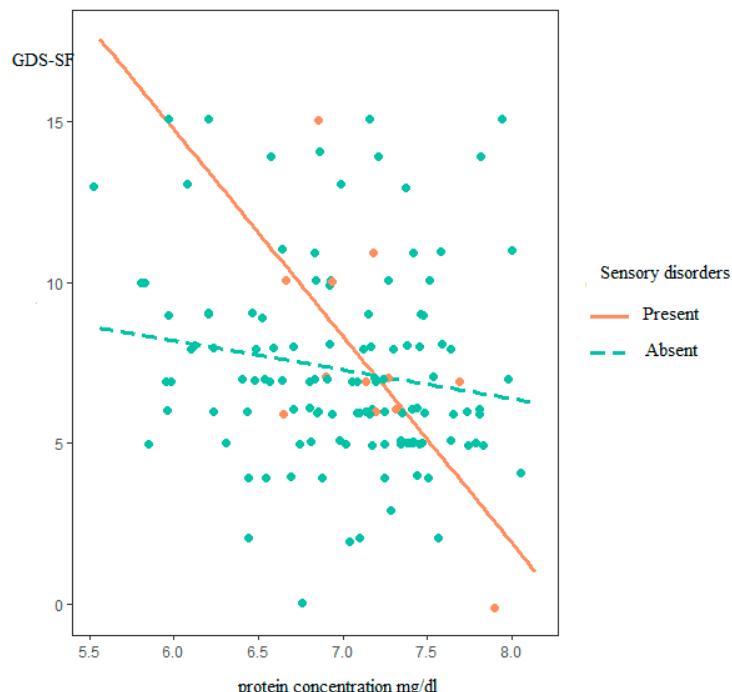
269 Among the comorbidities, sensory disorders, significantly affected the relationship between the
 270 total protein concentration, and the risk of depression occurrence, estimated using the GDS-SF scale,
 271 increasing a part of the explained variability to 8% ($\Delta R^2=0.035$) Figure 2. There was a stronger and
 272 significant association between the total protein concentration, and GDS-SF in participants with
 273 sensory disorders ($b = - 6.42$, 95% CI - 11.27 to - 1.58), in comparison to examined patients without
 274 such disorders ($b = - 0.90$, 95% CI - 1.89 to 0.08). However, in the model with interaction, both protein
 275 ($b = - 11.95$, 95% CI -21.67 to - 2.21), and the sensory disorders ($b = - 39.7$, 95% CI 23.75 to -162.29),
 276 were significantly related to the dependent variable. This means, that outside of the significant
 277 interaction, main effect (protein concentration) was also significant.

278 In the case of the other analysed diseases, there was no significant moderation effect observed.

279 **Table 3.** Moderation effect tested for the correlation between depression and protein concentration.

Model	Moderator coefficient (95% CI)	Protein coefficient (95% CI)	Interaction coefficient	F
Gender				
$R^2=0.04$	2.28 (-13.40; 17.96)	- 0.77 (-3.75; 2.21)	0.27 (-2.50; 1.97)	1.81
$\Delta R^2=0.0004$	0.42 (- 0.86; 1.69)	-1.11 (-2.09; - 0.13)		2.71
Age				
$R^2=0.037$	0.06 (-56.86; 77.60)	- 0.44 (-9.94; 9.06)	- 0.008 (- 0.12; 0.11)	1.66
$\Delta R^2=0.0001$	0.0002 (-0.065; 0.065)	-1.1 (-2.15; - 0.056)		2.49
Comorbidities				
Respiratory system				
$R^2=0.06$	- 0.67 (-18.78; 17.43)	- 0.89 (-5.73; 3.95)*	- 0.10 (-2.69; 2.48)	2.86
$\Delta R^2=0.000$	-1.41 (-2.89; 0.08)	-1.09 (-2.005; 0.12)*		4.33
Endocrine system				
$R^2=0.05$	-8.34 (-22.14; 5.46)	-2.84 (-5.97; .28)	1.17 (- 0.80; 3.14)	2.16
$\Delta R^2=0.01$	-0.18 (-1.26; 0.90)	-1.08 (-2.07; - 0.10)		2.55
Nervous system				
$R^2=0.04$	6.86 (-9.29; 23.01)	0.51 (-3.72; 4.73)	- 0.93 (-3.26; 1.39)	2.01
$\Delta R^2=0.005$	0.39 (-0.81; 1.59)	-1.14 (-2.13; - 0.16)*		2.71
Sensory				
$R^2=0.075$	-39.7 (-74.85; -4.55)	-11.95 (-21.69; -2.21)	5.52 (0.58; 10.47)	3.44
$\Delta R^2=0.035*$	-0.49 (-2.33; 1.35)	-1.12 (-2.10; -0.14)*		2.64
Psychiatric				
$R^2=0.043$	0.09 (-13.69; 13.87)	-1.04 (-4.33; 2.24)	- 0.05(-2.01; 1.92)	1.89
$\Delta R^2=0.000$	-0.24 (-1.29; 0.82)	-1.12 (-2.08; - 0.16)*		2.86
Rheumatic				
$R^2=0.046$	1.59 (-12.31; 15.49)	- 0.93 (-3.92; 2.07)	- 0.14 (-2.12; 1.83)	2.05
$\Delta R^2= .0002$	0.58 (-0.49; 1.64)	-1.13 (-2.11; - 0.16)*		3.08

280 R^2 -coefficient of determination, ΔR^2 – increase R^2 due to interaction, F – test static from model



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Figure 1. Moderation analysis.

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3.4. Mediation Model

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Table 4 shows the result of testing the mediation effect with models of linear regression. Multipleregression was used in order to assess whether laboratory parameters, anthropometric parameters, and the results of the Barthel index were mediators of the relationship between total protein concentration, and the assessment of the risk of depression occurrence (GDS-SF). Among the examined, indirect effect of 25 (OH)D vitamin concentration ($b = -0.162$, 95 % CI -0.452 to -0.015), was significant, as well as for the Barthel index ($b = -0.22$, 95% CI -0.54 to -0.05). In both cases, direct effect was statistically insignificant (because there was no statistically significant relationship between total protein concentration, and GDS-SF, after controlling for 25 (OH)D vitamin (coefficient for protein: $b = -0.88$, 95% CI -1.87 to 0.11), as well as after controlling values of Barthel index (coefficient for protein: $b = -0.79$, 95% CI -1.78 to 0.21) - (direct effect). Therefore, we can speak about full mediation of the relationship between GDS-SF, and total protein concentration through 25 (OH)D vitamin and the Barthel index. This means, that the relationship between total protein concentration, and the GDS-SF is transmitted through assessment of the Barthel index and 25 (OH)D vitamin (mediator).

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Table 4. Mediation effect tested for correlation between depression and protein concentration.

	Mediator (M)	Direct effect		Indirect effect		
		b	95% CI	b	95% CI	
Laboratory parameters:						
25 (OH)D vitamin	- 0.05	(- 0.10; - 0.007)	- 0.786	(-1.78; 0.21)	- 0.162	(- 0.45; - 0.01)
B12 vitamin	- 0.0003	(- 0.004; 0.003)	-1.09	(-2.12; - 0.06)	- 0.012	(- 0.31; 0.13)
Folic acid	0.045	(-0.05; 0.14)	-1.135	(-2.12; - 0.15)	0.032	(- 0.04; 0.23)
Total Cholesterol	- 0.003	(- 0.01; 0.007)	-1.078	(-2.06; - 0.09)	- 0.027	(- 0.25; 0.04)
HDL Cholesterol	-0.027	(- 0.06; 0.006)	- 0.908	(-1.91; - 0.09)	- 0.197	(- 0.63; 0.01)
LDL Cholesterol	0.0004	(- 0.13; 0.014)	- 1.10	(-2.08; - 0.12)	- 0.0013	(- 0.13; 0.09)
Triglycerides	0.001	(- 0.008; 0.01)	-1.132	(-2.13; - 0.13)	0.027	(- 0.12; 0.30)
Result in the Barthel index	-0.02	(- 0.04; - 0.0002)	- 0.882	(-1.87; 0.11)	- 0.223	(- 0.54; - 0.05)
Anthropometric variables:						
Calf circumference (cm)	-0.073	(- 0.17; 0.02)	-1.039	(-2.01; - 0.07)	- .035	(- 0.35; 0.07)
Arm circumference (cm)	-0.141	(- 0.25; - 0.035)	- 0.900	(-1.86; 0.06)	- 0.174	(- 0.50; 0.02)
Tibia bone length from foot base to the knee (cm)	-0.011	(- 0.11; 0.09)	-1.082	(-2.06; - 0.10)	0.008	(- 0.12; 0.24)
Thickness of under the shoulder skinfold (cm)	0.05	(- 0.01; 0.11)	-1.014	(-1.99; - 0.04)	- 0.059	(- 0.50; 0.06)
Arm triceps muscle skinfold	0.05	(- 0.01; 0.12)	-1.014	(-1.99; - 0.04)	- 0.600	(- 0.47; 0.07)

305

b - coefficient from linear regression

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307

308

309 **4. Discussion**

310 Our study results concern a certain group of patients from an older age group with physical
311 function impairment in the Barthel index (0-85 points) with comorbidities, requiring assistance of
312 carers (professional and informal) concerning activities of daily living in home conditions, with a
313 medium time span of using long-term care (3.91; SD = 2.61). Literature review shows, that in this
314 group of patients, the studies are scarce and results of empirical studies are not published
315 frequently.

316 Physical function impairment and hypokinesis connected with it, is reflected in reaction
317 disorders in physiological and biochemical reactions of human organism. Ferrando et. al. [13] shows
318 that the loss of protein in the organism, caused by lack of physical activity, connected with patients
319 long-term time spent in bed, is mainly the result of decrease in muscle protein synthesis, and that the
320 decrease is reflected both in the systemic parameters, and in skeletal muscles. The decrease in
321 protein synthesis was connected with the corresponding decrease in the sum of intracellular
322 amino-acid composition connected with protein decomposition and internal transport.

323 In our study, in the group of patients with physical function impairment, we found a mediating
324 relationship between total protein concentration, and intensification of the depression risk GDS-SF.
325 The mediators of this relationship were a low function impairment of the patients in the Barthel
326 index, and low levels of 25 (OH)D vitamin. The theory named by the authors *Social Signal*
327 *Transduction Theory of Depression* connects psychological factors (psycho-social), immune system
328 response, and depression. Depression is connected with emergence concerning chronic state of
329 immune response of moderate strength, and compensative activation of the system counter
330 measuring the inflammation (CIRS, *anti-inflammatory reflex system*) [23]. It is accompanied by
331 increased oxidative stress, and creation of antibodies aimed by protein, modified as a result of its
332 action [24-26].

333 In clinical practice of psychiatric care, and home family care. the knowledge of the particular
334 types of cells, and protein engaged in immune response, is not the matter of daily practice, and the
335 examination concerning levels of particular pro-inflammatory cytokines, is unlikely to become a
336 standard for the early diagnosis of depression. Our study showed that simple test on total protein of
337 blood serum in patients with physical function impairment, can significantly affect the diagnosis of
338 early depression symptoms, and the moderating factor of this correlation might also be a low level of
339 25 (OH)D vitamin.

340 A frequent reason for vitamin D deficiency is the decrease of synthesis of active form of vitamin
341 D in kidneys. It can be said, that this is a physiological phenomenon concerning older adults, but
342 also people with mobility disorders, frequently suffering from kidney disorders. Additionally, those
343 people avoid exposition to sunlight [27]. The receptor for vitamin D is located in the brain, its
344 deficiency not only impair mental functions, but also the development of selected psychiatric
345 disorders, such as schizophrenia and depression [28, 29]. Zanetidou et al. [30] believes that vitamin D
346 supplementation in older patients with coexisting dementia, can result in the decrease of depression
347 symptoms.

348 In the study of possible mediating effect of depression symptoms (GDS-SF M = 3.5; SD = 3.2),
349 correlation between ADL results (M = 96.2, SD = 8.1), and quality of life QOL in ambulatory
350 community of older adults over the age of 65 (n = 490), it was discovered that depression is the
351 mediator of the relationship between the modified Barthel index (MBI), and physical, psychological
352 and environmental quality of life facets WHOQOL-BREF [31]. Because our study concerned the
353 patients with impaired physical function (the Barthel index M = 43.20; SD = 27.06), and higher
354 intensity of depressive disorders (M = 7.34; SD = 3.1), they show slightly different model of
355 mediation effect, with the involvement of, at least, two similar variables (depression and function
356 impairment).

357 Initial conclusion is that the mediation effect can be true and deserves further confirmation.
358 This discovery may suggest, that in older adults with physical function impairment under home
359 care, the risk of depression development might be controlled, if the simple biochemical blood

360 parameters, such as total protein and 25 (OH)D vitamin, would be appropriately managed in clinical
361 practice of long-term care.

362 *4.1. Implications of the present findings*

363 The study also has implications for policy concerning people with physical function
364 impairment under care in home conditions. Depression symptoms significantly affect the quality of
365 life of both, the patient, and the informal carer, including the environment of care provision, and
366 social relations. This discovery means that there is a need for public effort in every possible aspect to
367 effectively manage the risk of depression occurrence in this group of patients.

368 *4.2. Limitations*

369 The limitations of the current study are as follows. First of all, the participants were recruited
370 intentionally from communities with long-term care, rather than selected under randomisation.
371 Cross-sectional character of the study, limits its strength to causal inference. Secondly, results of the
372 studies are based on a relatively small sample group, which might also affect the generalisation of
373 the obtained study results. A prospective, longitudinal, supplementary study is recommended in a
374 future programme, in order to confirm the mediation effect of the risk of depression symptoms in
375 relation to coefficient of total protein in blood serum, registering of parameters of physical function
376 of the patients and the levels of the 25 (OH)D vitamin.

377 **5. Conclusions**

378 Risk of depression particularly affects older adults with chronic diseases and physical function
379 impairment under home care. What is more, mediation effect of depression symptoms stays in
380 relation to the levels of total protein in blood serum, and the mediators are probably low physical
381 function, and low level of 25 (OH)D vitamin. Moreover, screening tests of simple laboratory blood
382 indices, and their control, may decrease the risk of depression in older adults with physical function
383 impairments. A cohort, control study is suggested to confirm our hypothesis.

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385 analyzed the data and contributed to its interpretation. G.J.N. interpreted the data and wrote the original draft.
386 G.J.N. and B.Ś. were involved in writing-review & editing. G.J.N., B.Ś. and M.Ł. was responsible for funding
387 acquisition and supervision. All Authors were involved in critically revising the manuscript, and have given
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