Leptin concentration and risk of impaired physical function in older adults: the Seniors-ENRICA cohort

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ABSTRACT

*Background***:** Leptin resistance, which may develop during the aging process, stimulates the production of proinflammatory cytokines and insulin resistance that could impair the muscle function. However, the role of leptin on physical functioning among older adults has not yet been elucidated.

*Objective***:** To examine the association between serum leptin levels and physical function impairment in older adults.

*Design and setting***:** Prospective study of 1,556 individuals aged ≥60 years from the Seniors-ENRICA cohort, who were free of physical function limitation at baseline.

Main outcome measure: Serum leptin was measured in 2008-2010, and incident functional limitation was assessed through 2012. Self-reported limitations in agility and mobility were assessed with the Rosow and Breslau scale, limitation in the lower extremity function was measured with the Short Physical Performance Battery, and impairment in the overall physical performance with the physical component summary of the SF-12.

*Results***:** After adjustment for potential confounders, and compared to individuals in the lowest quartile of leptin concentration, those in the highest quartile showed increased risk of impaired physical function; the odds ratio (95% confidence interval) and p-trend was: 1.95 (1.11-3.43), p=0.006 for self-reported impaired mobility; 1.76 (1.08-2.87), p=0.02 for self-reported impaired agility; 1.48 (1.02-2.15), p=0.04 for limitation in the lower extremity function; and 1.97 (1.20-3.22), p=0.01 for decreased overall physical performance. These associations were only modestly explained by C-reactive protein and insulin resistance. Moreover, the associations held across groups with varying health status and were independent of estimated total body fat.

*Conclusions***:** Higher leptin concentration was associated with increased risk of impaired physical function. Preserving metabolic function during the old age could help delaying physical function decline.

INTRODUCTION

Life expectancy has rapidly increased in the last decades, but healthy life expectancy has grown more slowly [1,2]. Healthy ageing is a process which enables older people to take an active part in society and to enjoy an independent and high quality of life [3]. A well-known threat to healthy ageing is the impairment of physical functioning. Unfortunately, the understanding of the biological factors that lead to impaired physical function in the old age is still limited [4].

There is evidence of the detrimental effect of obesity on physical function in older adults [5,6,7,8,9]. In other studies, underweight has also been associated with functional decline [10]. A plausible explanation is that adiposity, in addition to body weight, has a pivotal role on physical functioning [11]. In fact, older adults with sarcopenia or obesity are at higher risk of impaired physical function [12] probably because fat infiltrated in the muscle induces oxidative stress and chronic inflammation, which at the same time decreases skeletal muscle mass and strength [13,14]. Furthermore, other long term effects of adiposity, such as insulin resistance, could also have an impact on physical function [15]. However, the association between sarcopenic obesity and impairment in physical functioning has only been modestly explained by C-reactive protein (CRP) and insulin sensitivity [12], suggesting that other metabolic factors could be involved, such as adipokines.

Leptin is the first adipokine discovered and one of the best characterized. It is mainly secreted by the white adipocyte tissue and it acts predominantly through the central nervous system, contributing to the regulation of appetite and several neuroendocrine pathways, like glucose homeostasis [16]. But leptin has also peripheral effects, some of which are associated with the production of proinflammatory cytokines and insulin resistance [17]. Obese subjects can develop a state of central leptin resistance followed by increased serum leptin levels. In this state, the peripheral effects of leptin could prevail over its central action [18]. It has been suggested that leptin resistance could develop with the aging process [13]. In fact, one prospective study among middle-aged women has recently found that higher leptin concentration predicts impairments in mobility [19]. Nevertheless, its role on physical functioning among older adults has not yet been elucidated, specially whether or not is

mediated by inflammation or insulin resistance. Thus, we hypothesize that the increase in serum leptin levels associated with ageing could lead to functional limitations. Thus, the aim of this study was to examine the prospective association between serum leptin levels and the incident impairment of physical function among older adults.

METHODS

Study design and participants

Baseline data for this prospective study were obtained from the ENRICA cohort, which was established in 2008-2010 and involved 12,948 individuals representative of the non-institutionalized adult population of Spain [20]. At baseline, information on sociodemographic characteristics, lifestyle, health status and morbidity was collected through a telephone interview. In two subsequent home visits, trained research staff also obtained dietary information, conducted a physical examination and obtained blood and urine samples for several biochemical and hormonal determinations. In 2012, we performed a second wave of data collection among the participants aged 60 and older (n=2,614), which comprise the Senior-ENRICA cohort; given that 95 (3.6%) individuals passed-away during the follow-up period, updated information was obtained only for the remaining 2,519 subjects. The Clinical Research Ethics Committee of the *'La Paz'* University Hospital approved the study protocol. All study participants gave written informed consent.

Study variables

Leptin and other biomarkers

Baseline serum leptin concentration (ng/mL) was determined by enzyme-linked immunoassay (Diagnosis Biochem Canada) using a BEST2000 robot. The sensitivity of this test was 0.5 ng/dL and the coefficients of variation intra- and inter-assay were 7.5 and 9.6%, respectively. We also measured levels of insulin resistance and inflammation because they could be associated with leptin concentration. The homeostatic model assessment for insulin resistance index (HOMA-IR) was

calculated by multiplying glucose by insulin and dividing by 405. Glucose (mg/dL) was measured by the glucose oxidase method, and insulin (µU/mL) by immunoradiometric assay. Finally, CRP (mg/L) was determined by latex-enhanced nephelometry. The coefficients of variation intra- and inter-assay were <4% and <4% for glucose, 5.2 and 6.9% for insulin and 3.2 and 5.9% for CRP.

Physical function

We considered three basic domains of physical function: self-reported agility and mobility and an objective measure of lower extremity function, as well as a measure of overall self-reported physical performance. Limitation in self-reported agility was defined by answering "*a lot*" to the following question from the Rosow and Breslau scale [21]: "*On an average day with your current health, would you be limited in bending and kneeling?*"; whose categories of response were "*yes, a lot*", "*yes, a little*" and "*not at all*". In the same way, limitation in self-reported mobility was defined by answering "*a lot*" to any of the following questions from the Rosow and Breslau scale [21]: "*On an average day with your current health, would you be limited in the following activities: 1) picking up or carrying a shopping bag?; 2) climbing one flight of stairs?; 3) walking several city blocks (a few hundred meters)?*". Limitation in the lower extremity function was assessed using the Short Physical Performance Battery (SPPB). The SPPB combines the results of three measurements: the gait speed across 2.44 meters, balance using three hierarchical tandem tests, and the ability to rise from a chair five times consecutively [22]. The score with the sum of these three components ranges from 0 to 12 (highest level of function). Participants were considered to have limited function when they scored ≤9 points in the SPPB; of note is that this test was only measured in 2012. Lastly, limitation in self-reported overall physical performance was deemed to exist when the score of the physical component summary (PCS) of the 12-Item Short-Form Health Survey (SF-12) decreased at least 10 points from baseline to follow-up. We used this cut-off point because a 10-point lower score has been associated with severe adverse health outcomes [23,24]. Moreover, in medical practice, a 10-point change in individual patients is considered as a clinically relevant alarm signal [25].

7

Other variables

We also collected data on several potential confounders of the study association. These included sociodemographic variables and health behaviors such as age, sex, educational level, tobacco smoking, alcohol intake, time spent watching TV and physical activity during leisure time (using the EPIC-Spain validated questionnaire) [26]. Additionally, we considered two dietary variables derived from a validated diet history [27]: adherence to the Mediterranean diet, according to the Trichopoulou index [28], and total energy intake. Regarding adiposity, we estimated the percentage (%) of body fat using the CUN-BAE equation, which is based on sex, age, weight and height [29]. Finally, we obtained information on morbidity. Blood pressure was measured under standardized conditions and hypertension was defined as having a systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or being under drug treatment. Being diabetic was defined as having a medical diagnosis, or fasting serum glucose ≥126 mg/dl, or being treated with insulin or other hypoglycemic agents. Individuals also reported whether they had been diagnosed with cardiovascular disease, chronic obstructive pulmonary disease, cancer at any site, osteomuscular disease (including osteoarthritis, arthritis and hip fracture) or depression requiring pharmacological treatment. Moreover, cognitive function was measured using the Mini-Mental State Examination (MMSE), defining cognitive impairment as a MMSE score of <23 [30]. Finally, the Lawton-Brody index was used to ascertain limitations in instrumental activities of daily living (IADL) [31].

Data analysis

Of the sample comprising 2,519 individuals, we excluded 24 subjects with baseline medical diagnosis of dementia, Alzheimer or Parkinson disease. We excluded 658 participants who lacked data on mobility (n=184), agility (n=3), SPPB (n=447) or PCS score (n=24), 6 individuals without leptin determination, and 54 with missing data on potential confounders. Additionally, we excluded the participants with basal limitations in physical functioning: 91 with self-reported impaired mobility, 75 with self-reported impaired agility and 55 with fatigue, as a proxy of overall limitation in physical functioning. Baseline fatigue was measured by asking respondents how much time during the past 4

weeks they felt tired; responses of "*all of the time*" or "*most of the time*" were considered positive [**32**]. Thus, the analyses were conducted with 1,556 individuals.

Logistic regression models were used to estimate the odds ratios (OR) and the 95% confidence interval (CI) of the association between the serum concentration of leptin and incident limitation in physical function. Given that leptin concentration did not follow a normal distribution, we used the log-transformed values. Participants were classified into sex-specific quartiles, because leptin concentration was significantly higher in women (30.9 ng/dL ±18.9) than in men (12.3 ng/dL ±9.5). Several regression models and sensitivity analyses were built (please see a detailed description in **Appendix 1**). Moreover, to summarize the study associations we repeated all the analyses using an increment of one standard deviation (SD) of leptin concentration as the independent variable. We tested if the main results varied with sex by using interaction terms. Since the results were similar in each sex and the interactions did not reach statistical significance, the results are reported for the total study sample. Statistical analyses were performed with the STATA software (version 13.0; Stata Corp., College Station). A 2-tailed $p<0.05$ was considered statistically significant.

RESULTS

During a mean follow-up period of 3.5 years, the incidence of limitations in mobility was 12.5%, in agility 20.4% and in overall physical performance 16.7%. Furthermore, 54.8% of the individuals had impaired lower extremity function at the end of follow-up. **Table 1** shows the socio-demographic, behavioral and clinical characteristics of the participants at baseline, according to categories of functional impairment. With regards to leptin, the mean ± standard deviation of serum concentration was 21.2±17.5 ng/mL, and it was significantly higher among subjects with any incident functional impairment.

Compared to individuals in the lowest quartile of leptin concentration, those in the highest quartile showed increased risk of impaired physical function; in model 3, the ORs (95% CI) and p-trend were: 1.95 (1.11-3.43), p=0.006 for self-reported impaired mobility, 1.76 (1.08-2.87), p=0.02 for self-

reported impaired agility, 1.48 (1.02-2.15), p=0.04 for lower extremity function, and 1.97 (1.20-3.22), p=0.01 for decreased overall physical performance **(Table 2)**. The additional adjustment for CRP and HOMA-IR only modestly reduced the association found. Moreover, analyses using the increment of one SD of leptin instead of the quartiles of the concentration gave similar statistically significant results (**Table 2**). Additional adjustment for length of follow-up did not materially change the results (data not shown)**.**

Table 3 shows the association between leptin concentration and impairment in physical function among subgroups of participants with better health status. Per each increment of one SD of leptin concentration, we observed a higher incidence of limitations in all the domains, especially in self-reported impaired mobility, with a range of 55-62% of risk increment, followed by a 22-41% for impaired overall physical performance, 21-38% for self-reported impaired agility, and 10-18% for impaired lower extremity function. In stratified analyses, the association between leptin concentration and the impairment of physical function was statistically significant only among individuals with less physical activity, more hours watching TV and higher % body fat (please see **Appendix 2**). Nevertheless, the study association did not significantly vary across the strata (p for interaction >0.08 in all cases).

DISCUSSION

In this prospective study of community-dwelling older adults, higher serum leptin concentration was associated with greater risk of impairment in mobility, agility, lower extremity function and overall physical performance. These associations were observed in groups with varying health behaviors and status and were independent of the estimated body fat, which suggests that the impact of leptin on physical function is not totally explained by adiposity.

Several studies have found that body composition plays a role in the age-associated decline of physical function and the occurrence of frailty in older adults [5,6,7,8,9,10,14]. This could be due to several closely-related mechanisms. First, a sedentary lifestyle may contribute to muscle weakness and

atrophy, with muscle being progressively infiltrated by fat tissue. In turn, impaired physical function may lead to greater sedentariness. Moreover, excess adiposity induces inflammation and metabolic dysfunction, which also contribute to reduce muscle quality. These factors could partially explain the clustering of various body phenotypes, such as obesity and sarcopenia, which leads to sarcopenic obesity [12]. On the other hand, there is evidence that weight loss in older adults predicts disability [33], especially among those who were obese at entering old age [34].

Leptin contributes to energy balance mostly by reducing food intake and increasing energy expenditure, and it also has a role in vascular function and in the regulation of serum glucose and insulin [35,36]. However, in obese and older people, high leptin levels may reflect a state of resistance, in which vascular function and insulin sensitivity are altered. Moreover, given that leptin concentration reflects the metabolic activity of body fat, the mechanisms for the association between leptin and functional limitations could also entail an increased energy demand due to excess body fat and the subsequent metabolic dysfunction. In addition, leptin is a proinflammatory adipokine. Thus, given that higher leptin is associated with higher risk of cardiovascular disease and diabetes [37,38], which are both linked to impaired physical function, we adjusted the analyses for cardiovascular disease and diabetes. The fact that the results held after adjustment, and that they were also observed among individuals free of these diseases, suggests that other mechanisms may account for the leptin-functional impairment association. Moreover, our results also held after adjustment for CRP and HOMA-IR, which were used as a nonspecific proxy of the inflammation process and insulin resistance, respectively. Both conditions have often been related to functional impairment, but our findings suggest that leptin could be a prior step of the causal pathway.

Our results concur with those of Karvonen-Gutierrez et al [19] showing that leptin concentration predicts poorer physical functioning. These are the first investigations to report an association between leptin and physical function, thought their study was conducted among middle-aged women and ours among older men and women. Specifically, in the study of Karvonen-Gutierrez et al [19], leptin was prospectively associated with longer stair climb, sit-to-rise and 2-pound lift times, and shorter reach distance. Contrary to our findings, Karvonen-Gutierrez et al did not observe an association between

11

leptin and worse results in the walking test or leg strength; although leptin has deleterious effects on muscle [39], a lack of an association in their study might be due to including younger individuals. Our results are of particular importance for the older population because they showed an association of leptin with lower extremity performance, which is a good predictor of disability, hospitalization and mortality [22].

Our study has several strengths, including the relatively large sample size and the fact that most variables, including leptin and the components of physical functioning, were ascertained using standardized and validated methods. Also, the analyses were adjusted for a good number of well-measured confounders, and the results were robust in several sensitivity analyses. The main limitation was the lack of measurement of the soluble receptor of leptin, which has shown a stronger relation than leptin with some health outcomes. Also, we did not measure other adipokines of potential interest, such as adiponectin [19]; however, we attempted to partially account for this limitation by adjusting the analyses for our estimation of subjects' body fat, as a proxy for adipokines secretion, although the use of an objective measure of % body fat would have been desirable. We also lacked data about lean mass, which would have allowed us to characterize those individuals with sarcopenic obesity. Another limitation was the use of self-reported information as a proxy for mobility, agility and overall physical performance; however, we combined it with an objective assessment of lower extremity function, to achieve a more complete measurement of impaired physical function. Moreover, functional impairment was evaluated at the end of the follow-up, so that temporality and development of impairments during the interval period could not be fully ascertained. Finally, as in most observational studies, certain residual confounding cannot be ruled out, despite adjustment for many variables.

CONCLUSION

In conclusion, in community-dwelling older men and women, we found a significant association between higher leptin concentration and an increased risk of impaired physical function, which was

independent of the estimated body fat. The mechanisms of this association should be elucidated, but preserving metabolic function during the old age could help to delay physical function decline and subsequent disability.

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CONFLICT OF INTEREST

The authors have nothing to disclose.

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Table 1. Participants' characteristics at baseline according to physical function impairment (N=1,556)

	Self-reported impaired mobility		Self-reported impaired agility		SPPB score ≤ 9 points		10-point decrease in PCS	
	No	Yes	No	Yes	No	Yes	No	Yes
Participants, n	1,361	195	1,239	317	703	853	1,296	260
Leptin, ng/mL	19.6 (15.7)	32.2 (24.1)[c]	18.9 (15.3)	30.1 (22.1)[c]	20.4 (16.7)	25.1 (20.5)[c]	18.1 (14.4)	23.8 (19.2)[c]
Age, y	67.7 (5.8)	70.1 (6.4)[c]	67.6 (5.8)	69.7 (6.4)[c]	66.3 (5.2)	69.4 (6.2)[c]	67.8 (5.8)	68.9 (6.4)[b]
Men, %	55.7	28.7[c]	57.6	31.6[c]	59.7	46.2[c]	53.2	48.1
Primary education, %	47.8	65.1[c]	45.2	68.5[c]	42.1	56.4[c]	48.1	59.2[b]
Current smoker, %	12.5	11.8[b]	12.8	11.0[c]	13.8	11.3	12.7	10.8
Heavy drinker, %	9.3	5.1 [b]	9.2	7.3[a]	11.2	6.8[c]	9.0	7.7
Physical activity, MET-h/wk	23.5 (15.6)	18.8 (13.6)[c]	24.0 (15.8)	18.5 (13.1)[c]	24.8 (15.8)	21.3 (15.0)[c]	23.1 (15.6)	21.7 (14.5)
TV watching, h/wk	16.9 (10.4)	20.2 (11.4)[c]	16.5 (10.1)	20.3 (11.9)[c]	16.3 (10.3)	18.1 (10.9)[b]	17.0 (10.3)	18.8 (12.2)[a]
Trichopoulou index score	4.6 (1.5)	4.3 (1.6)[a]	4.6 (1.5)	4.4 (1.6)[a]	4.7 (1.5)	4.4 (1.5)[c]	4.6 (1.5)	4.5 (1.6)
Energy intake, kcal/d	2,121 (769)	1,892 (538)[c]	2,131 (784)	1,943 (564)[c]	2,165 (826)	2,032 (671)[b]	2,100 (750)	2,054 (735)
Estimated body fat, %	35.7 (6.7)	40.6 (4.5)[c]	35.2 (6.5)	40.7 (7.0)[c]	35.0 (6.4)	37.4 (7.2)[c]	36.1 (6.9)	37.7 (6.9)[b]
Morbidity, %								
Diabetes	13.3	19.0[a]	13.0	18.0[a]	9.5	17.7[c]	13.9	16.6
Hypertension	63.9	66.2	63.8	65.9	61.6	66.4	63.4	68.1
Cardiovascular disease	3.2	7.2[b]	3.2	5.7[a]	3.1	4.1	3.6	4.2
Chronic lung disease	6.3	11.8[b]	5.7	12.3[c]	5.4	8.3[a]	6.2	11.2[b]
Cancer	1.6	2.1	1.5	2.2	1.4	1.9	1.5	2.3
Osteomuscular disease	39.5	71.8[c]	36.5	71.0[c]	36.9	48.9[c]	41.6	53.1[b]
Depression	5.7	12.3[b]	5.0	12.6[c]	4.6	8.2 [b]	5.6	11.5[b]
Incident morbidity during follow-up, %	24.0	36.4[c]	23.6	33.1[b]	21.9	28.5[b]	28.5[b]	35.0[c]
IADL disability, %	6.2	14.9[c]	5.6	13.9[c]	4.8	9.2[b]	6.9	8.9
MMSE score	28.3 (1.9)	27.1 (2.6)[c]	28.3 (1.8)	27.4 (2.5)[c]	28.4 (1.6)	27.9 (2.3)[c]	28.2 (1.9)	27.8 (2.3)[b]
C-reactive protein*, mg/L	0.17 (1.03)	0.21 (1.10)[a]	0.17 (1,03)	0.22 (1.07)[c]	0.16 (1.04)	0.19 (1.04)[b]	0.17 (1.03)	0.20 (1.08)[a]
HOMA-IR	2.3 (0.1)	3.1 (0.3)[c]	2.3 (2.1)	2.9 (2.7)[c]	2.4 (2.3)	2.4 (1.6)	2.3 (2.1)	2.6 (2.3)[a]

PCS: Physical component summary of the SF-12; SPPB: Short physical performance battery; MET; Metabolic equivalent; MMSE: Mini-Mental State Examination.
[a]p<0.05; [b]p<0.01; [c]p<0.001
For continuous variables, the mean (standard deviation) is reported.
*Geometric mean (standard error of the geometric mean).

Table 2. Odds ratios (95% confidence interval) for the association between sex-specific quartiles of serum concentration of leptin[a] and physical function impairment during a 3.5 year follow-up. (N=1,556)

	Leptin concentration				p-trend	Per 1 SD increase of leptin
	Quartile 1 (n=394)	Quartile 2 (n=388)	Quartile 3 (n=391)	Quartile 4 (n=383)		
Self-reported impaired mobility						
Cases	39	37	45	74		
Model 1[b]	1.00	0.98 (0.61-1.60)	1.20 (0.76-1.91)	2.18 (1.42-3.34)	<0.001	1.48 (1.30-1.70)
Model 2[c]	1.00	0.84 (0.50-1.42)	1.07 (0.65-1.76)	1.82 (1.13-2.93)	0.004	1.42 (1.22-1.66)
Model 3[d]	1.00	0.88 (0.51-1.51)	1.12 (0.65-1.92)	1.95 (1.11-3.43)	0.006	1.55 (1.30-1.85)
Model 4[e]	1.00	0.82 (0.47-1.43)	1.01 (0.57-1.80)	1.69 (0.92-3.13)	0.03	1.53 (1.27-1.85)
Self-reported impaired agility						
Cases	53	69	79	116		
Model 1[b]	1.00	1.45 (0.97-2.16)	1.68 (1.14-2.49)	2.87 (1.97-4.18)	<0.001	1.44 (1.28-1.62)
Model 2[c]	1.00	1.32 (0.85-2.04)	1.57 (1.02-2.40)	2.36 (1.55-3.59)	<0.001	1.32 (1.16-1.61)
Model 3[d]	1.00	1.14 (0.73-1.80)	1.28 (0.80-2.02)	1.76 (1.08-2.87)	0.02	1.21 (1.04-1.42)
Model 4[e]	1.00	1.14 (0.71-1.81)	1.24 (0.76-2.03)	1.62 (0.95-2.76)	0.06	1.17 (1.00-1.39)
SPPB score ≤ 9 points						
Cases	201	202	204	246		
Model 1[b]	1.00	1.08 (0.80-1.46)	1.03 (0.77-1.38)	1.67 (1.24-2.26)	0.001	1.22 (1.09-1.27)
Model 2[c]	1.00	1.02 (0.75-1.38)	0.95 (0.69-1.27)	1.36 (0.99-1.27)	0.05	1.13 (1.01-1.27)
Model 3[d]	1.00	1.05 (0.77-1.44)	1.00 (0.71-1.38)	1.48 (1.02-2.15)	0.04	1.18 (1.03-1.35)
Model 4[e]	1.00	1.04 (0.75-1.43)	1.00 (0.70-1.43)	1.48 (0.98-2.23)	0.11	1.18 (1.02-1.38)
10-point decrease in PCS[e]						
Cases	46	66	68	80		
Model 1[b]	1.00	1.63 (1.08-2.46)	1.71 (1.13-2.57)	2.18 (1.46-3.26)	0.001	1.29 (1.14-1.46)
Model 2[c]	1.00	1.51 (0.98-2.32)	1.62 (1.06-2.48)	1.92 (1.25-2.94)	0.004	1.23 (1.08-1.41)
Model 3[d]	1.00	1.53 (0.98-2.37)	1.64 (1.04-2.57)	1.97 (1.20-3.22)	0.01	1.25 (1.07-1.46)
Model 4[e]	1.00	1.68 (1.06-2.64)	1.82 (1.12-2.94)	2.12 (1.24-3.62)	0.02	1.25 (1.06-1.48)

PCS: Physical component summary of the SF-12; SPPB: Short physical performance battery
[a]Sex-specific quartile cut-off points for leptin levels were 5.5, 9.8 and 16.6 ng/mL in men and 18.0, 29.1 and 39.5 ng/mL in women.
[b]Model 1: logistic regression model adjusted for sex and age (years).

[c]Model 2: model 1 additionally adjusted for educational level (≤primary, secondary, university), smoking behavior (never, former, current), alcohol consumption (none, moderate, heavy drinker), leisure-time physical activity (quartiles of MET-h/wk), TV watching (tertiles of h/d), Mediterranean diet score (tertiles), energy intake (quartiles of Kcal/d), diabetes, hypertension, cardiovascular disease, cancer, chronic lung disease, osteomuscular disease, depression, incident chronic disease during follow-up, and MMSE score (quartiles).

[d]Model 3: model 2 additionally adjusted for % body fat (quartiles).

[e]Model 4: model 3 additionally adjusted for C-reactive protein (quartiles) and HOMA-IR (quartiles).

[e]Additionally adjusted for basal PCS.

Table 3. Odds ratios (95% confidence interval)[a] of physical function impairment during a 3.5 year follow-up per 1 SD increase of serum concentration of leptin among subgroups of participants with better health status.

	Self-reported impaired mobility	Self-reported impaired agility	SPPB score ≤ 9 points	10-point decrease in PCS[b]
No basal diabetes (n=1,338)				
Cases	158	260	222	702
OR (95 % CI)	1.55 (1.27-1.89)	1.27 (1.07-1.51)	1.24 (1.04-1.47)	1.18 (1.02-1.37)
No basal osteomuscular disease (n=879)				
Cases	55	92	122	436
OR (95 % CI)	1.62 (1.20-2.21)	1.38 (1.06-1.78)	1.41 (1.11-1.78)	1.10 (0.92-1.32)
No basal IADL disability (n=1,443)				
Cases	166	273	237	774
Model 2	1.56 (1.29-1.89)	1.23 (1.05-1.45)	1.22 (1.04-1.44)	1.16 (1.01-1.34)
No incident cognitive impairment (n=1,524)				
Cases	183	299	249	825
OR (95 % CI)	1.55 (1.29-1.85)	1.21 (1.03-1.42)	1.23 (1.04-1.43)	1.18 (1.03-1.36)
No incident morbidity (n=1,159)				
Cases	124	212	169	610
OR (95 % CI)	1.60 (1.26-2.03)	1.21 (1.00-1.47)	1.24 (1.01-1.52)	1.17 (1.01-1.38)

IADL: Instrumental activities of daily living; PCS: Physical component summary of the SF-12; SPPB: Short physical performance battery. OR: odds ratio; CI: Confidence interval.
[a]Analyses adjusted as model 2 in table 2.
[b]Additionally adjusted for basal PCS.

Supplementary data. Appendix 1

Data analysis

The first regression model was adjusted for sex and age (years). The second model was additionally adjusted for educational level (primary or less, secondary or university), smoking (never, former or current), alcohol consumption (abstainer, moderate, or heavy drinker, with the threshold between moderate and heavy drinking established as 20 g/d in men and 10 g/d in women), TV watching (tertiles of h/d), leisure physical activity (quartiles of METs-h/week), adherence to Mediterranean diet (tertiles of the score), energy intake (quartiles of kcal/day), hypertension, diabetes, cardiovascular disease, chronic obstructive lung disease, cancer, osteomuscular disease, depression, incident morbidity between 2008 and 2012, and cognitive function (quartiles of MMSE score). The third model was additionally adjusted for the estimation of % body fat (quartiles), as a proxy for the concentration of adipokines other than leptin, and a fourth model was also adjusted for HOMA-IR and CRP (modeled in quartiles) to assess if these biomarkers could partially explain the association between leptin and functional impairment. We additionally included the length of follow-up as a covariate in a fifth model. Moreover, analyses regarding changes in PCS score were also adjusted for the basal PCS. To investigate the linear dose-response relationship, we estimated the p for trend by modeling leptin as a continuous variable.

Additionally, we performed some sensitivity analyses by excluding participants with diabetes, osteomuscular disease, IADL limitation, and cognitive impairment at baseline or with incident morbidity during the follow-up. Moreover, because the physiology of leptin may differ according to body adiposity and physical activity [**16**], we conducted analyses stratified by the median of % body fat, METs-h/d in leisure activity and hours of TV watching. To assess if the study association varied across the strata we built interaction terms as the product of leptin quartiles by the stratification variables, and then used the likelihood ratio test to compare models with and without interaction terms.

Supplementary data. Appendix 2

Table Appendix 2. Odds ratios (95% confidence interval)[a] of physical function impairment during a 3.5 year follow-up per 1 SD increase of serum concentration of leptin, stratified by physical activity, time spent watching TV and % body fat.

	Self-reported impaired mobility	Self-reported impaired agility	SPPB score ≤ 9 points	10-point decrease in PCS[b]
Physical activity ≥ median (n=766)				
Cases	74	124	122	384
OR (95 % CI)	1.37 (1.02-1.86)	1.05 (0.81-1.34)	1.13 (0.89-1.46)	1.27 (1.02-1.58)
Physical activity < median (n=790)				
Cases	121	193	138	469
OR (95 % CI)	1.78 (1.40-2.27)	1.32 (1.07-1.64)	1.35 (1.09- 1.69)	1.12 (0.93-1.35)
P-interaction	0.15	0.48	0.84	0.25
TV watching < median (n=850)				
Cases	86	141	134	427
OR (95 % CI)	1.46 (1.10-194)	1.24 (0.97-1.59)	1.21 (0.96-1.53)	1.08 (0.90-1.30)
TV watching ≥ median (n=706)				
Cases	109	176	126	426
OR (95 % CI)	1.55 (1.23-2.01)	1.24 (1.01-1.53)	1.29 (1.03-1.61)	1.32 (1.06-1.63)
P-interaction	0.59	0.75	0.12	0.75
% Body fat < median (n=778)				
Cases	58	91	116	371
OR (95 % CI)	1.35 (0.91-2.02)	1.17 (0.85-1.62)	1.25 (0.95-1.65)	1.05 (0.85-1.30)
% Body fat ≥ median (n=778)				
Cases	137	226	144	482
OR (95 % CI)	1.58 (1.28-1.96)	1.22 (1.01-1.47)	1.26 (1.03-1.53)	1.25 (1.03-1.51)
P-interaction	0.65	0.71	0.78	0.08

PCS: Physical component summary of SF-12; SPPB: short physical performance battery; OR: Odds ratio; CI: Confidence interval.

[a] Analyses adjusted as model 2 in table 2

[b] Additionally adjusted for basal PCS.