Obestatin is associated to muscle strength, functional capacity and cognitive status in old women

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Abstract Obestatin has been proposed to have anorexic and anti-ghrelin actions. The objective was to study obestatin concentrations in relation to handgrip strength, functional capacity and cognitive state in old women. The prospective study included 110 women (age, 76.93 ± 6.32) from the Mataró Ageing Study. Individuals were characterized by anthropometric variables, grip strength, Barthel and assessment of cognitive

impairment [Mini Cognoscitive Examination (MCE) Spanish version], depressive status by the Geriatric Depression Scale (GDS) and frailty by the Fried criteria. Obestatin was measured by IRMA. Obestatin showed negative correlation to handgrip at basal time point ($r = -0.220$, $p = 0.023$) and at 2-year follow-up ($r = -0.344$, $p = 0.002$). Obestatin, divided into quartiles, showed a negative lineal association with handgrip: 11.03 ± 4.88 kg in first, 8.75 ± 4.08 kg in second, 8.11 ± 3.66 kg in third and 7.61 ± 4.08 kg in fourth quartile ($p = 0.018$). Higher obestatin levels were associated to increased weakness (categorized by handgrip of frailty criteria): 2.24 ± 0.42 ng/ml in weak vs. 1.87 ± 0.57 ng/ml in non-weak ($p = 0.01$). The decrease of either MCE or Barthel scores at 2-year follow-up was significantly higher in individuals in the fourth quartile of obestatin in comparison with individuals in the first quartile ($p = 0.046$ and $p = 0.019$, respectively). No association was found between obestatin and GDS score and neither with frailty as a condition. Obestatin is associated to low muscle strength, and impaired functional and cognitive capacity in old women participating in the Mataró Ageing Study.

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Introduction

Obestatin (Zhang et al. 2005) is a gastrointestinal hormone which originates from the same precursor

as ghrelin (Kojima et al. 1999), the preproghrelin (Gualillo et al. 2006). Ghrelin is a well-known hormone with two major functions: to enhance the release of GH and to stimulate the hypothalamic ‘hunger centre’ (Rosicka et al. 2002; Wren et al. 2001). While ghrelin is mostly an orexigenic hormone which promotes appetite and increases food intake in humans (Nakahara et al. 2008; Wren et al. 2001), and activates gastrointestinal motility and gastric emptying, obestatin is believed to have the opposite effect, reducing food intake and gastric emptying and preventing body weight gain (Zhang et al. 2005). The role of obestatin has not been fully confirmed in humans, and some of its effects show no universal reproducibility (Nogueiras et al. 2007). Ghrelin gene products may be implicated in a complementary manner in energetic homeostasis and body weight control in which obestatin would play its role as an indirect modulator of the orexigenic action of ghrelin, although final demonstration of such relationship is still necessary.

GH and IGF-I decline is part of the ageing process and is probably etiologically involved in the changes in metabolism, body composition and functional capacity observed in old people (Ceda et al. 2005; Puig-Domingo et al. 2008). Moreover, a decline in plasma ghrelin with age has also been reported (Serra-Prat et al. 2007), and several groups, including ours, have investigated the role of ghrelin in the elderly in relation to metabolic syndrome (MS), hunger and satiety (Rigamonti et al. 2002; Serra-Prat et al. 2009a, b). Ghrelin circulating concentrations decline in old people may contribute to the phenomenon called ‘anorexia of ageing’ and influence nutritional impairment development. In addition, an association between ghrelin and its decline with ageing, muscle strength and functional capacity, probably mediated by GH–IGF-I axis, has also been described (Serra-Prat et al. 2010). However, no information is available concerning the role of obestatin in muscle strength and functional capacity in the elderly.

Ghrelin has also an extra-hypothalamic role, promoting learning and memory by activating plasticity in the hippocampus, enhancing reward and motivation and having a neuroprotective role (Andrews 2011). Interestingly, obestatin has also been observed to improve memory in rats (Carlini et al. 2007); however, little information is available related to the role it plays in memory and ageing in humans.

As previous studies have demonstrated that development of frailty over time is higher in women (Mora et al. 2012b), we were interested in studying the role of obestatin in women’s ageing. The purpose of the present study was to investigate if obestatin circulating concentrations are related to ageing phenotype in a cohort of non-institutionalized old women at baseline and after 2-year follow-up period.

Subjects and methods

Study population

All subjects of the present study were participating in the Mataró Ageing Study, a population-based prospective cohort study designed to identify risk factors for frailty and successful ageing condition among old people. The study group was selected from the inhabitants of Mataró and Argentona (Barcelona, Spain), and has previously been described elsewhere (Puig-Domingo et al. 2008). Among the 313 individuals who participated in the Mataró Ageing Study, 160 were women. One hundred and ten women from this cohort, in which all data collection and biological samples for obestatin were available, were finally included. The Ethic Committee of the Consorci Sanitari de Maresme approved the study protocol, and all subjects signed an informed consent before entering.

Data collection

A geriatric assessment was performed by a specialized team composed of ten general practitioners specifically trained in evaluating the aged condition. This assessment was carried out in two Primary Health Care centres, and it comprised of a physical examination and questionnaires to evaluate cognitive, nutritional, depression status and functional capacity as per the study protocol.

According to the study protocol, a review of the electronic medical history of the individual and/or a first-time evaluation in case of no previous registration at the Consorci Sanitari de Maresme medical history database was done at the start of the study. Chronic and previous diseases, life style factors, education and physical examination were recorded. Blood samples were collected, biochemical and hormonal

determinations were performed and results were recorded in the database.

Grip strength was measured at the non-dominant hand using a handheld dynamometer (Jamar model). Guralnik index and the unipodal balance test (good balance was considered when participants could stand on one foot for 5 s or more) were performed to assess functional capacity. Functional capacity was also evaluated by determining Barthel score (Mahoney and Barthel 1965) and considered optimal when score = 100. The Spanish version of the Mini Mental State Examination [Mini Cognoscitive Examination (MCE) Spanish version] was used to assess cognitive function; subjects with ≥ 24 points were considered as having no deterioration of the cognitive function (Lobo et al. 1999). Depressive status was evaluated performing the Spanish version of the Geriatric Depression Scale (GDS; Fernandez-San et al. 2002). Frailty was evaluated by using the Fried criteria (Fried et al. 2001) and according to this definition; frailty was present if three or more of the following conditions were present: unintentional weight loss (4.5 kg in the past year), self-reported exhaustion, weakness (by grip strength measurement), slow walking speed and low physical activity.

Hormonal measurements

Blood samples for all measurements were obtained after a 12-h night time fasting period. Glucose and lipids were analysed by enzymatic techniques. Serum was obtained after centrifugation and stored in aliquots at -70°C until assayed. Serum obestatin was measured by a commercially available enzyme immunoassay (YK231 Human Obestatin EIA, Yanaihara Institute, Fujinomiya-SHI, Shizuoka, Japan). The intra- and inter-assay coefficients of variation were 2.62 % and 7.3 %, respectively. The detection limit of the assay was 0.231 ng/mL. As ghrelin, IGF-I and 25-hydroxy-vitamin D have shown to be related to obestatin in previous studies, these were also measured in the present investigation and used if necessary as confounders for multivariate analysis. Total plasma ghrelin concentrations were measured with a human radioimmunoassay (RIA) kit (Linco Research Inc., St. Charles, MO, USA). The detection limit was 93 pg/mL with intra- and inter-assay variation coefficients of 11.1 % and 14.7 %, respectively. Total IGF-I was measured by using a two-side immunoradiometric

assay [Immunotech IGF-I kit, Immunotech-Beckman, Marseille, France; intra-assay variation coefficients (CV), <6.3 %; inter-assay CV, 6.8 %; sensitivity, 30 ng/mL]. Plasma 25-hydroxy-vitamin D concentration was measured using the LIAISON 25 OH vitamin D total assay (DiaSorin Inc., Stillwater, MN, USA), a competitive chemiluminescent immunoassay with the LIAISON DiaSorin automated analyzer. Intra-assay and inter-assay coefficients of variation (CV) were <6.3 % and 9.1 %, respectively, and the assay sensitivity was 4 ng/mL.

Data analysis

Categorical variables were expressed as percentages, and continuous data as mean (standard deviation). Kolmogorov–Smirnov test was used to assess normality of continuous variables. To compare means, *t* Student's test, Mann–Whitney *U* or ANOVA was used. Association of continuous variables was evaluated by using Pearson or Spearman correlations; multiple regressions and lineal regression analysis were used to adjust the variables when correlation was significant in the univariate analysis. Categorical variables were studied using Chi-square test. We considered statistical significance for association with a *p* value <0.05.

Results

Subject characteristics and obestatin hormonal relationships

One hundred and ten women with an age ranging from 69.41 to 101.14 were evaluated at basal time point. At 2-year follow-up, four women had died, and the other 20 were not available for evaluation (3 were not found, 11 did not accept to participate in the follow-up and 6 had logistical problems to attend the research site). Characteristics of individuals at first visit and at follow-up are described in Table 1. Forty-eight per cent of women were illiterate, 50.5 % were married and 43 % were widowed; 28 % lived alone, 40.2 % with their husbands, 11.2 % with their husband and offspring, and 16.8 % only with their offspring; 49.5 % presented with some visual impairment and 32.7 % with some deafness; and 93.5 % had never smoked. In our sample of women of the Mataró

Ageing Study, mean obestatin levels were 1.93 ± 0.57 ng/ml (0.49–3.77); mean IGF-I, 100.61 ± 31.04 ng/ml (36–193); mean ghrelin, $1,041 \pm 399.62$ pg/ml (465–2,477) and mean 25-hydroxy-vitamin D, 16.51 ± 8.02 ng/ml (5.30–41.70). Obestatin was positively correlated to ghrelin levels ($r=0.220$, $p=0.022$), and IGF-I was positively associated to ghrelin ($r=0.272$, $p=0.004$); however, no association was found between obestatin and IGF-I ($r=0.025$, $p=0.797$). No association was found between IGF-I, obestatin or ghrelin levels and age. 25-Hydroxy-vitamin was correlated to age ($r=-0.345$, $p<0.001$) but not with ghrelin, obestatin and IGF-I.

Muscle strength and obestatin

Table 1 shows the changes of muscle strength measurements over time. Handgrip was correlated to IGF-I ($r=0.189$, $p=0.051$) and age ($r=-0.350$, $p<0.001$), but no association was found with vitamin D levels. Obestatin was negatively correlated to handgrip at first evaluation ($r=-0.220$, $p=0.023$). As a univariate

association was found between handgrip and age, and handgrip with IGF-I, we used multiple regression to adjust obestatin association with handgrip. After adjusting for age and IGF-I, obestatin remained significantly associated to handgrip ($\beta=-0.263$, $p=0.003$, $R^2=0.237$). We also found an association between handgrip at 2 years follow-up and obestatin ($r=-0.344$, $p=0.002$; after adjustment, $\beta=-0.352$, $p=0.001$, $R^2=0.232$; Fig. 1). When obestatin was divided into quartiles (first quartile, <1.49 ng/ml; second quartile, 1.49 – 1.91 ng/ml; third quartile, 1.91 – 2.98 ng/ml and fourth quartile, ≥ 2.98 ng/ml), a statistically significant negative lineal association was observed in relation to handgrip: 11.03 ± 4.88 kg in first quartile, 8.75 ± 4.08 kg in second quartile, 8.11 ± 3.66 kg in third quartile and 7.61 ± 4.08 kg in fourth quartile ($p=0.018$), being the difference between first and fourth quartile also significant ($p=0.007$, $p=0.003$ after adjusting for age and IGF-I, $R^2=0.236$; Fig. 2a). Similar results were found in relation to handgrip at 2 years follow-up: 10.92 ± 3.86 kg in first quartile, 9.56 ± 3.62 kg in second quartile, 7.07 ± 3.25 kg in

Table 1 Characteristics of sample at first and second visit

	First visit (basal), <i>n</i> =110	First visit (basal), <i>n</i> =86 (with follow-up)	Second visit (follow-up), <i>n</i> =86	<i>p</i>
Age	76.93 $\pm$ 6.32	76.97 $\pm$ 5.92	79.21 $\pm$ 5.91	<0.001
Co-morbidities				
Depression	31.8 %	29.3 %	31.4 %	0.924
Cancer	12.1 %	12.9 %	11.6 %	0.908
Arthrosis	72 %	71.8 %	82.6 %	0.116
Chronic lung disease	11.2 %	10.6 %	10.5 %	0.940
Asthma	8.4 %	5.9 %	7 %	0.925
Diabetes	27.1 %	22.4 %	24.4 %	0.792
Hypertension	56.1 %	54.1 %	65.1 %	0.259
Stroke	15 %	12.9 %	11.6 %	0.631
Ischaemic heart disease	11.2 %	9.4 %	15.1 %	0.553
Falls (last 12 months)	30.8 %	31.8 %	37.2 %	0.430
Hours walking outdoor	2.16 $\pm$ 7.13	2.06 $\pm$ 7.33	0.66 $\pm$ 0.74	0.090
Guralnik	8.44 $\pm$ 2.11	8.54 $\pm$ 2.10	7.26 $\pm$ 5.91	<0.001
Barthel	95.90 $\pm$ 5.22	96.43 $\pm$ 5.00	94.24 $\pm$ 7.34	<0.001
GDS	1.57 $\pm$ 1.37	1.54 $\pm$ 1.44	1.29 $\pm$ 1.38	0.105
MCE	28.39 $\pm$ 6.05	28.95 $\pm$ 5.53	27.80 $\pm$ 5.34	0.001
Handgrip (kg)	9.55 $\pm$ 4.46	9.12 $\pm$ 4.54	8.69 $\pm$ 3.94	0.028
Strength in left leg 120° (kg)	94.60 $\pm$ 39.01	94.10 $\pm$ 41.58	91.11 $\pm$ 32.83	0.550
Strength in right leg 120° (kg)	95.92 $\pm$ 35.08	94.64 $\pm$ 36.51	104.34 $\pm$ 36.47	0.029

Bolded values mean they have statistical significance

Fig. 1 Correlation between obestatin (nanograms per millilitre) and handgrip at basal time (**a**) and handgrip at follow-up (**b**). After adjusting for age and IGF-I, obestatin remained significantly associated to handgrip both at basal

time ($\beta=-0.263$, $p=0.003$, $R^2=0.237$) and at 2 years follow-up and obestatin ($r=-0.344$, $p=0.002$; after adjustment, $\beta=-0.352$, $p=0.001$, $R^2=0.232$)

third quartile and 7.45 ± 3.86 kg in fourth quartile, being the difference between first and fourth quartile significant ($p=0.006$, $p=0.010$ after adjusting for age and IGF-I, $R^2=0.192$; Fig. 2b).

Higher obestatin levels were associated to pathological handgrip according to frailty criteria: 2.24 ± 0.42 ng/ml in weak handgrip vs. 1.87 ± 0.57 ng/ml in non-weak ($p=0.01$). No significant differences in obestatin levels were observed in relation to the presence of frailty either at first evaluation or at follow-up (2.24 ± 0.71 ng/ml in frail vs. 1.94 ± 0.60 ng/ml in non-frail, and at 2 years, 2.03 ± 0.52 ng/ml in frail vs. 1.93 ± 0.66 ng/ml in non-frail). Pathological handgrip was

associated to obestatin [47.4 % in fourth quartile vs. 0 % in first quartile, $p=0.022$; lineal association, $p=0.004$; OR, 9.16 (2.32–36.18; $p=0.002$) between fourth vs. first after adjusting for age and IGF-I, $R^2=0.191$]. Obestatin concentrations at basal time point were not associated to loss of handgrip over time ($r=-0.010$, $p=0.935$).

Functional status and frailty

Table 1 shows the evolution of functional capacity measurements over time. No association was found between baseline obestatin concentrations and absolute

Fig. 2 Association between obestatin in quartiles and handgrip at basal time (**a**) and handgrip at follow-up (**b**)

values of MCE, Barthel, Guralnik, unipodal balance test or GDS and obestatin, either at the beginning or at follow-up. Absolute and relative decreases in MCE were significantly higher in individuals in the fourth quartile of obestatin in comparison with individuals in the first quartile (relative decrease, 7.25 ± 12.03 % in fourth quartile vs. -0.09 ± 10.48 % in first quartile; $p = 0.046$). Moreover, absolute and relative decreases in Barthel score were also significantly higher in individuals in the fourth quartile of obestatin in comparison with individuals in the first quartile (relative decrease, 3.77 ± 5.77 % in fourth quartile vs. 0.46 ± 3.19 % in first quartile; $p = 0.019$). Absolute or relative modifications of Guralnik, unipodal balance test and GDS values were not related to obestatin concentrations.

In relation to frailty according to Fried criteria, 0.9 % fulfilled the first criteria (weight loss), 17.9 % the second criteria (exhaustion), 17.8 % had pathological handgrip strength, 28 % had low gait speed and 36.8 % had poor physical activity. Thus, 40 % of women had not one single frailty condition, 25.5 % had one, 27.3 % had two and 7.3 % had three; therefore, 7.3 % of women were frail according to Fried criteria at initial evaluation, while 34.5 % were frail at 2 years of follow-up. Frailty development according to Fried criteria was not associated to obestatin, while it was with basal IGF-I concentrations (89.07 ± 30.6 ng/ml in frail developers vs. 113.18 ± 30.21 ng/ml in non-frail developers; $p = 0.01$).

Discussion

Obestatin, one of the two main products of the ghrelin gene, has been discovered and begun to be studied after a decade of investigation mostly devoted to ghrelin (Gualillo et al. 2006). Both in human health and disease states, as well as in animal models, obestatin seems to exert different effects in comparison to ghrelin. Its participation in the regulation of energetic homeostasis in humans has been suggested, and initial data pointed towards the idea that obestatin was driving an anorexigenic message in contraposition to the orexigenic effect of ghrelin. However, various recent studies have shown conflictive results regarding this (Nakahara et al. 2008; Nogueiras et al. 2007; Zhang et al. 2005; Zizzari et al. 2007). In a recent study, we found that obestatin was not associated to weight loss or diminished appetite or decreased eating

behaviour frequently observed in the ageing phenomenon and referred to as anorexia of aged people (Mora et al. 2012a). As ghrelin has important interactions with the somatotrophic axis, it could be interesting to explore whether these pro-somatotropic and therefore anabolic effects of ghrelin may be counteracted or influenced by obestatin. Recent studies have shown that obestatin does modulate ghrelin actions and thus reversing its excitatory effects on hypothalamic GHRH neurons (Feng et al. 2011). As there are few predictive biomarkers of frailty development or loss of function whilst ageing, in the present work, we aimed to investigate if obestatin was associated to components of frailty condition or was able to predict frailty as a geriatric syndrome in the group of women participating in the Mataró Ageing Study. We found that muscle strength was diminished in those individuals with higher circulating obestatin at initial evaluation and at 2 years follow-up, independently of IGF-I circulating levels. No individuals with decreased handgrip strength or other components of frailty condition had obestatin levels in the first quartile. Moreover, impaired evolution of either mental state or functional capacity assessed by MCE and Barthel scores were observed more frequently at follow-up in individuals in the fourth quartile of obestatin. The overall data of the present study indicate that high obestatin may be considered as a biological marker of loss of health status over time in women older than 75 years, at least in our population, and among other biomarkers. Ghrelin has been considered as an anabolic hormone for its ability to promote the activation of somatotrophic axis activity and to stimulate orexigenic behaviour. These actions are thought to be performed by the so-called active form of the ghrelin molecule, acyl ghrelin, which is the only ghrelin isoform able to bind the GHSR1a receptor, while des-acyl ghrelin, the much more abundant ghrelin circulating isoform, is not able to bind this receptor. By virtue of these effects, we previously showed that individuals with higher circulating ghrelin had a better handgrip while ageing (Serra-Prat et al. 2010). However, the mechanisms by which ghrelin may influence muscle function may not only be related to an indirect endocrine action GH-mediated. It has recently been described that ghrelin, either acyl and des-acyl ghrelin, may act at the muscle level through direct mechanisms not involving GHSR1a receptor which is not present in myocytes (Baldanzi et al. 2002; Filigheddu et al. 2007;

Gnanapavan et al. 2002; Sheriff et al. 2012). Filigheddu et al. (2007) have shown that both ghrelin and des-acyl ghrelin stimulate tyrosine phosphorylation of several muscle proteins and activate ERK-1/2 and Akt, indicating that both ghrelin isoforms could exert a biological activity on muscle cells. These results strongly suggest that these actions at the muscle level are mediated by a yet unidentified receptor, common to both acylated and unacylated peptide and distinct from GHSR1a and reinforces similar evidence for common receptors for ghrelin isoforms previously reported in several cells, including a cardiomyocyte-derived cell line (Baldanzi et al. 2002).

All these data may explain that individuals with higher circulating ghrelin levels may have better muscle functional capacity reflected by a better muscle strength. Interestingly, we found that, conversely, individuals with high levels of obestatin were those that performed worse in terms of muscle strength, thus pointing towards the proposed opposite actions of obestatin in relation to ghrelin, which at least seems to be confirmed with respect to muscle strength. Whether obestatin has its own mechanisms of direct action upon muscle cells or acts through modulating ghrelin effects at the myocyte level is yet to be established. The latter may be probably more plausible as all data published on obestatin physiology seems to indicate that obestatin is more a ghrelin regulator rather than a peptide with true hormonal properties (Garg 2007), although a potential interaction with this yet unidentified ghrelin receptor cannot be completely discarded.

This detrimental action of obestatin on muscle function expressed as reduced muscle strength in individuals with highest obestatin quartile is extended to other relevant functions whilst ageing, as exemplified by the findings of the present study of impaired mental and functional capacity over time in those particular subjects bearing obestatin in the upper range. Little is known about the role of obestatin in cognition, as opposed to ghrelin where there is more information available (Frago et al. 2011). Experimental studies in rats have demonstrated the neuroprotective role of ghrelin (Carlini et al. 2002, 2004), as well as of obestatin (Carlini et al. 2007). In humans, little data is available in relation to the influence of both ghrelin and obestatin in cognitive function. The only study

published so far in the elderly showed a negative correlation between ghrelin and cognitive domains, just the opposite of what was found in experimental studies (Spitznagel et al. 2010). We have obtained similar results in relation to obestatin, as women in the highest quartile of obestatin showed a higher loss in MCE.

Why these findings are not seen in younger individuals warrant further studies to clarify this opposed behaviour of the ghrelin gene products. A coherent explanation would be that loss of ghrelin action in old people over time would lead to a net increase in the negative modulation of obestatin on ghrelin effects; however, this possibility, as well as others, remains very speculative at present. Finally, although a causal participation of the ghrelin/obestatin binomium in the development of frailty condition while ageing is an attractive possibility, all these findings may just correspond to epiphenomena of the ageing process itself.

Other biological markers associated to deterioration of health, probably even more potent than ghrelin/obestatin, have also been confirmed in our study; specifically, low IGF-I identified quite robustly subjects at risk of functional and mental impairment, as well as those with a pathologic handgrip at follow-up.

The strengths of our study are that the sample is a homogeneous one obtained from a specific geographical area with a common cultural background that warrants a similar lifestyle and evaluated in a prospective manner. In addition, the phenotypic characterization of the subjects is quite extensive. However, our study also has several limitations. First, although it is a population-based study where non-institutionalized individuals were selected, the inclusion of a younger sample of subjects would have overcome the problem of the overrepresentation of certain phenotypes due to survival bias. The number of subjects included was also restricted to the recruitment possibilities in the specific geographical areas involved in the study; a higher number of participants would have allowed a stronger statistical power. Moreover, these results are only referred to women, so that conclusion cannot be extrapolated to men. Additionally, the measurement of acylated ghrelin rather than total ghrelin, as well as amidated obestatin instead of total obestatin, would have helped a more in-depth explanation of potential relationships not found with our current methodology.

In summary, we found that obestatin is associated to low muscle strength and impaired functional and

cognitive capacity in old women participating in the Mataró Ageing Study. The mechanistic explanation of this association and the apparent opposite actions of these two peptides derived from the ghrelin gene require further in-depth studies for its characterization.

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Authors' contributions

M. Mora analysed the data, wrote the paper and had primary responsibility for final content. M.L. Granada conducted research and provided essential materials. E. Palomera analysed the data. M. Serra-Prat designed and conducted research. M. Puig-Domingo designed and conducted research, wrote the paper and had primary responsibility for final content.