Sarcopenic obesity in free-living older adults detected by the ESPEN-EASO consensus diagnostic algorithm: Validation in an Italian cohort and predictive value of insulin resistance and altered plasma ghrelin profile

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ABSTRACT

Aging and obesity are synergistic sarcopenia risk factors (RF). Their association in sarcopenic obesity (SO) enhances morbidity and mortality, but consensus on SO diagnostic criteria is limited. ESPEN and EASO issued a consensus algorithm for SO screening (obesity and clinical SO suspicion) and diagnosis [low muscle strength by hand-grip (HGS) and low muscle mass by BIA], and we investigated its implementation in older adults (>65-years), as well as SO-associated metabolic RF [insulin resistance (IR: HOMA) and plasma acylated (AG) and unacylated (UnAG) ghrelin, with predictive value also assessed from 5-year-prior observations]. Older adults with obesity from the Italian MoMa study on metabolic syndrome in primary care (n = 76) were studied. 7 of 61 individuals with positive screening had SO (SO+; 9 % of cohort). No individuals with negative screening had SO. SO+ had higher IR, AG and plasma AG/UnAG ratio ($p < 0.05$ vs negative screening and SO-), and both IR and ghrelin profile predicted 5-year SO risk independent of age, sex and BMI. The current results provide the first ESPEN-EASO algorithm-based investigation of SO in free-living older adults, with 9 % prevalence in those with obesity and 100 % algorithm sensitivity, and they support IR and plasma ghrelin profile as SO risk factors in this setting.

1. Introduction

Although excess body fat is its defining derangement [1], obesity is often associated with metabolic and nutritional alterations that impair the ability to preserve skeletal muscle mass and function, thereby leading to altered body composition and a sarcopenic obesity phenotype [2–4].

Co-existence of low skeletal muscle mass and function in persons with obesity is a strong risk factor for complications and adverse clinical outcomes [5–10]. Despite its increasingly recognized clinical relevance, awareness of sarcopenic obesity remains limited, and its screening and diagnosis are not part of routine obesity management. A major reason for low awareness has been lack of consensus on how to define and diagnose sarcopenic obesity in clinical research and clinical practice [7,11]. To address this unmet clinical need, the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity (EASO) have recently promoted an international initiative resulting in a sarcopenic obesity screening, diagnostic and staging algorithm [12], with screening based on body mass index (BMI) or waist circumference (WC) and clinical history, whereas skeletal muscle function and mass can be assessed for diagnosis through simple methods also potentially available in non-specialized outpatient clinics [12].

While loss of muscle mass and function in obesity may occur at any age, particularly in the presence of comorbidities [5,8,10,13], older age is an additional strong and independent risk factor for sarcopenia and negative outcomes [14–16]. In the current study, we therefore implemented the ESPEN-EASO algorithm in a cohort of older adults (age > 65

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years) from the epidemiological North-East Italy MoMa study carried out in a primary care setting [17–19]. The goals of the study were: 1) to test the applicability and sensitivity and specificity of the ESPEN-EASO algorithm, and thereby identify the algorithm-based prevalence of sarcopenic obesity (SO) in free-living older adults; 2) to identify further potential metabolic risk factors for SO, and their potential predictive value from 5 year-prior observations. Since unacylated ghrelin has been recently identified as a potential regulator of insulin sensitivity and skeletal muscle mass [18,20–24], and its plasma concentration may be low in persons with obesity [25,26], plasma ghrelin profile was also investigated.

2. Methods

2.1. Patients and ESPEN-EASO algorithm

Patients were recruited in the context of the MoMa epidemiological study, performed by researchers from the University of Trieste in collaboration with local Primary Care (PC) physicians in the PC outpatient clinic in the municipalities of Montereale Valcellina and Maniago, Pordenone, in the Friuli-Venezia Giulia Region in North-East Italy, by random selection from the general population public registries (age 18–70 years) [17–19]. The competent Pordenone Hospital Ethics Committee and Regional Ethics Committee approved the study protocol (prot. 41121/DS June 19, 2006 and 2016-Os-078-UnivTS), and each subject gave written informed consent to participate before recruitment. The protocol included collection of clinical history, medical examination and blood samples for biochemical and hormonal measurements [17–19]. Five years after initial visit, a follow-up evaluation with identical data collection and added measurement of handgrip strength and body composition by bioelectrical impedance analysis (BIA) was performed in a randomly selected representative subgroup (Supplementary Fig. 1 and Supplementary Table 1). The ESPEN-EASO algorithm for screening and diagnosis of SO in clinical practice was implemented in a subgroup of 76 individuals including all older adults in the follow-up cohort matching inclusion criteria (age $>$ 65 and obesity, defined as BMI $\geq$ 30 kg/m^2 or WC $\geq$102 cm or $\geq$88 cm for males and females, respectively) (Supplementary Fig. 1).

According to the algorithm, an initial screening step is followed by diagnostic procedures in patients with positive screening, and by staging in patients diagnosed with sarcopenic obesity [12]. Positive screening is defined by the presence of high BMI in the obesity range or waist circumference equal or above 102 or 88 cm for male or female patients respectively, associated with clinical suspicion for low muscle function [12] (main suspicion factors are history of disability, falls, comorbidities with potential muscle-catabolic metabolic derangements). Positive screening mandates diagnostic procedure with assessment of muscle function through validated tools (including handgrip strength [HGS] or sit-to-stand test); in the presence of low muscle function, diagnosis should be confirmed by body composition measurement through validated techniques including BIA [12]. SO diagnosis should be followed by staging, with stage I or stage II defined by absence or presence of complications attributable to altered body composition and muscle function, respectively. In the current study, screening was accordingly performed in the 76 recruited individuals followed by diagnosis and staging as applicable. In order to test the sensitivity and specificity of the ESPEN-EASO screening in detecting all cases of sarcopenic obesity, diagnostic procedures with HGS and BIA were also exceptionally performed in individuals with negative screening. HGS measurement was measured by dynamometry (Hydraulic Hand Dynamometer, Jamar Technologies, Horsham, PA), taking the average of 3 independent measures per arm [27]. Body composition was measured by single-frequency (50 kHz) bioimpedance analysis (BIA101, Akern, Italy) and Muscle and Fat masses calculated using manufacturer's proprietary software (BodyGram 3.0, Akern, Italy) and expressed as percentage of body weight (BW). For low skeletal muscle mass (SMM) to body weight (BW) ratio (SMM/BW), cut-off values of 37 and 27.6 were used for males and females, respectively [28]. Appendicular Skeletal Muscle Mass (ASMM) was estimated using the equation of Sergi and colleagues [29] and normalized by BW. Cut-off values of 37 and 27.6 were used for males and females as suggested by ESPEN-EASO [12].

2.2. Additional risk factors and predictive factors identification

In order to identify potential metabolic and hormonal risk factors for SO and their potential predictive value, we further analyzed metabolic and hormonal parameters and their association with the ESPEN-EASO algorithm-based SO diagnosis. In particular, we analyzed associations between SO and metabolic syndrome components as well as their underlying metabolic determinant insulin resistance [5,30,31], since the MoMa study was designed to monitor prevalence of metabolic syndrome in the Friuli-Venezia Giulia region. In addition, we hypothesized a potential role for plasma ghrelin profile, since ghrelin forms have been differentially associated with insulin resistance itself and the regulation of skeletal muscle protein-anabolic pathways. A direct association was reported between the acylated ghrelin form and insulin resistance in persons with overweight and metabolic syndrome [25], whereas the unacylated form was reported to directly enhance skeletal muscle insulin action and protein-anabolic pathways in obesity and chronic disease models [20,21,24]. In the current study, insulin resistance was assessed through the Homeostasis model assessment (HOMA) [18,32] calculated with the following formula: HOMA = (FPG $\times$ FPI) / 22.5, where FPG and FPI are fasting plasma glucose (mmol) and fasting plasma insulin (μU/ml), respectively. Plasma total (TG) and acylated ghrelin (AG) were measured using radio immuno assay (Linco, St. Charles, MO) [18,33]; unacylated ghrelin (UnAG) was calculated by subtracting AG from TG [18]. HOMA and ghrelin forms were also available from the 5-year prior evaluation performed in the setting of the MoMa study, and these parameters were used to investigate their potential predictive value on actual SO diagnosis at the time of the current investigation.

2.3. Statistical analysis

Data distributions for continuous variables was assessed by Shapiro-Wilk test and appropriate transformations used for non-normal data. Sex-subgroup comparison was performed by independent sample *t*-test or Mann-Whitney as appropriate. Assessment of subgroup representativeness was performed comparing subgroup -included vs -non-included subjects. In multiple subgroup analysis, differences were evaluated by ANOVA or Kruskal–Wallis followed by post-hoc Tukey or Mann-Whitney comparisons for normally or non-normally distributed variables, respectively. p values were corrected for multiple comparisons according to Benjamini and Hochberg when appropriate. Univariate associations between variables were evaluated by Pearson point-biserial correlation. Multiple stepwise logistic regression analysis was performed to assess association between SO diagnosis and continuous parameters in different models. Analysis was performed by SPSS v.17 software (SPSS, Inc., Chicago, IL). Sensitivity, specificity and predictive values of the screening step of the EASPEN-EASO algorithm were calculated using MedCalc Software Ltd. Diagnostic test evaluation calculator available at https://www.medcalc.org/calc/diagnostic_test.php (Version 20.210; accessed December 27, 2022). Sensitivity was considered as the probability that a test result will be positive when the disease is present (true positive rate), specificity as the probability that a test result will be negative when the disease is not present (true negative rate), positive predictive value as the probability that the disease is present when the screening test is positive, negative predictive value as the probability that the disease is not present when the test is negative, accuracy as the overall probability that a patient is correctly classified. Sensitivity, specificity, prevalence, and predictive values as well as accuracy are expressed as percentages. Confidence intervals for sensitivity, specificity

and accuracy are calculated according to Clopper-Pearson (exact intervals). p values <0.05 were considered statistically significant. Continuous variables are presented as mean ± standard deviation. In multiple comparisons post-hoc analyses, for all variables with the same letter, alone or in combination, the difference is not significant.

3. Results

3.1. ESPEN-EASO algorithm implementation and SO prevalence

All participants underwent screening and diagnostic procedures at the local outpatient primary care clinic in Montereale Valcellina or Maniago. 76 individuals aged >65 years meeting the ESPEN-EASO algorithm BMI or WC thresholds for positive screening were identified and participated in the study. They were admitted under overnight fasting conditions and BMI, WC and arterial blood pressure were measured as previously reported [17–19]. Blood samples were also collected for metabolic and hormonal profiling, followed by HGS and BIA measurement. Primary care physicians, Trieste University researchers and nurses performed the procedures. Sixty-one individuals had positive screening (EES+), with main suspicion factors represented by metabolic syndrome (n = 37), chronic diseases (n = 18), diabetes (n = 14) and age > 70y (n = 7), with more than one factor often observed in the same subject. EES+ and EES- had comparable sex, BMI and WC as well as metabolic syndrome parameters, while age was lower by algorithm in EES- (Table 1). Seven out of sixty-one EES+ individuals (12 % of EES+; 9 % of total cohort) had SO based on the diagnostic procedure, with both low HGS and low skeletal muscle mass as determined by BIA-based SMM/BW or ASMM/BW (Table 1). EES+ individuals with (SO+) or without SO (SO-) had comparable age, sex, BMI or WC and all metabolic syndrome parameters (p > 0.5; Table 1). Interestingly, SO+ individuals had lower HGS, a pre-requisite for SO diagnosis, but SMM/BW and ASMM/BW statistically comparable to SO-, thereby suggesting overlap in body composition and muscle mass among groups with low or normal HGS. Finally, all persons with SO had stage 2 SO based on the presence of metabolic syndrome, considered as a metabolic complication whose risk may be directly enhanced by SO [12].

In order to investigate sensitivity, specificity and accuracy of the screening step, skeletal muscle function and mass were also measured in individuals without sarcopenic obesity according to screening. No individuals in this group had SO based on HGS and BIA. Predictive values of the screening step are described in Table 2. The screening step showed 100 % sensitivity and negative predictive value, with no individuals with SO after negative screening. Specificity and positive predictive value were lower with high level of EES+ without SO and an overall accuracy of 28.8 %.

3.2. ESPEN-EASO algorithm-based SO diagnosis and metabolic and hormonal risk and predictive factors

SO+ had selectively higher HOMA index, AG and plasma AG/UnAG ratio compared to both screening-positive SO- and screening-negative groups (p < 0.05) (Table 1). Besides HGS and SMM/BW or ASMM/BW, HOMA index, AG and AG/UnAG ratios were the only investigated parameters to be significantly associated with SO diagnosis at the time of

Table 2
ESPEN-EASO algorithm for SO diagnosis test assessment.
Sensitivity, specificity, negative and positive predictive values and overall accuracy with 95 % confidence intervals of the screening (EES) step of the ESPEN-EASO algorithm for sarcopenic obesity (SO) diagnosis. Statistical data are expressed in percentage with relative confidence interval (CI).

Contingency table				
	ESPEN-EASO SO diagnosis			
		Negative	Positive	Total
EES	Negative	15	0	15
	Positive	54	7	61
	Total	69	7	76

Statistics	
	Value (CI) [%]
Sensitivity	100.0 (59.0–100.0)
Specificity	21.7 (12.7–33.3)
Positive predictive value	11.2 (10.0–12.5)
Negative predictive value	100.0
Accuracy	28.8 (19.0–40.3)

Table 1
Clinical and biochemical profile.
Gender, age, body mass index (BMI), waist circumference (WC), hand grip strength (HGS), body fat (FM), skeletal muscle (MM) and appendicular skeletal muscle mass (ASMM) relative to body weight (BW), HOMA insulin resistance index, acylated (AG), unacylated (UnAG), total ghrelin plasma levels and form ratio in the studied cohort (all and separately for males and females) and subgroups according to ESPEN-EASO screening (EES) and sarcopenic obesity (SO) criteria. Results are reported as mean ± standard Deviation. Among EES screening and diagnosis subgroups, data not sharing a letter is different (p < 0.05) for the same parameter.

	All			EES screening and diagnosis subgroups		
	All	Males	Females	EES-	EES+ SO-	EES+ SO+
	76	35	41	15	54	7
Sex (M/F)	35/41	35/0	0/41	7/8	25/29	3/4
Age [years]	71.13 ± 3.35	71.07 ± 3.07	71.17 ± 3.61	68.73 ± 1.06 a	71.55 ± 3.15 b	73.02 ± 5.45 b
BMI [kg/m^2]	31.75 ± 4.62	32.15 ± 4.21	31.41 ± 4.70	31.04 ± 4.88 a	32.07 ± 4.63 a	30.80 ± 4.32 a
WC [cm]	107.43 ± 11.88	114.17 ± 10.98	101.68 ± 9.41*	104.33 ± 12.37 a	108.15 ± 11.94 a	108.57 ± 10.81 a
HGS [kg]	26.71 ± 9.42	33.89 ± 7.27	20.42 ± 5.94*	29.69 ± 9.38 a	27.54 ± 8.28 a	13.98 ± 8.75 b
FM/BW [%]	37.15 ± 5.77	33.76 ± 4.19	40.04 ± 5.37*	35.86 ± 6.47 a	37.53 ± 5.48 a	37.00 ± 6.91 a
MM/BW [%]	38.72 ± 4.91	41.24 ± 3.64	36.57 ± 4.86*	39.64 ± 5.37 a	38.52 ± 3.80 a	34.47 ± 3.88 a
ASMM/BW [%]	23.35 ± 2.31	25.02 ± 1.41	21.92 ± 1.94*	24.11 ± 2.36 a	23.42 ± 2.05 a	21.13 ± 3.11 b
Systolic arterial pressure	144.20 ± 18.27	144.89 ± 18.98	143.61 ± 17.85	136.67 ± 17.99 a	142.98 ± 18.17 a	138.86 ± 15.94 a
Diastolic arterial pressure	85.67 ± 8.74	85.54 ± 9.66	85.78 ± 8.00	83.20 ± 9.26 a	86.17 ± 8.41 a	87.14 ± 10.49 a
Triglycerides [mg/dl]	136.16 ± 72.33	147.49 ± 94.86	126.49 ± 44.20*	96.47 ± 30.37 a	150.78 ± 78.23 b	108.43 ± 49.26 ab
HDL-cholesterol [mg/dl]	62.50 ± 18.34	57.34 ± 15.67	66.90 ± 19.47*	66.73 ± 18.34 a	60.07 ± 16.91 a	72.14 ± 26.24 a
HOMA	6.07 ± 6.37	6.97 ± 7.59	5.30 ± 5.08	4.18 ± 1.78 a	5.70 ± 4.67 a	12.99 ± 15.51 b
Acylated ghrelin [pg/ml]	77.01 ± 77.89	77.21 ± 71.48	76.84 ± 83.46	61.62 ± 22.61 a	72.43 ± 72.77 a	158.30 ± 158.10 b
Unacylated ghrelin [pg/ml]	1080.45 ± 758.65	998.98 ± 676.77	1152.33 ± 827.59	1165.61 ± 605.76 a	1013.99 ± 714.47 a	1394.39 ± 1304.67 a
Total ghrelin [pg/ml]	1156.77 ± 797.13	1076.11 ± 689.37	1227.94 ± 885.61	1227.23 ± 619.29 a	1086.41 ± 765.51 a	1531.77 ± 1304.92 a
AG/UnAG	0.085 ± 0.091	0.094 ± 0.129	0.071 ± 0.082	0.064 ± 0.040 a	0.080 ± 0.006 a	0.173 ± 0.278 b

* p < 0.05 between females vs males in the whole study cohort.

the current examination ($p < 0.05$; Table 3). In addition, 5 years before the current examination HOMA and ghrelin ratio were also associated with the development of SO diagnosis (Table 3). Associations were also independent of age, sex and BMI in multiple logistic regression analysis (Table 4). Both HOMA and ghrelin profile also intriguingly predicted 5-year SO risk independent of age, sex and BMI (Table 4).

4. Discussion

The ESPEN-EASO algorithm [12] was effectively implemented in free-living older adults in a primary care setting, and the current data demonstrate a 9 % SO prevalence in the overall cohort. Based on the algorithm, insulin resistance and altered plasma ghrelin profile with higher AG/UnAG ratio are additional risk factors for age-related SO.

4.1. ESPEN-EASO algorithm implementation and SO prevalence

The study demonstrated the feasibility of implementing the recently proposed ESPEN-EASO algorithm for SO screening and diagnosis in an outpatient primary care setting, in an older cohort of free-living persons with obesity. 9 % SO prevalence from this cohort indicated a relatively common condition, considering that no individuals were institutionalized, and all were free-living and free of non-metabolic major comorbidities including cancer, chronic heart and kidney failure, chronic pulmonary disease and recent hospitalizations (based on clinical history and plasma biochemical profile including normal plasma creatinine and liver enzymes). A recent meta-analysis reported a global SO prevalence of 11 % in very heterogeneous populations (with and without comorbidities and various age groups) with heterogeneous assessment methods [34]. The current findings therefore support the ability of the ESPEN-EASO algorithm to diagnose SO with reliable prevalence, specifically in the important at-risk group of free-living older adults, where early diagnosis of SO may be possible before the onset of functional impairment associated with loss of autonomy, thereby allowing for potential prevention of disabilities and other complications through early prevention and treatment strategies.

Despite strong evidence that combined high fat and low skeletal muscle mass and function have major negative impact on major clinical outcomes [5–10], awareness and identification of sarcopenic obesity in clinical practice remain unacceptably low [7]. It is well recognized that use of heterogeneous tools for clinical research as well as lack of diagnostic criteria with large consensus and acceptable simplicity are strong contributing factors to low awareness and low clinical implementation [11]. The ESPEN-EASO algorithm was recently proposed by an international expert panel promoted by the European Societies for both Clinical Nutrition and Obesity, in the context of a collaborative ongoing initiative to define scientific and clinical gaps, with the major goal to propose consensus-based and acceptably simple diagnostic criteria [12,35]. Continuing international efforts among stakeholders are being promoted for validation in the context of comparable data, and further algorithm refinement as appropriate [36]. In the current study, all procedures were carried out in primary care outpatient clinics, thereby confirming the simplicity and feasibility of the ESPEN-EASO algorithm in this setting. All information and clinical data were collected by primary care physicians and nurses, with the exception of BIA and handgrip strength that were performed in collaboration with Trieste University personnel in the same outpatient clinic. Training of primary care personnel for BIA and handgrip strength assessment was rapid and successful and is most likely feasible in most settings. Primary care is highly relevant for early identification of SO in the general population, and based on this preliminary study the ESPEN-EASO algorithm may represent a suitable tool for this relevant clinical goal. It is important to point out that sensitivity and negative predictive value of the screening phase were 100 % in this setting, indicating that this step is able to identify all persons with SO for further diagnosis. Specificity was predictably lower, the screening step aiming by definition at inclusiveness, and being expected to also identify non-affected subjects for further analyses. Future studies should verify the possibility to improve screening specificity without affecting sensitivity, in order to reduce workload for involved healthcare professionals [36]. Nevertheless, we

Table 3
Association analysis.
Association between ESPEN-EASO Sarcopenic obesity (SO) diagnosis and male gender, age, and contemporary or 5 years before parameters. BMI: body mass index; WC: waist circumference; HGS: hand grip strength; FM: body fat mass; MM: muscle mass; ASMM: appendicular skeletal muscle mass; BW: body weight; HOMA: homeostasis model assessment for insulin resistance index; AG: acylated ghrelin plasma levels; UnAG: unacylated ghrelin plasma levels. Analyses are presented for the whole study population (n = 76).

	ESPEN-EASO SO diagnosis	
	r_{PB}	p
Current		
Sex (M)	−0.020	0.861
Age	0.181	0.118
BMI	−0.066	0.571
WC	0.031	0.790
HGS	−0.452	<0.001
FM/BW	0.104	0.371
MM/BW	−0.228	0.048
ASMM/BW	−0.229	0.046
Systolic arterial pressure	−0.94	0.421
Diastolic arterial pressure	0.054	0.643
Triglycerides	−0.123	0.290
HDL-cholesterol	0.169	0.144
Glucose	0.145	0.211
Insulin	0.249	0.030
HOMA	0.348	0.002
Acylated ghrelin	0.255	0.026
Unacylated ghrelin	0.134	0.248
Total ghrelin	0.153	0.187
AG/UnAG	0.313	0.006
5 years before		
BMI	−0.130	0.263
WC	−0.082	0.481
HOMA	0.231	0.045
Acylated ghrelin	0.060	0.607
Unacylated ghrelin	0.065	0.577
Total ghrelin	0.069	0.554
AG/UnAG	0.265	0.021

Table 4
Multiple regression analyses.
Multiple logistic regression analyses between ESPEN-EASO sarcopenic obesity (SO) diagnosis and current or 5 years before HOMA, and acylated/unacylated (AG/UnAG) ghrelin form ratio in the whole study population (n = 76) in different statistical adjustment models. B: coefficient, SE: standard error; z: Wald test.

		ESPEN-EASO SO diagnosis			
		B	SE	Wald	p
Current parameters					
HOMA	Model 1	0.101	0.044	5.251	0.022
	Model 2	0.103	0.045	5.226	0.022
AG/UnAG	Model 1	6.652	4.217	4.489	0.034
	Model 2	7.694	4.487	5.623	0.018
5 years before parameters					
HOMA	Model 1	0.562	0.361	4.414	0.036
	Model 2	0.559	0.386	4.096	0.043
AG/UnAG	Model 1	5.599	3.712	4.276	0.039
	Model 2	6.373	3.994	4.547	0.032

Data adjustments (contemporary or 5 years before as appropriate):
Model 1: age, gender.
Model 2: Model 1 + BMI.

also suggest that measurement of skeletal muscle function by handgrip strength in individuals with positive screening may carry independent clinical value even in the absence of confirmed SO diagnosis, since muscle function is an independent predictor of major outcomes including survival [37,38].

Low muscle function as assessed by muscle strength was indeed by definition a key feature of SO, with significantly lower handgrip strength in affected individuals. Interestingly, muscle mass as assessed by BIA and calculated SMM/BW or ASM/BW was instead comparable in SO+ and SO- individuals after positive screening, indicating large overlap in these parameters in the current free-living cohort of older persons living with obesity. The above combined observations indirectly support the view that the selection of low muscle strength as a preliminary requisite for SO diagnosis may be justified not only by its clinical implications, but also by a natural history of muscle derangements in this context. Further studies will be needed to directly test this hypothesis.

Staging was based on the presence of SO-related complications as indicated by the ESPEN-EASO algorithm [12], including metabolic diseases, functional disabilities and cardiorespiratory complications that may all be directly caused or worsened by low skeletal muscle mass. In the current cohort, metabolic syndrome was the key staging criterion, in the absence of disabilities and known cardiorespiratory complications. It should be however pointed out that metabolic syndrome also represents a clinical suspicion factor leading to positive screening [12], and patients entering assessment with metabolic syndrome as screening suspicion factor are bound to be classified with stage II SO if SO is diagnosed during the diagnostic procedure. A dual role of metabolic syndrome and metabolic diseases at large, as both risk factor and complication for SO, seems however only partly surprising, given the potential vicious cycle between muscle-catabolic obesity-induced metabolic derangements and the role of muscle derangements to induce or worsen metabolic complications. Future longitudinal studies should further elucidate the relationships between these clustered alterations and the potential differential clinical impact of their differential combinations in different patient subgroups.

4.2. Risk factors

The current study also allowed to investigate the potential role of additional metabolic and hormonal parameters as risk factors associated with SO at the time of screening and diagnosis. In addition, their predictive role for future SO was retrospectively investigated from a previous evaluation performed five years earlier, during the first baseline assessment of the MoMa study. Based on the current findings, insulin resistance and altered plasma ghrelin profile with relative excess of the acylated form were independently associated with the risk of SO.

Systemic insulin resistance is a fundamental metabolic derangement associated with obesity, and it may play a primary role in further development or worsening of hyperglycemia and type 2 diabetes [30,39]. Insulin resistance may also be exacerbated by low muscle mass since skeletal muscle is a major insulin target tissue and a major glucose utilizer at the whole-body level [5,30,40]. Insulin resistance may however most importantly play a key causal role in the development of sarcopenic obesity through its protein-catabolic impact in skeletal muscle [5,14,30] including both enhanced protein degradation and impaired protein anabolism [4,8,14]. The current combined findings from cross-sectional and prospective associations support a relevant role of systemic insulin resistance also in the onset of SO in free-living older adults before the onset of disabilities and relevant systemic comorbidities.

Although ghrelin has been originally identified as a gastric orexigenic hormone in its circulating acylated form [41–43], a role of unacylated ghrelin has subsequently emerged to independently and positively modulate skeletal muscle and systemic insulin sensitivity, involving regulation of mitochondrial dynamics and mitophagy, energy metabolism, oxidative stress and inflammation [20,21,44–46]. In addition, obesity-associated alterations in plasma ghrelin profile have been demonstrated with relative or absolute deficiency of unacylated ghrelin [25,26,47]. In a rodent model of diet-induced obesity, unacylated ghrelin overexpression conversely completely normalized the above clustered muscle metabolic alterations [21], and unacylated ghrelin treatment was able to completely prevent loss of muscle mass and mitochondrial abnormalities in kidney disease-induced sarcopenia [20]. The current novel association between altered plasma ghrelin profile with relative unacylated hormone deficiency and sarcopenic obesity in humans is therefore consistent with previous pre-clinical reports [20,22,23]. Longitudinal associations also further suggest a potential primary role for relative unacylated ghrelin deficiency that may precede and potentially contribute to the onset of sarcopenia in older persons with obesity. The potential role of muscle-derived hormonal signals and their potential interplay with insulin signalling and ghrelin forms should be directly investigated in future studies.

4.3. Limitations and strengths

The current study is limited by relatively low number of individuals. We employed the validated HOMA-IR for assessment of insulin resistance, as commonly used for clinical research in cohort studies; while this tool allowed identifying a potential predictive role for insulin resistance in the current study in a real-life primary care setting, more sophisticated methods potentially including the hyperinsulinemic-euglycemic clamp technique may be needed in more mechanistic or pre-clinical studies. The current study was not designed to test interventions for treatment or preventions of sarcopenic obesity; all patients were self-reported weight stable at the time of both assessments. On the other hand, lack of interventions and attempts to implement systematic dietary modifications may have contributed to homogeneity of the study sample. Lack of handgrip strength and BIA with consequent lack of SO assessment at initial evaluation may also limit interpretation of the role of insulin resistance and ghrelin profile as primary, causal contributors to the onset of subsequent SO. Finally, the current conclusions cannot be extended to other ethnic groups, or to other high-risk groups for sarcopenic obesity, such as patients with chronic or recent acute non-metabolic diseases, as well as non-geriatric populations at large. On the other hand, homogeneity of the study cohort allows for reliable conclusions for the older free-living adult population, and for potential interplay between SO and obesity-associated metabolic and hormonal derangements in this setting, where early diagnosis before functional impairment may have a key role in preventing the onset of disabilities and other complications.

4.4. Conclusions

In conclusion, we report here on implementation of the new ESPEN-EASO consensus algorithm for diagnosis of SO in an outpatient primary care setting in a cohort of older adults. The current results support implementation of the algorithm, and they indicate a 9 % prevalence of SO, with excellent screening sensitivity. In the current cohort of free-living individuals, SO appears to precede the onset of clinically relevant disabilities affecting autonomy, and to be independent of major non-metabolic comorbidities. Insulin resistance and altered plasma ghrelin profile, with relative acylated hormone excess and unacylated deficiency, may be early indicators of SO and potential risk factors for its future onset.

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CRediT authorship contribution statement

Gianluca Gortan Cappellari: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **Annamaria Semolic:** Conceptualization, Investigation, Writing – review & editing. **Michela Zanetti:** Conceptualization, Writing – review & editing. **Pierandrea Vinci:** Conceptualization, Investigation, Writing – review & editing. **Mario Ius:** Conceptualization, Writing – review & editing. **Gianfranco Guarnieri:** Conceptualization, Writing – review & editing. **Luca Busetto:** Conceptualization, Writing – review & editing. **Lorenzo Maria Donini:** Conceptualization, Formal analysis, Writing – review & editing. **Rocco Barazzoni:** Conceptualization, Methodology, Investigation, Supervision, Writing – original draft.

Declaration of competing interest

The authors have no conflict of interest to declare with regard to the topic of this paper.

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