Plasma Inflammatory Biomarkers and Anorexia of Ageing among Community-Dwelling Older Adults: An Exploratory Analysis of the MAPT Study

J.L. Sánchez-Sánchez[1,2], S. Guyonnet[2,3], A. Lucas[4], A. Parini[4], Y. Rolland[2,3], P. de Souto Barreto[2,3]

1. Department of Health Sciences, Public University of Navarre, Pamplona, Spain; 2. Gerontopole of Toulouse, Institute of Ageing, Toulouse University Hospital (CHU Toulouse), Toulouse, France; 3. Maintain Aging Research Team, CERPOP, Inserm, Université Paul Sabatier, Toulouse, France; 4. Institute of Metabolic and Cardiovascular Diseases (I2MC), Inserm UMR 1297, University of Toulouse, Toulouse, France

Corresponding Author: Juan Luis Sánchez-Sánchez, CHU Toulouse Gérontopôle: Centre Hospitalier Universitaire de Toulouse Gerontopole, France, jl.sanchezs@hotmail.com

Abstract

Anorexia of aging and biological aging might share physiological underpinnings. The aim of this secondary analysis was to investigate the associations between circulating inflammation-related markers and anorexia of aging in community-dwelling older adults. C-reactive protein (CRP), tumor necrosis factor receptor-1 (TNFR-1), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and growth/differentiation factor-15 (GDF-15) were measured in plasma. Anorexia of aging was defined by the response "severe/moderate decrease in food intake" to the first item of the Mini-Nutritional Assessment. We included 463 subjects (median age=74y, IQR=71-78; 63.1% women). 33 subjects (7.1%) presented with anorexia at baseline, whereas 25 out of 363 (6.9%) developed it along 1-year follow-up. We found that TNFR1 (OR=1.74, 95%CI=1.27-2.39) and GDF-15 (OR=1.38, 95%CI=1.01-1.89) were associated with a significant increase in the odds of presenting with anorexia of aging cross-sectionally. No further significant associations were found. Biological aging mechanisms might be involved in the pathogenesis of anorexia of aging.

Key words: Biomarkers, anorexia of aging, inflammation, malnutrition.

Introduction

Anorexia of aging is defined as the reduction in appetite at older age (1). Secondary overt reductions in food intake may lead to derangements in the balance between quantity and quality of energy and nutrients ingested, and physiological demands (2). Consequently, anorexia of aging results in the development of detrimental outcomes at older age such as malnutrition, immune dysfunction, weight loss, frailty, cognitive decline, sarcopenia and increased morbidity and mortality (3-6). Therefore, it is of utmost importance to improve the understanding of the biological mechanisms contributing to anorexia of ageing in the context of aging physiology, in order to unveil therapeutic targets for the development of timely preventive and treatment options (7-9).

Given the close association between aging and reductions in food intake, experts have suggested that the aging process and anorexia of aging (similarly to other geriatric syndromes) might share biological underpinnings (10). Consequently, intervening and slowing down biological aging might as well positively impact anorexia of aging (11). Chronic low-grade inflammation, one of the so-called hallmarks of aging, characterized by high levels of circulating pro-inflammatory peptides (12), is gaining attention given its anorexigenic potential (13, 14) and its involvement in the pathogenesis of downstream consequences of anorexia, such as malnutrition, sarcopenia and frailty (15). Despite links between inflammation and appetite reductions have systematically been observed in conditions such as renal failure, cancer-related cachexia or among hospitalized older adults (16, 17), less is known on the role of chronic inflammation in the absence of severe disease.

Therefore, the aim of this study is to explore the association between plasma inflammation-related biomarkers (C-reactive protein [CRP], tumor necrosis factor receptor-1 [TNFR-1], interleukin-6 [IL-6], monocyte chemoattractant protein-1 [MCP-1] and growth/differentiation factor-15 [GDF-15] and both prevalence and incidence of anorexia of aging in community-dwelling older adults. We hypothesize that greater levels of circulating inflammation-related markers would be related to anorexia of aging and its development over a 1-year follow-up.

Methods

Study design

This is a secondary analysis of the MAPT study (Multidomain Alzheimer Preventive Trial, ClinicalTrials.gov [NCT00672685]). In summary, MAPT was a four-arm randomized clinical trial designed to explore the effectiveness of a multidomain intervention (nutrition and physical activity advice and cognitive stimulation) with/without omega 3 polyunsaturated fatty acid (PUFA) supplementation on the prevention of cognitive decline compared to placebo over a 3-year time frame among community-dwelling older adults (18). Subjects were followed-up for two years after the end of the intervention. Details of the design of the study can be found elsewhere (18). Present analyses include a subsample of the MAPT study with available data on both plasma-based biomarkers (measured at the 12-month visit) and anorexia-

Figure 1. Flowchart of the MAPT participants included in present analyses
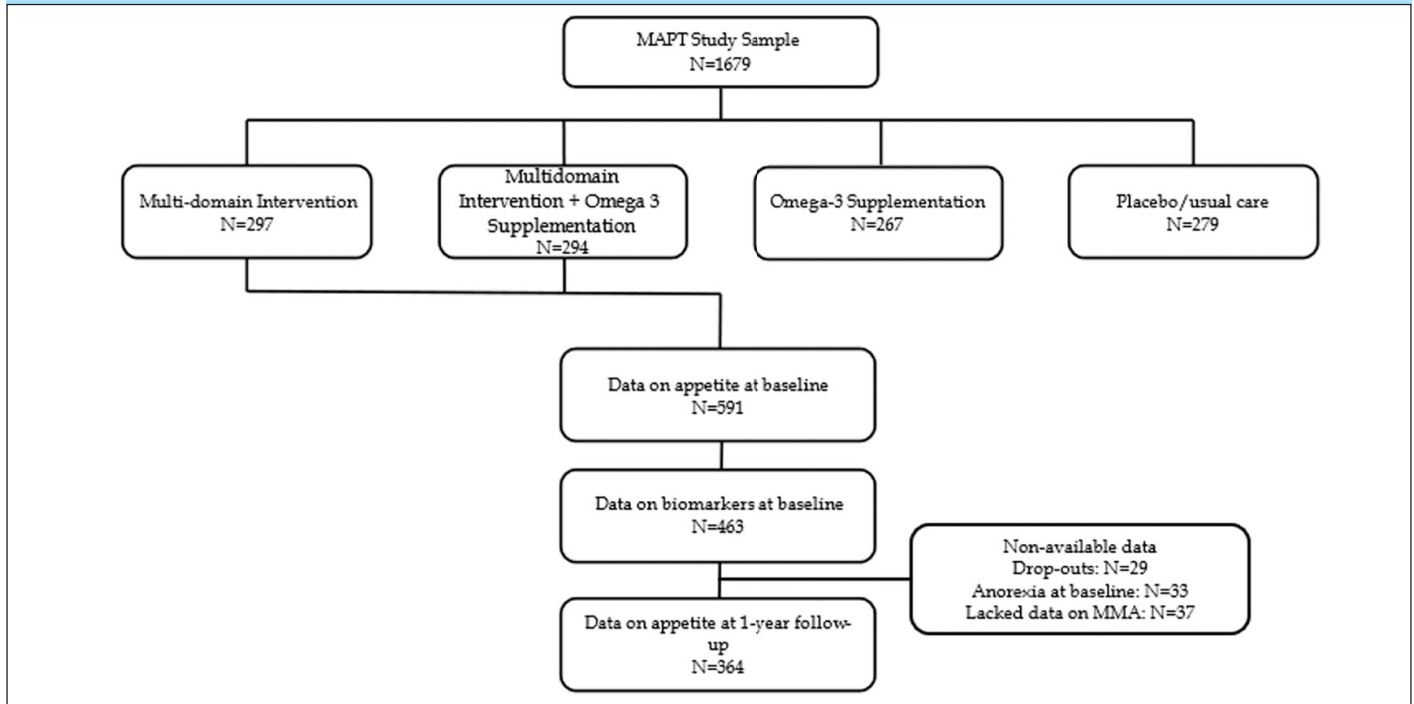
related information at 12-month and/or 24-month (when anorexia-related information was last measured) follow-up visits in MAPT. The present study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. The ethics committee in Toulouse (CPP SOOM II) approved MAPT protocol, that followed the Declaration of Helsinki. Participants provided written consent forms prior to participation.

Participants

MAPT study included community-dwelling older adults (age ≥70 years) presenting with spontaneous memory complaints, difficulty in one instrumental activity of daily living or low gait speed (<0.8 meters per second). Dependency for basic activities of daily living, contraindications for the intervention, a Mini-Mental State Examination score below 24 or having been supplemented with omega-3 PUFA in the 6 prior months constituted the exclusion criteria for participation.

Inflammation-related biomarkers assessment

CRP (in mg/mL) was measured by immunoturbidity according to standard protocols. Plasma levels of IL-6, TNFR-1, MCP-1 and GDF-15 were assessed using the fully automated immunoassay platform Ella (Bio-Techne, San Jose, CA, USA) as described elsewhere (19). The obtained protein concentrations were calculated by the internal instrument software and displayed in pg/mL.

Anorexia of ageing

Anorexia of ageing was defined based on the response to the first item in the Mini-Nutritional Assessment tool (20):

"Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?". Subjects reporting "severe/moderate decrease in food intake" were classified as presenting with anorexia of aging, whereas those reporting "no decrease in food intake" constituted the no anorexia group. Anorexia of aging incidence was defined as the evolution to a positive response at the 24-month visit (12 months after the clinical assessment and biological sampling) among those with absence of anorexia at baseline.

Confounders

Covariates consisted of age, sex, and the number of comorbidities (among cardiovascular diseases, chronic respiratory disease, cancer, type 2 diabetes, depression and dementia). The number of comorbidities was selected as a covariate given its potential association with increased levels of inflammatory markers.

Statistical analysis

Descriptive statistics (median ± interquartile range or frequencies and percentages, as appropriate) were used for the characterization of the study population. Baseline differences between people with anorexia and those without were ascertained by means of the Mann-Whitney's U test in the case of continuous, and the χ^2 test in the case of categorical variables.

Associations between levels of blood-borne markers and anorexia of aging were explored both cross-sectionally and longitudinally (incident anorexia at 1-year) by means of multivariate logistic regression models adjusted by age, sex

Table 1. Baseline characteristics of the population according to the anorexia of ageing status at baseline

Variables N (%) or mean (SD)	Whole population (N=463)	No anorexia of ageing (N=430)	Anorexia of ageing* (N=33)	Statistic Z-statistic or χ²	p-value
Age, years	74 (71-78)	74 (71-78)	77 (72-80)	-1.80	0.07
Female Sex, n (%)	292 (63.07%)	273 (63.49%)	19 (57.58%)	0.46	0.49
N of comorbidities	1 (0-1)	1 (0-1)	1 (0-2)	-2.073	0.04
TNFR1 pg/mL	1141 (962-1356)	1132 (949-1343)	1279 (1140-1883)	-3.773	<0.001
GDF-15, pg/mL	987 (809-1278)	976 (798-1269)	1154 (1011-1740)	-2.942	<0.01
IL-6, pg/mL	2.56 (1.79-3.72)	2.51 (1.78-3.6)	2.91 (2.33-4.35)	-2.050	0.043
MCP-1, pg/mL	205 (171-254)	205 (170-253)	201 (174-270)	-0.465	0.641
CRP, mg/L	1.6 (1-3.3)	1.7 (1-3.3)	1 (1-2.7)	1.485	0.137
MNA	28 (27-29)	28 (27-29)	25 (23.5-26.5)	7.21	<0.001

Abbreviations: TNFR1: Tumor necrosis factor receptor 1; GDF-15: Growth/differentiation factor 15; IL-6: Interleukin-6; MCP-1: Monocyte chemoattractant Protein 1; CRP: C-Reactive Protein; MNA: Mini-Nutritional Assessesment. *Subjects were classified as presenting with anorexia of ageing if reporting a severe/moderate decline in food intake in the past three months (question A of Mini Nutritional Assessment)

Table 2. Association between plasma inflammatory markers and prevalence (A) and onset (B) of anorexia of ageing

	A) Prevalence			B) Incidence		
	N	OR (95% CI)	p-value	N	OR (95% CI)	p-value
TNFR1 pg/mL	463	1.74 (1.27-2.39)	0.001	338	0.61 (0.25-1.47)	0.273
GDF-15, pg/mL	462	1.38 (1.01-1.89)	0.046	338	0.78 (0.36-1.71)	0.542
IL-6, pg/mL	462	1.06 (0.29-3.80)	0.923	337	1.11 (0.13-9.40)	0.144
MCP-1, pg/mL	463	1.01(0.72-1.44)	0.930	338	0.54 (0.24-1.23)	0.923
CRP, mg/mL	386	0.28 (0.06, 1.27)	0.101	280	1.03 (0.60, 1.75)	0.905

Logistic regression models were adjusted by age, sex and the number of comorbidities. Abbreviations: TNFR1: Tumor necrosis factor receptor 1; GDF-15: Growth/differentiation factor 15; IL-6: Interleukin-6; MCP-1: Monocyte chemoattractant Protein 1; CRP: C-Reactive Protein; OR (95%): Odds ratio, 95% confidence interval associated with a 1-SD increase in the levels of the inflammatory markers.

and the number of comorbidities reported. First, the odds ratio associated with a 1-SD increase in the biomarkers was computed. We further conducted a sensitivity analysis excluding subjects presenting with extreme values (defined as >4SD from the mean). A potential age-effect on significant associations was explored by including a two-way age-group (<75 vs. ≥75 years) x biomarker (as continuous) interactions. All analyses were performed using SAS 9.4 software. Statistical significance was set at an alpha value of 0.05.

Results

Characteristics of the study population

Out of 1679 subjects in the MAPT study whole sample, 463 (27.6%) were included in cross-sectional analyses, whereas 364 had data at follow-up (Figure 1). Median age was 74 (interquartile range=71-78) and 63.1% were female. 33 (7.1%) individuals presented with anorexia of aging at baseline. Subjects with anorexia of aging were older, had more comorbidities, presented significant higher values in plasma TNFR1, GDF-15 and IL-6 at baseline (Table 1), and a worse score in the Mini-Nutritional Assessment (Table 1). Among people without anorexia at baseline, 25 (6.9%) developed anorexia of aging at the 1-year follow-up.

Associations between plasma biomarkers and anorexia of aging

Table 2A displays the associations between plasma inflammation-related biomarkers and anorexia of aging in our sample. A 1-SD increase in TNFR1 (OR=1.74, 95%CI=1.27,2.39) and GDF-15 (OR=1.38, 95%CI=1.01,1.89) was associated with a significant increase in the odds of presenting with anorexia of aging. The association between TNFR1 and anorexia of aging was stronger in the ≥75 years age-group compared to younger counterparts (OR=1.80, 95%CI=1.31,2.45 vs. 1.62, 95%CI=1.07,2.46), and was restricted to the oldest in the case of GDF-15 (OR=1.46, 95%CI=1.08,1.97 vs. 1.32, 95%CI=0.86,2.02). No significant association arose from the analyses exploring associations between inflammation-related markers and the incidence of anorexia of aging at the 1-year follow-up visit (Table 2B). Table S1 shows the results of the sensitivity analyses excluding outliers, that remained virtually the same.

Discussion

Our exploratory analyses investigate the associations between inflammation-related biomarkers and anorexia of aging in a relatively healthy sample of older adults. We found a link between greater levels of TNFR1 and GDF-15 and

the odds of presenting with self-reported anorexia of aging cross-sectionally. Interestingly, these associations seemed to be stronger with increasing age, which might further support the biological aging-anorexia relationship.

Our findings are compatible with previous research showing associations between circulating levels of markers of chronic low-grade inflammation and appetite loss in samples of community-dwelling older adults. Specifically, Lee et al. (20) found a cross-sectional association between circulating TNFα and decreased appetite (OR=1.71, 95CI=1.23,2.34) in a sample (n=2169, 70-79 years old, 12% presenting with impaired appetite) of community-dwelling older adults. In the same study, the pleiotropic inflammatory marker IL-6 was not linked to greater anorexia of aging when covariates were taken into account, similarly to our findings and the observations by Chareh et al. (22) .

Overall, the involvement of inflammatory cytokines in anorexia of aging might occur through both peripheral (delayed gastric emptying and reduced intestinal motility, increased leptin -a potent appetite suppressor- expression) (23) and central levels (anorexigenic signals at hypothalamic and other brain neuronal circuitry areas) (24).

Mechanistically, processes underlying primary aging-mediated anorexia might differ from those observed among severely ill older adults or animal models of cachexia (16, 25), as suggested by the divergent findings between our study and previous ones. Our study failed at showing associations of IL-6 or CRP (an acute-phase protein elevated in severe illness or trauma) with anorexia of aging, contrary to some early reports (17). This might be explained by higher stability of TNFR-1 levels in blood (26), compared to the more volatile CRP and IL-6, which might be mostly elevated following acute stress/insult. According to these results, the TNFα/TNFR axis might be a predominant marker of anorexia related to inflammation and target of interventions oriented to preventing/treating this syndrome. Due to the scarcity of evidence, more mechanistic research should explore this hypothesis.

To our knowledge, our study is the first piece of evidence exploring the link between GDF-15 and anorexia of aging in humans. GDF-15 is a stress signaling cytokine elevated with aging, inflammation and mitochondrial stress that has shown anorexigenic potential in animal models (27). GDF-15 was also found to be elevated in older women showing impaired post-prandial appetite (28) and to be associated with weight loss in cachectic humans (29). Little is known around mechanisms underlying GDF-15-reduced appetite link at older age, but emerging evidence points to the regulation of its receptor at the level of brainstem and the downstream triggering of neurons in centers known to control feeding behavior (30), which is compatible with our observations. In any case, the evidence on the role of GDF-15 on anorexia of aging is its infancy, and more research is needed.

Our study presents strengths such as its longitudinal nature, the relatively large sample, and the assessment of multiple markers of inflammation. However, it presents with limitations. First, anorexia of aging was defined based on a single self-reported item of a questionnaire designed for malnutrition screening used to detect reductions in food intake by reasons

beyond appetite loss, such as dysphagia and digestive problems, which might poorly capture the construct. Future research might benefit by the use of scales specifically designed to assess appetite that are being incorporated to research, such as the Simplified Nutritional Assessment Questionnaire (31). In addition, we included a homogeneous subsample of relatively healthy and highly educated older adults who participated in a randomized clinical trial, which might limit our ability to investigate the association along the whole continuum of the exposure and the outcome. In fact, figures of anorexia of aging were smaller compared to those reported in literature, suggesting statistical power issues. In addition, results may differ in observational studies with demographically diverse populations and generalization of results should be cautious. We lacked data on markers previously related to reduced appetite in older adults (interleukin-1, interleukin-18 (17)). Finally, we cannot exclude the possibility that anorexia of aging could lead to worse inflammatory profile (reverse causality), or residual confounding by the contribution of diseases or habits contributing to appetite loss and inflammation.

In summary, our study shows cross-sectional associations between inflammation and stress, assessed via circulating levels of TNFR-1 and GDF-15, and reduced appetite in a sample of community-dwelling older adults, suggesting that biological aging mechanisms might be involved in the pathogenesis of anorexia of aging. Consequently, interventions oriented to slow down biological aging (32) might be effective in improving appetite in older adults and avoid its burdensome consequences. In any case, given the limitations of our study, further research should explore this hypothesis in the future.

References

1. Chapman IM, MacIntosh CG, Morley JE, Horowitz M. The anorexia of ageing. Biogerontology 2002;3:67–71.

2. Morley JE. Anorexia, body composition, and ageing. Curr Opin Clin Nutr Metab Care 2001;4:9–13.

3. Fielding RA, Landi F, Smoyer KE, Tarasenko L, Groarke J. Association of anorexia/appetite loss with malnutrition and mortality in older populations: A systematic literature review. J Cachexia Sarcopenia Muscle 2023;14:706–729.

4. Landi F, Russo A, Liperoti R, Tosato M, Barillaro C, Pahor M, Bernabei R, Onder G. Anorexia, physical function, and incident disability among the frail elderly population: results from the ilSIRENTE study. J Am Med Dir Assoc 2010;11:268–274.

5. Landi F, Liperoti R, Russo A, Giovannini S, Tosato M, Barillaro C, Capoluongo E, Bernabei R, Onder G. Association of anorexia with sarcopenia in a community-dwelling elderly population: results from the ilSIRENTE study. Eur J Nutr 2013;52:1261–1268.

6. Dagenais S, Fielding RA, Clark S, Cantu C, Prasad S, Groarke JD. Anorexia in Medicare Fee-for-Service Beneficiaries: A Claims-Based Analysis of Epidemiology and Mortality. J Nutr Health Aging 2023;27:184–191.

7. Picca A, Calvani R, Coelho-Júnior HJ, Landi F, Marzetti E. Anorexia of Aging: Metabolic Changes and Biomarker Discovery. Clin Interv Aging 2022;17:1761–1767.

8. Sánchez-Sánchez JL, Rolland Y (2023) Social Isolation and Loneliness: Overlooked Therapeutic Targets of Anorexia of Aging? J Nutr Health Aging. doi:10.1007/s12603-023-2011-0.

9. Chen L-K.Unveiling the hidden epidemic: Anorexia of aging and nutritional decline in older adults. Arch Gerontol Geriatr 2023;111:105064.

10. de Souto Barreto P. Poor Appetite & Aging: The Role of Physical Activity under a Geroscience Perspective. J Nutr Health Aging 2022;26:907–908.

11. Sierra F, Caspi A, Fortinsky RH, Haynes L, Lithgow GJ, Moffitt TE, Olshansky SJ, Perry D, Verdin E, Kuchel GA. Moving geroscience from the bench to clinical care

and health policy. J Am Geriatr Soc 2021;69:2455–2463.

12. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–254.

13. Yeh S-S, Blackwood K, Schuster MW. The cytokine basis of cachexia and its treatment: are they ready for prime time? J Am Med Dir Assoc 2008;9:219–236.

14. Laviano A, Meguid MM, Inui A, Muscaritoli M, Rossi-Fanelli F. Therapy insight: Cancer anorexia-cachexia syndrome--when all you can eat is yourself. Nat Clin Pract Oncol 2005;2:158–165.

15. Morley JE. Anorexia of ageing: a key component in the pathogenesis of both sarcopenia and cachexia. J Cachexia Sarcopenia Muscle 2017;8:523–526.

16. Sieske L, Janssen G, Babel N, Westhoff TH, Wirth R, Pourhassan M. Inflammation, Appetite and Food Intake in Older Hospitalized Patients. Nutrients 2019;11:1986.

17. Pourhassan M, Babel N, Sieske L, Westhoff TH, Wirth R. Inflammatory cytokines and appetite in older hospitalized patients. Appetite 2021;166:105470.

18. Andrieu S, Guyonnet S, Coley N, et al. Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. Lancet Neurol 2017;16:377–389.

19. Lu W-H, Gonzalez-Bautista E, Guyonnet S, Lucas A, Parini A, Walston JD, Vellas B, de Souto Barreto P, Group for the M. Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults. J Cachexia Sarcopenia Muscle 2023;14:930–939.

20. Vellas B, Guigoz Y, Garry PJ, Nourhashemi F, Bennahum D, Lauque S, Albarede JL The Mini Nutritional Assessment (MNA) and its use in grading the nutritional state of elderly patients. Nutr Burbank Los Angel Cty Calif 1999;15:116–122.

21. Lee JS, Kritchevsky SB, Tylavsky F, Harris TB, Ayonayon HN, Newman AB. Factors associated with impaired appetite in well-functioning community-dwelling older adults. J Nutr Elder 2006;26:27–43.

22. Chareh N, Kiesswetter E, Kob R, Hannink A, Brandl B, Skurk T, Hauner H, Sieber CC, Volkert D. Association Between Inflammation and Appetite in Healthy Community-Dwelling Older Adults-An enable Study. Front Aging 2022;3:826816.

23. Soenen S, Rayner CK, Jones KL, Horowitz M. The ageing gastrointestinal tract. Curr Opin Clin Nutr Metab Care 2016;19:12–18.

24. Molfino A, Rossi-Fanelli F, Laviano A. The interaction between pro-inflammatory cytokines and the nervous system. Nat Rev Cancer 2009;9:224–224.

25. Molfino A, Iannace A, Colaiacomo MC, Farcomeni A, Emiliani A, Gualdi G, Laviano A, Rossi Fanelli F. Cancer anorexia: hypothalamic activity and its association with inflammation and appetite-regulating peptides in lung cancer. J Cachexia Sarcopenia Muscle 2017;8:40–47.

26. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, Wallach D. Variation in serum levels of the soluble TNF receptors among healthy individuals. Lymphokine Cytokine Res 1992;11:157–159.

27. Johnen H, Lin S, Kuffner T, et al. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nat Med 2007;13:1333–1340.

28. Herpich C, Lehmann S, Kochlik B, Kleinert M, Klaus S, Müller-Werdan U, Norman K. The Effect of Dextrose or Protein Ingestion on Circulating Growth Differentiation Factor 15 and Appetite in Older Compared to Younger Women. Nutrients 2022;14:4066.

29. Morita-Tanaka S, Miyagawa-Hayashino A, Yamada T, Matsui Y, Morimoto K, Hiranuma O, Masuzawa N, Yoshimura A, Iwasaku M, Tokuda S, Kaneko Y, Kim YH, Konishi E, Takayama K. Significance of localized expression of full-length growth differentiation factor-15 in cachexia of advanced non-small cell lung cancer. Support Care Cancer Off J Multinatl Assoc Support Care Cancer 2023;31:308.

30. Raffin J, Rolland Y, Parini A, Lucas A, Guyonnet S, Vellas B, de Souto Barreto P, Group F the M. Association between physical activity, growth differentiation factor 15 and bodyweight in older adults: A longitudinal mediation analysis. J Cachexia Sarcopenia Muscle 2023;14:771–780.

31. Lau S, Pek K, Chew J, Lim JP, Ismail NH, Ding YY, Cesari M, Lim WS. The Simplified Nutritional Appetite Questionnaire (SNAQ) as a Screening Tool for Risk of Malnutrition: Optimal Cutoff, Factor Structure, and Validation in Healthy Community-Dwelling Older Adults. Nutrients 2020;12:2885.

32. Ruiz JG. Non-pharmacological Interventions in Anorexia of Aging. J Nutr Health Aging 2022;26:110–111.