Investigation of Age-Dependent Obesity and Its Consequences - Animal Studies and Meta-Analysis

University doctoral thesis (Ph.D.)

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1. Introduction

The prevalence of overweight and obesity has increased dramatically over the past decades. From 2016 to 2022, the number of overweight adults rose by 0.6 billion (*WHO, 2020; WHO, 2024a*). In 2016, approximately 650 million people were obese worldwide. Similar trends are observed among younger populations, including children, but obesity and overweight still affect middle-aged adults at the highest rates. Since overweight and obesity are associated with enhanced cardiometabolic risk, the prevention and management are important (*Meldrum et al., 2017; Koliaki et al., 2019*).

The loss of muscle strength and mass in older age, known as primary sarcopenia, also represents an increasing challenge for healthcare systems due to population aging (*United Nations, 2019; Eurostat, 2022*). Sarcopenia could also lead to severe complications in the elderly. It increases the risk of immobilization, frailty syndrome and bone fractures, thereby significantly reducing quality of life and markedly elevating mortality risk (*Reinders et al., 2017*).

Both age-related obesity and sarcopenia in older adults represent serious public health burden. Thus, the understanding of the underlying pathomechanisms is essential for prevention and treatment. It is a well-established fact that environmental, social and economic factors significantly influence body weight. Since similar age-dependent changes in body composition can be observed in certain mammals, regulatory mechanisms may also play a crucial role beyond these factors (*Kmieć et al., 2013*). Our pevious animal studies indicate that the effects of body weight–reducing neuropeptides exhibit characteristic biphasic changes with ageing, which may contribute to both age-related obesity and the development of anorexia, weight loss and sarcopenia in older age (*Rostás et al., 2016; Füredi et al., 2018*). However, only limited published data are available on age-dependent changes in neuropeptides that stimulate appetite and increase body weight. Among these, the strongest orexigenic hypothalamic neuropeptide, neuropeptide Y (NPY), was previously assumed to show a linear decline in activity with age (*Wang et al., 1997; Coppola et al., 2004; Akimoto & Miyasaka, 2010*). Previous studies examined only 2–3 age groups of animals. To clarify the age-dependent dynamics, analysis across more than three age groups is necessary, as our research group has demonstrated in studies of anorexigenic mediators, enabling the detection of their biphasic age-dependent changes.

One of the aims of my doctoral thesis was to investigate the age-dependent changes in hypothalamic NPY activity in an animal model, examining five distinct age groups in detail, and to explore the potential role of this peptide in the development of age-related obesity and sarcopenia. The concurrent presence of obesity and sarcopenia, termed sarcopenic obesity (SO), represents a combination of complications from both conditions, resulting in a worse prognosis compared to normal weight population or in sarcopenic non-obese (SNO) individuals (*Zamboni et al., 2008; Zhang et al., 2019*).

Based on available clinical studies, it remains unclear whether SO is associated with worse prognosis compared to SNO. Some studies have reported certain beneficial effects of obesity, a phenomenon referred to as the "obesity paradox" (*Donini et al., 2020b*). Therefore, as a second objective, I conducted a systematic literature review and meta-analysis of human studies to investigate whether the additional obesity in sarcopenic patients could influences the risk of mortality and comorbidities and worsen the quality of life.

1.1. Age-related changes in body composition - epidemiological implication

The prevalence of obesity has increased dramatically over the past decades. Currently, obesity affects middle-aged and older populations at the highest rates. In Hungary, 72% of individuals aged 65–74 are overweight or obese (*Eurostat, 2022*). Overweight and obesity pose a significant burden on healthcare systems, as they are associated with higher incidence of type 2 diabetes mellitus, hypertension and cardiovascular diseases (*Safaei et al., 2021*).

In addition to overweight and obesity, population aging represents a major challenge. Economic development and improvements in healthcare have increased life expectancy. In 2024, the average life expectancy reached 73.3 years. Based on 2023 data, there were 1.1 billion people aged 60 years or older (*WHO, 2024b*). The obesity pandemic affecting middle-aged and older populations is not solely attributable to an obesogenic environment but also correlates with age-dependent physiological changes. Body weight and composition physiologically change with ageing. From adulthood, visceral and intramuscular fat gradually increase, peaking between 55–70. Subsequently, age-related obesity shows a slight decline, primarily affecting subcutaneous fat mass (*Kelly et al., 2009; Stenholm et al., 2008*). In contrast, muscle mass and strength gradually decrease from the middle age (40–50 years) leading to aging sarcopenia by 70–75. Socioeconomic factors, comorbidities and age-related anorexia may also contribute to this decrease of muscle mass (*Pétervári et al., 2013*). According to a systematic review of 130 publications, primary sarcopenia affects 10–16% of individuals over 70 years of age (*Yuan et al., 2023*). Aging sarcopenia is associated with adverse health consequences including the risk of fractures, frailty, immobilization and reduces the quality of life (*Reinders et al., 2017; Yuen et al., 2023*). Sarcopenia is often accompanied by an increase in fat mass and this condition was defined as sarcopenic obesity (*Kelly et al., 2009; Gould et al., 2014; Ferrucci & Studenski, 2015*). This condition affects approximately one out of ten adults (*Liu et al., 2023*). Both sarcopenia and obesity have been shown to reduce health span and life span. In SO they may act synergistically presenting an increasing health burden in aging societies. According to some studies which have investigated the role of low muscle mass or high body fat separately, an increased fat mass may be more predictive of self-reported disability, functional limitation, and poor physical performances than a decreased muscle mass (*Zamboni et al., 2008; Zhang et al., 2019*). However, it is still unclear if the additional presence of obesity could effect on the risk of mortality and quality of life. Some studies suggest obesity may improve outcomes (*Liu et al., 2014*), while others report the opposite (*von Berens et al., 2020*).

Similar trends in body composition changes (overweight/obesity, sarcopenic obesity, sarcopenia) are observed in mammals, including laboratory rodents, suggesting that in addition to socioeconomic factors (nutrition, lifestyle, education, etc.), age-dependent regulatory changes in body weight regulation may contribute to middle-aged obesity and muscle loss in ageing (*Kmiec et al., 2013*). These regulatory processes are primarily mediated by the neuropeptides of the hypothalamus–adipose tissue axis playing a pivotal role in energy homoeostasis controlling the nutritional status and energy storage. Previous results from our Experimental Gerontology and Energetics Laboratory at the University of Pécs Medical School have demonstrated non-linear age-related changes in the central catabolic (appetite reducing and hypermetabolic actions leading to weight loss) effects of leptin, the main adiposity signal from fat tissue and in those of its hypothalamic target, the melanocortin system: a transient decline in their catabolic efficacy preceding middle-aged weight gain, which was followed by an enhancement preceding weight loss in older age groups (*Pétervári et al., 2010; Pétervári et al., 2011; Pétervári et al., 2014; Rostás et al., 2015; Rostás et al., 2016; Tenk et al., 2016; Füredi et al., 2018; Kovács et al., 2023*).

1.2. Age-related changes of the major regulatory peptides of the adipose tissue–hypothalamus axis

In terms of body weight, composition, and energy homeostasis, two major trends are observed with ageing in humans and many mammals and laboratory rodents such as our male Wistar rats. Initially, aging is associated with increased body weight and fat mass, followed by anorexia and weight loss (*Székely et al., 2013; Székely et al., 2018*). Animal experiments and human observations have demonstrated that the activity of the neuropeptides of the adipose tissue-hypothalamus axis shows characteristical changes with ageing. These changes can significantly influence the long-term development of body weight and body composition.

1.2.1. Catabolic neuropeptides and their age-related changes

Leptin is one of the main catabolic (anorexigenic and hypermetabolic) neuropeptide produced mainly in the adipocytes in proportion to fat mass could cross the blood-brain barrier. Leptin stimulates central melanocortin system and inhibits neurons in the hypothalamic arcuate nucleus (ARC) producing neuropeptide Y (NPY), a major anabolic (appetite inducing action with decreased energy expenditure leading to weight gain) hypothalamic neuropeptide (*Pétervári et al., 2014; Rostás et al., 2016*).

In high-fat fed animals and also in human obesity a chronic elevation of leptin level could be observed leading to leptin resistance (*Jackson & Ahima, 2006; Seth et al., 2020; Obradovic et al., 2021*), which cuold further increase obesity. Based on previous studies, responsiveness to exogenous leptin declines with ageing. However, our earlier experimental results indicate a biphasic, age-dependent change: leptin sensitivity decreases in middle age and increases again in older ones. In the 12-month-old, middle-aged male Wistar rats, the anorexigenic effect of the intracerebroventricularly injected leptin was significantly weaker compared to both younger and older groups. In the old 24-month-old rats leptin responsiveness to exogenous leptin was increased again (*Rostás et al., 2016*). A similar pattern was observed during a 7-day intracerebroventricular (ICV) administration of leptin infusion (*Pétervári et al., 2014*).

Administration of the strong anorexigenic, hypermetabolic α-melanocyte-stimulating hormone (alfa-MSH) also resulted in biphasic age-related changes (*Rostás et al., 2015*). *In vitro* analyses of peptide and receptor production and their gene expression confirmed these *in vivo* findings (*Füredi et al., 2018; Rostás et al., 2016*). In conclusion, age-related changes in leptin and its hypothalamic target, the melanocortin system, may contribute to middle-age obesity and also to ageing anorexia and muscle loss.

In addition to these key catabolic neuropeptides, peripheral anorexigenic cholecystokinin (CCK) and corticotropin-releasing factor (CRF) - a hypothalamic neuropeptide of secondary neurons - also show similar biphasic changes. Their anorexigenic effect is reduced in middle age and enhanced in older animals following exogenous administration (*Balaskó et al., 2013; Tenk et al., 2016; Tenk et al., 2017*). Another endogenous ligand of the CRF2 receptor, urocortin 2, showed age-dependent changes in gene expression as well as in its anorexigenic and weight-reducing effects following ICV injection as seen after administration of leptin and alfa-MSH (*Kovács et al., 2023*).

In contrast to male Wistar rats, females do not show age-related obesity rather maintain their stable low body weight throughout life (*Tenk et al., 2016*). Female rats do not experience human-like menopause, their reproductive senescence is characterized by moderate to high estradiol levels (Koebele et al., 2016). Although the role of several catabolic mediators has been established, age-related changes in orexigenic peptides and their potential role in middle-aged obesity and ageing anorexia have not yet been examined in detailed.

1.2.2. Anabolic neuropeptides and their age-related changes

One of the most potent orexigenic, i.e. food intake–stimulating and anabolic hypothalamic neuropeptides is NPY, a 36–amino acid peptide (*Adrian et al., 1983; Mercer et al., 2011; Székely et al., 2016*), which was first isolated from porcine brain in 1982 (*Tatemoto, 1982; Mercer et al., 2011*). NPY is mainly produced in the ARC and released along neuronal projections in the paraventricular, perifornical, and ventromedial hypothalamic nuclei, as well as in the lateral hypothalamic area. Leptin inhibits NPY-producing neurons in the ARC. Conversely, food deprivation increases hypothalamic NPY mRNA expression (*Grove et al., 2003*), NPY levels (*Calzá et al., 1989*) and NPY receptor expression (*Xu et al., 1998*). By promoting energy storage, NPY acts on secondary hypothalamic neurons to induce hyperphagia and hypometabolism (*Zhang et al., 2014*). In contrast, overfeeding suppresses hypothalamic Npy mRNA expression (*McMinn et al., 1998*). These effects are mainly mediated via Y1

and Y5 receptors (*Nguyen et al., 2012; Shi et al., 2017*). Several studies suggest a critical role of NPY for the beneficial anti-aging effects of caloric restriction (*Botelho et al., 2015*).
Additionally, NPY decreases processes triggered by sympathetic activity: nonshivering thermogenesis in brown adipose tissue and lipolysis in white adipose tissue. They are associated with enhanced adipogenesis shifting fuel utilization to carbohydrates to preserve fat reserve (*Zhang et al., 2014; Su et al., 2016*). Accordingly, a chronic central NPY infusion was shown to induce increased adiposity in rats by hyperphagia and reduced thermogenesis (*Baran et al., 2002*).
Beyond feeding regulation, NPY plays important roles in several other physiological systems, including cardiovascular regulation (*Tan et al., 2018*), immune function (*Farzi et al., 2015*), sleep and circadian rhythms (*Dyzma et al., 2010*) and stress and anxiety responses (*Heilig, 2004*). Given its involvement in multiple processes, NPY is also implicated in human diseases such as obesity (*Loh et al., 2015*), hypertension (*Zhu et al., 2015*), atherosclerosis (*Zhu et al., 2015*), epilepsy (*Cattaneo S et al., 2024*) and stress-related or behavioral disorders (*Rasmusson, 2017*).
The NPY acts through G-protein-coupled receptors of which several subtypes exist. Animal studies indicate that the Y1 and Y5 receptors are the most relevant in metabolic and body weight regulation (*Yi et al., 2018*). Human and other mammalian receptors show high homology (*Babilon et al., 2013*), enabling the identification of potential therapeutic targets in animal models that may later could translate into human applications. Promising results have been obtained with NPY analogs preferentially targeting human Y1 receptors in breast cancer (*Pedrazzini et al., 2003; Li et al., 2015*). Selective NPY receptor ligands also show potential in obesity pharmacotherapy. The Y2- and Y4-receptor agonist TM30338 (a synthetic analog of peptide YY and pancreatic polypeptide), administered subcutaneously, safely and effectively reduced food intake in humans with well-tolerated side effects (*Kamiji et al., 2007*). Similarly, MK-0557, a selective Y5 receptor antagonist, showed modest but clinically negligible weight reduction in trials (*Erondu et al., 2006*). These results suggest that Y5 receptor antagonists alone are insufficient for effective weight reduction but may be useful in combination therapies. Intranasal administration of NPY analogs has also been suggested as an effective alternative to oral formulations, supported by studies where intranasal NPY analogs proved effective in depression and post-traumatic stress disorder (*Mathé et al., 2020*).
Human and animal studies indicate that hypothalamic NPY production and its orexigenic effect decline in old age (*Kmieć et al., 2013*) potentially contributing to age-related anorexia and weight loss. Available data suggest a predominantly linear decrease in the effect of NPY with ageing; however, it should be noted that most studies examined only two or three age groups and did not specifically address the hypometabolic effects of NPY. To understand the age-related changes in NPY activity is important since it can be a relevant pharmacological target.
Less is known about agouti-related protein (AgRP), an orexigenic neuropeptide co-expressed with NPY and an antagonist of melanocortin receptors. According to our previous results, AgRP gene expression did not show significant changes in middle-aged animals (*Füredi et al., 2018*), despite the expectation of elevated levels due to age-related leptin resistance. Ghrelin, a peripheral stomach-derived hormone with effects opposite to leptin in the hypothalamus, is responsible for hunger signaling. For both ghrelin and orexins, which mediate the orexigenic effects of NPY, only age-related decreases have been reported (*Toshinai et al., 2007; Akimoto & Miyasaka, 2010*). However, detailed investigations covering more than three age groups have not yet been performed for orexigenic, anabolic mediators.

1.3. Age-related obesity and muscle loss: sarcopenic obesity

With aging fat deposition in the body is increasing (leading to age-related obesity). Later it can be combined with aging sarcopenia defined as a condition of progressive loss of muscle mass and strength

with functional impairment. Sarcopenia is often accompanied by an increase in fat mass and this condition was defined as sarcopenic obesity (SO) (*Kelly et al., 2009; Gould et al., 2014; Ferucci és Studenski, 2015*).

Several studies have suggested that obesity may exacerbate sarcopenia by promoting intramuscular fat deposition, which further impairs muscle function. In addition, proinflammatory cytokines and adipokines released from adipocytes may contribute to subclinical inflammation, impairing muscle function and increasing the risk of comorbidities through chronic low-grade inflammation (*Kalinkovich and Livshits, 2017*). Accordingly, the presence of obesity in sarcopenia is generally regarded as an additional risk factor.

Although obesity is a well-known risk factor for many cardiometabolic diseases (e.g., metabolic syndrome, heart failure, hyperlipidemia, hypertension, diabetes mellitus), paradoxically, in certain cases it may confer survival advantages compared to normal body weight (*Bosello and Vanzo, 2021*). This called *"obesity paradox"* which was first described in 2002. In overweight and obese patients with coronary artery disease, lower surgical complication rates and reduced mortality were observed compared to normal-weight individuals (*Gruber et al., 2002*). Several reports have confirmed similar findings especially in older populations. Other studies suggest that the obesity paradox is apparent only when BMI is used as a diagnostic measure, since higher BMI may reflect increased muscle mass rather than increased fat mass (*Choi, 2016; Donini et al., 2020b; Bosello and Vanzo, 2021*).

From adulthood fat mass gradually increases, reaching its peak 55-70 years of age, then it declines (*Baumgartner et al., 1998; Ferrucci and Studenski, 2015; Ding et al., 2007; Kelly et al., 2009*). This peak typically occurs at around 50 years in men and 60 years in women (*Roubenoff et al., 1995*). Before menopause, estrogens protect against obesity by reducing appetite and enhancing energy expenditure. Moreover, estrogens promote subcutaneous fat deposition while inhibiting visceral fat accumulation. During menopause, estrogen levels decline, leading to a shift toward visceral fat deposition, which increases metabolic risk, similar to patterns observed earlier in men (*Palmer et al., 2015*). With aging, fat distribution undergoes substantial changes: visceral and intramuscular fat mass progressively increase, whereas subcutaneous fat decreases. This trend observed in both sexes (*Stenholm et al., 2008*). Muscle mass begins to decline gradually from the age of 40–50 years, and this reduction, together with decreased muscle strength and function, contributes to the development of age-related (primary) sarcopenia (*Kelly et al., 2009; JafariNasabian et al., 2017; Pétervári et al., 2013*). Sarcopenia is diagnosed when at least two of the following criteria are met: reduced muscle strength (e.g. handgrip strength, knee extension), reduced muscle mass (dual-energy X-ray absorptiometry [DEXA], bioelectrical impedance analysis [BIA], or computed tomography [CT]) and impaired muscle function (chair rise test, gait speed, timed up-and-go test [TUG], or short physical performance battery [SPPB]) (*Cruz-Jentoft et al., 2019*). While women generally have lower muscle mass and strength in youth, aging affects them in a similar manner (*Coelho-Júnior et al., 2024*). Sarcopenia in old age is associated with numerous adverse outcomes and comorbidities, including increased fall risk (*Tanimoto et al., 2014)*, higher fracture incidence (*Steihaug et al., 2017*), reduced physical activity (*Benjumea et al., 2018*), greater prevalence of depression (*Chang et al., 2017*), poorer quality of life (*Giglio et al., 2018*), more frequent hospitalizations (*Cawthon et al., 2017*) and increased mortality risk (*Liu et al., 2014; Zhang et al., 2019*).

When sarcopenia is accompanied by increased fat mass this condition termed *sarcopenic obesity* (SO) (*Baumgartner, 2000*). The prevalence of SO is increasing worldwide, particularly among the elderly population, where it may affect one out of ten individuals (*Batsis and Villareal, 2018; Gao et al., 2021; Liu et al., 2023*). Reported prevalence rates vary widely, ranging between 2.75% and 20%, depending on the diagnostic criteria and methodologies applied (*Donini et al., 2020a*). Both sarcopenia and obesity

independently shorten healthspan and lifespan, reducing quality of life. Their coexistence may act synergistically. Some studies suggest that obesity has a greater impact on self-reported mobility limitation, decreased physical activity, and impaired function than sarcopenia alone (*Rolland et al., 2009; Zamboni et al., 1999*).

Although the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity published new consensus-based diagnostic criteria for SO in 2022 (*Donini et al., 2022*), the definitions and diagnostic methods in the literature remained heterogeneous (*Donini et al., 2020a; Gao et al., 2021)*. The BMI is the most commonly used method to assess obesity, but it does not always reflect the actual fat mass (*Bloomfield et al., 2006; Oud, 2013*). Therefore, the new consensus recommends BMI only as a screening tool (*Donini et al., 2022*). Nevertheless, in sarcopenic populations, a higher BMI often does correspond to increased fat mass. Waist circumference (WC) is also frequently applied, serving as a reliable indicator of visceral adiposity (*Lim et al., 2018*). Other approaches include fat percentage and fat mass index, assessed by DEXA or BIA, which allow quantification of total fat mass but not its distribution. Accurate estimation of visceral fat requires CT imaging (*Donini et al., 2020a*).

In patients with cancer or other severe illnesses, muscle mass loss is often more pronounced and correlates more strongly with mortality than in otherwise healthy, community dwelling elderly individuals. In hospitalized older patients, the coexistence of sarcopenia and obesity significantly increases mortality risk compared with non-sarcopenic, non-obese adults (*Zhang et al., 2019*). However, other studies have reported that obesity improves survival in sarcopenic elderly and cancer patients (*Liu et al., 2014; Lodewick et al., 2015*). These findings raise the question whether obesity truly exacerbates mortality risk and impairs quality of life in sarcopenic individuals.

2. Objectives

1.) Hypothesis: The long-term, age-dependent change of NPY is not linear but may show the opposite direction to catabolic neuropeptides: first, it increases in middle-aged contributing to obesity, while it decreases with ageing leading to anorexia, weight loss, and muscle mass reduction.

Few data are available regarding the long-term, age-dependent changes of anabolic neuropeptides. Based on earlier studies, a linear decrease of NPY, the most potent orexigenic hypothalamic neuropeptide, could be assumed with advancing age (*Wang et al., 1997; Coppola et al., 2004; Akimoto & Miyasaka, 2010*). However, these studies compared at most three age groups, which does not allow conclusions about the precise dynamics of these changes.

Therefore, the aim of the thesis was to examine in detail the age-dependent alterations in hypothalamic NPY activity and effects in an animal experimental model across five different age groups.

2.) Hypothesis: The quality of life of SO patients is poorer; however, their mortality risk does not differ from that of the SNO population, particularly in the elderly. Although both sarcopenia and obesity increase morbidity and mortality, the literature is inconsistent regarding how concomitant overweight/obesity affects health status and modifies mortality risk in the presence of sarcopenia. There is a controversy surrounding the effects of overweight and obesity on health outcomes and mortality depending on the age, sex, health status of the investigated population and on the tool used for the assessment of SO (*Atkins & Wannamathee, 2020; Bosello & Vanzo, 2021; Schetz et al., 2019; Lennon et al., 2016; Liu et al., 2014*). A number of studies suggest that overweight and obesity, measured by body mass index (BMI) are associated with a lower mortality risk in the older adults and in the severely ill or cancer patients known as „the obesity paradox“ (*Zamboni et al., 2005; Dorner & Rieder, 2012; Veronese et al., 2015; Marcks et al., 2021*). Since BMI does not distinguish between fat and lean body

mass, it is considered to be a poor marker of obesity (*Allison et al., 2002*). Higher BMI values are frequently associated with higher fat-free mass and not necessarily with an increase of fat mass, therefore the term „BMI paradox" was introduced (*Donini et al., 2020b*). However, studies applying different measures of body fat also confirmed the obesity paradox in severely ill patients (e.g. heart failure) and even in the sarcopenic older adults (Horwich et al., 2018; Liu et al., 2014).
Thus, based on current literature, it remains unclear how the presence or absence of obesity influences the quality of life and mortality risk of sarcopenic individuals. Some studies suggest that obesity improves survival (Liu et al., 2017), while others report that it worsens mortality risk among sarcopenic patients (von Berens et al., 2020). Therefore, my aim was to investigate, through a systematic literature search and meta-analysis, how obesity affects the mortality and quality of life of individuals with sarcopenia.

3. Experimental work and meta-analysis

3.1. Investigation of the role of neuropeptide Y (NPY) in the development of age-dependent obesity – animal study

Compared to young rodents or humans, both the level of NPY and its hyperphagic effect decrease in older age groups, which may contribute to age-related anorexia. Therefore, an age-dependent linear decline could be assumed. There is no study that examined NPY activity in more than three age groups. According to our hypothesis, the age-related change in NPY is not linear but may shift in the opposite direction of catabolic neuropeptides: an initial increase could contribute to middle-aged obesity, followed by a decrease leading to ageing anorexia, weight loss, and muscle mass reduction.
To test this hypothesis, it was necessary to establish a suitable animal model including more than three age groups and reflecting trends in body composition changes characteristic of the human population. By assessing body composition, muscle strength, and food intake (FI), we developed an animal model using male Wistar rats. We investigated the orexigenic and hypometabolic effects of intracerebroventricularly (ICV) administered NPY *in vivo*, as well as endogenous NPY activity in intact animals *in vitro* by measuring mRNA and peptide levels in the ARC region across five age groups (3–24 months).

3.1.1. Methods

Male Wistar rats from the colony of the Institute for Translational Medicine, University of Pécs, Hungary were used: 3, 6, 12, 18, and 24 months of age, corresponding to human young adult, younger or older middle-aged, aging, and old populations, respectively. Our protocols and procedures were approved by the Animal Welfare Committee of the University of Pécs and by the National Scientific Ethical Committee on Animal Experimentation of Hungary. The license was granted by the Government Office of Baranya County (BA02/2000-6/2020). They were also in accordance with the directives of the European Union (86/609/EEC, Directive2010/63/EU) and the rules of the Hungarian Government (40/2013.II.14.) on the protection of animals used for scientific purposes. This study was reported in accordance with the ARRIVE guidelines (*Percie du Sert et al., 2020*).

<u>Surgeries and drug administration</u>

Upon reaching the appropriate age, 22-gauge stainless-steel guide cannula were implanted into the right lateral cerebral ventricle. During the acute tests, a single ICV injection of NPY dissolved in PFS or PFS as control was given in random. For the chronic tests, at least one week before the ICV cannula implantation the rats underwent the implantation of biotelemetric transmitters. The ICV cannula was connected to an Alzet osmotic minipump (model 2001) which was placed under the skin of the neck and

contained solution for a 7-day-long infusion (NPY or PFS).

Assessment of acute orexigenic effects of NPY

Two weeks before the experiments, rats were transferred individually to chambers of the automated FeedScale system (Columbus, OH, USA). Powdered chow was used in order to avoid hoarding. Food consumption was recorded every 10 min. After the ICV NPY or PFS injections, the follow-up lasted for 24 h.

Assessment of chronic effects of NPY on energy homeostasis

In our biotelemetric system, the implanted transmitters recorded Tc, HR (for indirect assessment of metabolic rate) and spontaneous horizontal locomotor activity continuously in freely moving animals. The receiver was placed under the MiniMitter cage. Data were sampled every 5 min and averaged for 12 h periods by the computer (VitalView software). One mean value was generated for the night (active period) and one for the daytime (inactive period). Daily FI and BW were measured manually

Assessment of age-related differences in muscle strengths of intact rats

For the assessment of skeletal muscle strength, a grip strength meter for rats (model 47200; Ugo Basile, Italy) was used. A rat, held by the tail, was allowed to reflexively grasp with both forelimbs the T-shaped bar attached to the force transducer. The experimenter pulled the rat by the tail gently till the animal lost the grip. The maximum force generated before the loss of grip was automatically registered. The data were analyzed using DCA software (version1.1, Ugo Basile, Italy). Forelimb grip strength scores were obtained by averaging the force (g) of three readings for each animal, taken by the same two researchers to decrease variation. The score was adjusted to body weight of the rat (g/100 g BW).

***Post mortem* body composition analysis**

Body composition of intact rats euthanized for *in vitro* tests were determined to reveal age-related differences. Adiposity index was assessed by the measurement of bilateral epididymal and retroperitoneal fat pads and expressed as % of actual BW (*Sinitskaya et al., 2007*). To obtain an indicator of muscle mass we introduced a novel muscle index: the left tibialis anterior, soleus, extensor digitorum longus, and extensor hallucis longus muscles were removed, and their wet weights were calculated for 100 g BW.

RNAscope ISH combined with immunofluorescence

Our *in vitro* studies were conducted in collaboration with the Institute of Anatomy and the Institute of Pharmacology and Pharmacotherapy at the University of Pécs Medical School. An independent cohort of naive male Wistar rats (n=5/age group with BW similar to age-matched animals of the *in vivo* experiments) was intraperitoneally anesthetized, transcardially perfused with 50 mL 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by 250 mL 4% paraformaldehyde in Millonig's buffer. Brains were dissected, post-fixed and five series of thirty µm coronal Vibratome (Leica Biosystems, Wetzlar, Germany) sections between − 1.5 mm and − 3.5 mm to the bregma were collected and stored in anti-freeze solution at − 20 °C. Four representative ARC sections per animal were selected and subjected to a modified pretreatment for RNAscope ISH, optimized for 30 µm sections, as we recently published (*Ujvári et al., 2022*). . Npy mRNA was visualized by Cy3 (1:3000) using a rat Npy probe (Cat No: 450971-C2, Advanced Cell Diagnostics, Newark, CA, USA, ACD). After channel development, the sections were rinsed with PBS and treated with a polyclonal sheep NPY antiserum (1:48:000, FJL #14/3A, generous gift of Dr. Istvan Merchenthaler) overnight, at room temperature. After PBS washes,

an Alexa Fluor 647-conjugated donkey anti-sheep secondary antiserum (1:500, RRID: AB_2340750, Cat No: 713-605-003, Jackson Immunoresearch Europe Ltd., Cambridgeshire, UK) was applied for 3 h. Finally, after washes, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and covered with antifade medium. The sensitivity of the test was confirmed in randomly selected ARC sections, hybridized with triplex positive (Cat No: 320891, ACD) and negative control (Cat No: 320871, ACD) probes. The positive control emitted obvious cytoplasmic fluorescence, while no signal puncta were seen in the negative control. The specificity and sensitivity of the NPY serum in the rat brain tissue was tested earlier by others and in our laboratory (*Füzesi et al., 2007; Füredi et al., 2016*). No immunosignal was seen in sections where the primary serum was omitted or replaced with normal sheep serum. Four ARC cross-section areas per animal were digitalized using Olympus FluoView 1000 confocal microscope with 40x (NA:0.8) objective. The excitation and emission of fluorophores were chosen according to the built-in settings of the FluoView software (Fv10-ASW; Version0102). Blue (DAPI), red (Cyanine 3) and white (Alexa Fluor 647) virtual colors were assigned to the dyes. 1024×1024 pixel images were taken in sequential scanning mode with an optical thickness of 3.5 μm. The density of cytoplasmic Npy mRNA signal dots was measured in five ideally cut ARC cells, per section. The density of the NPY immunoreactivity was also measured, but because the cell bodies do not contain reliably detectable amount of NPY peptide without slowing down the axonal transport by colchicine treatment (*Füredi et al., 2016*), the assessment was performed in the NPY-containing nerve fibers in the ARC. Four non-edited digital images per animal by two independent researchers (D.K.K. and Sz.E.) were quantified using the ImageJ software (version 1.52a, NIH). The four values were averaged, and this number represented one animal in the statistics. For publication, selected representative images were cropped, contrasted using Adobe Photoshop software.

Statistical analysis

Repeated-measures ANOVA or one-way ANOVA with Tukey's post hoc tests were used (SPSS for Windows 25.0). One-sample t-test was applied for the analysis of 24-h BW lost upon NPY injection. The significance was set at the level of p

3.1.2. *In vivo* results

Body composition

In our male Wistar rats, development in body weight (BW) and adiposity (indicated by adiposity index) show a continuous age-related rise until 18 months of age. A rapid growth period until 6 months of age is followed by a moderate rise reaching the peak at 18 months. Pronounced BW decline is observed in the oldest 24-month-old animals, which is combined with a significant loss of both fat and muscle mass. Interestingly, this muscle index was also significantly reduced in the 18-month-old rats demonstrating a remarkable age-related muscle loss already at the age of the highest BW and body fat. Their high BW itself does not explain this reduction of muscle index, since the absolute wet weights of their dissected muscles were also significantly lower than in the 12-month-old (middle-aged) rats with similar BW (1.47±0.04 g vs. 1.81±0.10 g, p=0.012, one-way ANOVA with Tukey's post hoc test). In summary, fat along with muscle mass increases until the age of 12 months, then the rats accumulate excess fat during the following 6 months, while starting to lose muscle. Nevertheless, the forelimb grip strength was maintained in the 18-month-old animals. Only the oldest 24-month-old rats exhibited reduced grip strength. This reduction in muscle strength and mass indicates aging sarcopenia. At this age their spontaneous daily FI also decreases indicating aging anorexia.

***In vivo* results – acute and chronic effect of ICV administered NPY injection**

We investigated the effect of NPY on FI upon ICV injection or during a 7-day ICV infusion. Administration of pyrogen-free saline (PFS) injection did not induce significant change in daytime FI of control rats. Except for the oldest animals, the acute orexigenic effect of a central NPY injection on 1-h FI as compared with agematched PFS-treated controls was significant and changed with aging. The amount of NPY-induced chow of the 12-month-old animals was significantly higher than those of 6-, 18-, 24-months-old ones. The oldest rats consumed less than the three youngest age groups.

We applied the ratio of NPY-induced cumulative FI to the corresponding spontaneous daily FI and found significantly higher effect in 12-month-old than all other age groups. Compared to the young adult 3-month-old rats, the effect of the peptide showed an increase in middle-aged animals reaching a peak in the 12-month-old group, then it decreased in older animals. Thus, the age-related rise in the orexigenic responsiveness to NPY occurred before the peaks of BW and adiposity observed at 18 months, whereas the declined responsiveness of the 18-month-old group precedes the appearance of weight loss and sarcopenia by 24 months.

The feeding activity was maximal in the first 60-min period following the NPY injection. This hyperphagia was followed by a rebound anorexia, which attenuated nocturnal feeding and resulted even in a slight transient weight loss (restricted to the first 24 h). This change in 24-h BW expressed as % of initial BW was significant only in 3- and 12-month-old rats ($-3.4\pm0.9\%$ and $-2.5\pm0.9\%$, $p=0.004$, 0.036, respectively, one-sample t-test).

Effects of chronic 7-day ICV infusion of NPY on daily FI, BW and the circadian changes in heart rate (HR), core temperature (Tc), spontaneous horizontal locomotor activity were measured in our biotelemetric system. Control animals treated with PFS did not change their FI, HR, Tc, activity during the tested 7-day period. The pre-infusion (baseline) values did not differ in NPY- vs. age-matched PFS-treated groups. Infusion of NPY significantly increased the daily FI in all age-groups as compared with their age-matched controls. Except for the 24-month-old group, we observed significant differences between NPY-treated rats and their age-matched controls during the whole infusion period. The hyperphagia lasted only for 4 days in the oldest animals.

In order to analyze NPY-induced FI changes among different age groups, we compared mean daily FI during the NPY infusion with the mean value of the pre-infusion daily FI (average for three pre-infusion days as own baseline) of the same animals in all age groups. Except for the oldest rats, this difference was significant in all age groups. The 12-month-old animals ate significantly more than the oldest ones ($p < 0.001$, one-way ANOVA with Tukey's post hoc test). The rate of increase in NPY-induced FI in relation to baseline showed similar age-related pattern as seen in acute experiments and it was significantly higher in the 12-month-old group than in other age groups. They raised their FI by 74% indicating a strong hyperphagic effect of NPY. Chronic 7-day ICV infusion of NPY suppressed metabolic rate as shown by the reduced HR of the free-moving animals especially during the active nighttime period when their metabolic rate is already higher.

The mean nighttime HR values (maxima of the circadian rhythm) decreased significantly in all age-groups as compared with their age-matched PFS-treated controls. This difference was significant during the first 4 days in the youngest group, and for 7 days in other age groups. This suppression of HR was strongest from day 1 to day 3. When comparing the mean of HR maxima on these first 3 days with the mean of their pre-infusion period (baseline HR) in each age group), the difference was significant only in 6-, 12- and 18-monthold groups. Baseline HR of the oldest rats was significantly lower than that of 6-month-old animals indicating a decline with aging ($p <0.001$, one-way ANOVA with Tukey's post hoc test). The NPY-induced fall in relation to baseline) increased with age until 12 months (on some days reaching a maximal reduction exceeding 67 beats per minute) followed by a decline in the efficacy of NPY. Analysis of all groups by one-way ANOVA followed by Tukey's post hoc test did not show any

significant age-related difference. However, when we compared only two groups directly by one-way ANOVA, HR reduction was significantly stronger in 12-month-old rats than in the 3- or the 24-month-old group ($p=0.011$ or 0.049, respectively). Except for the 6-month-old rats, NPY infusion also induced a moderate, but significant reduction of the mean daytime HR (nadir of the circadian rhythm) in all age groups as compared with their age-matched PFS-treated controls. . The difference was significant to day 5 in 3- and 24-month-old rats, the reduction remained significant throughout the 7-day period in 12- and 18-month-old groups. The suppression of the mean HR minima during the first 3 days (compared with their baseline values) seemed most pronounced in the oldest three groups, but the Tukey's post hoc test failed to show statistically significant age-related differences ($p>0.05$). A direct comparison of the HR reduction in 12-month-old rats with the 3-month-old group revealed a significantly stronger suppression (-19 ± 6 vs. -3 ± 2 beats per minute, $p=0.004$, one-way ANOVA).

The hypometabolic effect of NPY assessed indirectly by the reduction in HR resulted in hypothermia. The mean nighttime Tc decreased significantly only in 3-, 12- and 18-month-old groups as compared with their age-matched controls. This suppression of Tc maxima lasted for 7 days and it was most pronounced from day 2 to day 6. Compared to the mean value of the pre-infusion period (baseline nighttime Tc), mean Tc maxima on the days 2–6 of the NPY infusion were reduced in the 3- and the 12-month-old groups, this fall exceeded eventually 1 °C, it was more moderate in 18-month-old rats. The decrease in Tc in relation to baseline was significantly larger at 3 or 12 months than at 24 months of age. Centrally administered NPY induced significant reduction of the daytime Tc values (nadir of the circadian rhythm) from day 3 to day 7 of the infusion only in 3-month-old animals as compared with their age-matched controls.

During the NPY infusion the nighttime spontaneous horizontal locomotor activity failed to show significant changes in any group. Surprisingly, despite daytime hypometabolism and hypothermia, the NPY-treated 3- and 12-month-old animals showed significantly higher daytime activity than their age-matched controls.

The difference remained significant throughout the 7-day period. Moreover, this change was associated with disrupted circadian rhythm in 12-month-old animals since their mean daytime activity values exceeded the nighttime ones. This phenomenon could result from their high feeding activity even during the daytime period.

In contrast to other parameters, changes in BW showed age-related differences even in PFS-treated control The youngest rats gained weight (from 389.3 ± 5.4 g to 397.5 ± 4.3 g by the 7th day) corresponding to their normal growth rate that was missing in all older animals. The surgery induced a marked weight loss in the three oldest age groups, their BW started to be normalized only after day 4. Baseline BW was similar in control and in age-matched animals treated with NPY (Supplementary Table). The overall anabolic (i.e. hyperphagic and hypometabolic) effects of NPY resulted in weight gain. Except for the 6-month-old rats, the NPY infusion significantly increased the BW in all age-groups as compared with their age-matched controls. This effect was most pronounced from day 4 to day 6. Comparison of the NPY-induced mean BW changes for days 4–6 in relation to baseline with those of PFS-infusion showed age-dependent effects. In this case, the NPY-induced weight gain was significant only in 12-month-old animals. Thus, the anabolic effect of NPY shows biphasic pattern: it increases in middle-aged animals and promotes weight gain before the peak of adiposity observed at 18 months of age. Then the responsiveness of the 18-month-old group declines followed by weight loss at 24 months of age.

Aging-related dynamics in the Npy mRNA expression and NPY peptide content of the ARC

In accordance with our functional results, the RNAscope ISH combined with immunofluorescence revealed a significantly higher NPY specific signal strength density (SSD) value in the 12-month-old

group than in younger 6-month-old and older 18- or 24-month-old rats. Thus, the highest values were found in the middle-aged animals. Interestingly, an opposite age-related dynamics was observed in the Npy mRNA expression. A significant decrease in the younger middle-aged 6-month-old group was followed by a gradual increase with the course of aging.

3.1.3. Conclusions

Our experiments confirmed that the activity and anabolic effect of the central NPY system change with age in a non-linear manner: in young adult (3-month-old) and 6-month-old middle-aged rats, the orexigenic response remained preserved, but the hypometabolic effect was attenuated, therefore the anabolic effect of NPY was not observed in the 6-month group. In contrast, in 12-month-old middle-aged rats, the effect of NPY was strongest, both in terms of orexigenic and hypometabolic responses, leading to enhanced body weight gain, fat accumulation, and disruption of circadian rhythm. Endogenous NPY activity was also elevated in this group, partly due to reduced inhibitory effects of the leptin and melanocortin systems. In aged (18–24 months old) animals, NPY hypersensitivity was attenuated, contributing to late-life weight loss, muscle mass reduction, and sarcopenia, while fat mass and ectopic fat deposition continued to influence muscle condition. Our findings emphasize the age-dependent, complex role of the NPY system in the regulation of energy balance and body weight, and highlight its potential therapeutic relevance in the treatment of age-related anorexia, while targeted application of NPY antagonists may be relevant in age-dependent obesity.

3.2. Investigating the impact of obesity in sarcopenia – mortality and morbidity risk – systematic literature review and meta-analysis

To date, no meta-analysis has directly compared the impact of SO on mortality or quality of life to that of sarcopenia without obesity (SNO). We, therefore, aimed to review the literature complemented by a meta-analysis to evaluate the impact of the additional obesity in sarcopenic patients on health outcomes. We also aimed to investigate the potential influence of age on mortality in community-dwelling adults or in severely ill patients and that of the tools used for the assessment of obesity. Since assessment of muscle functional parameters (primarily based on muscle strength) is a fundamental component of SO in the diagnostic process according to the new consensus (*Donini et al., 2022*), we also took into consideration the diagnostic methods for sarcopenia. We hypothesized, that despite their worse quality of life, the mortality of SO adults does not exceed that of SNO ones, especially in the older population.

3.2.1. Methods

Search strategy, selection and data extraction

Our search was conducted in MEDLINE, EMBASE, Scopus, and CENTRAL databases until 20th February 2023 independently by two investigators (S.E., K.D.). The search query was: (sarcopeni* OR sarcopaeni*) AND (obes* OR overweight* OR adipos* OR ”fat mass”) without any restriction. The symbol ’* ’ represents truncation. All search results were combined in and duplicates were removed with a reference manager software, EndNote X9 (Clarivate Analytics, Philadelphia, PA, US) and manually. We manually searched the reference lists of eligible articles and relevant reviews to identify additional studies. The cited and citing (by Google Scholar search engine) articles were also screened for eligible articles. No supplementary information was obtained from investigators of the original clinical studies, only published data were used. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42020162748).

After screening for title and abstract, all potentially relevant studies were retrieved for full-text

evaluation. The included studies had to provide data about at least one health outcome (e.g. mortality, adverse events or comorbidities related to SO, markers of inflammation and parameters describing physical or mental function) in SO and SNO groups of adults and these data have to be sufficient for the direct comparison of these groups. The exclusion criteria were the followings: animal experiments, no clear diagnostic criteria of sarcopenia or obesity. If duplicate studies providing the same type of outcomes were found within the same data source, the larger population was selected. We extracted the following data from each study: publication year, sample size, type (design) and location (country) of the study, sex and age of the participants, presence of tumor or severe illness, method of measurement of sarcopenia and overweight/obesity, control for confounding, outcome measures with corresponding measures of precision (e.g. standard deviation) and follow-up period. Data from those studies which applied different methods to determine sarcopenia and obesity were extracted according to each method. The values adjusted for most confounders were extracted.

Risk of bias assessment

Risk of bias for each study was assessed using the nine-star Newcastle-Ottawa Scale (NOS) adapted for cohort studies (*Stang, 2010*) and cross-sectional studies (*Herzog et al., 2013*).

Data analysis

We analyzed continuous, survival and binary outcome types. For continuous outcomes we used the mean difference (MD) as effect size measure. To calculate MD and its variance, we extracted the mean value, the standard error, the standard deviation, the confidence interval (CI) limits and the sample size in SO and SNO groups.

We presented our findings on forest plots reporting the average effect size, its 95% CI and the result of the statistical test. If it was applicable, we reported the 95% summary prediction interval (PI), following the recommendation of IntHout (*IntHout et al., 2016*). For all analyses we used the random effects model with the Hartung-Knapp adjustment (*Hartung and Knapp, 2001*) to prevent false positive findings. Statistical significance was considered if p value < 0,05. Due to weighting methods, data with low participant numbers were assigned with lower weights during the analysis. We carried out a given analysis if data from at least three studies were available. For the summary estimate of OR where the raw data were available, we applied the Mantel-Haenszel method. To assess statistical heterogeneity Q test and I 2 statistics were calculated. We considered the Q test significant if $p < 0.1$. I-squared statistics represents the percentage of effect size heterogeneity that cannot be explained by random chance. Heterogeneity could be interpreted as moderate (30–60%), substantial (50–90%) or considerable (above 75%) (*Higgins et al., 2019*).

We carried out separate analyses

1) for the community-dwelling adults, for severely ill/hospitalized patients and for cancer patients,
2) for different follow-up periods,
3) for different applied methods to define obesity.

The available data allowed sensitivity analyses for the mean age of the population and for the levels of obesity: we also performed the tests without those studies in which 1) the mean age was lower than 65 years; 2) SO group included not only adults with obesity but also adults with overweight. If the available data allowed, we performed additional sensitivity analyses by excluding those studies, in which sarcopenia was diagnosed only based on reduced muscle mass. We used the meta-regression model to explore the potential effect of the mean age (if it was not reported, the median age) of the analyzed populations on the effect size (HR of mortality). In each case, we tested the aggregate age (SO and SNO) as covariate and reported the regression coefficients, 95% CI-s, and the p value. To test the presence of

publication bias (small-study effect) we assessed the symmetry of the funnel plots visually. All statistical analyses were performed with R program (R core Team 2020, V4.0.3) using meta (version 6.1–0) and metafor (version 3.0–2) packages.

3.2.2. Results

Search, selection and characteristics of the studies

The systematic search identified 15060 records. After removing duplicates, screening of titles, abstracts and full-text papers for eligibility, 112 studies were included in the qualitative synthesis, 47 of which were not appropriate for meta-analysis.

The available number of studies allowed the analysis of the following parameters as outcomes:

- all-cause mortality,
- cardiovascular disease (CVD) events and mortality risk,
- comorbidities related to SO (metabolic syndrome, diabetes mellitus, hypertension, hyperlipidemia, stroke, other heart disease),
- serum level of lipids,
- fasting glucose,
- C-reactive protein (CRP, inflammatory marker used as a predictor of mortality, Crimmins et al., 2008),
- blood pressure (BP),
- cognitive functions assessed by the Mini-Mental Scale (MMSE),
- depression,
- physical function (TUG, experience of falls, arthritis, osteoporosis, bone mineral density).

Study quality

Scores ranged from 4 to 9 (mean score of cohort studies = 7.1, mean score of cross-sectional studies = 7.0). Quality of the individual studies was relatively high: 68% of the cohort studies and 70% of the cross-sectional studies were of high quality, the remaining 32% and 30% received medium scores, respectively.

All-cause mortality in sarcopenic community-dwelling populations with obesity vs. without obesity

The analysis of studies that investigated all-cause mortality in community-dwelling adult populations showed significantly lower risk of mortality in the SO compared to the SNO group (HR: 0,92, 95% CI 0,85-1,0, p = 0,04). Sensitivity analysis by excluding data items of those studies), in which adults with overweight were also considered as SO patients, indicated similar risk of mortality in the SO compared to the SNO group (HR: 0.94, 95%CI 0.84, 1.05, p = 0.213; I^2 = 20%, p = 0.260). Only four publications considered grip strength in the definition of SO. Analysis of their data yielded similar result (HR: 0.95, 95%CI 0.82, 1.09, p = 0.382; I^2 = 32%, p = 0.183).

The separate analysis of these older adults (mean age over 65 years in each study) also showed significantly lower risk of mortality (by around 15%) in the SO compared to the SNO group. Even the sensitivity analysis by including only data items of studies, in which obesity, but not overweight was considered as SO, indicated significantly lower mortality of the SO vs. SNO older adults. With regard to the prediction intervals shown in our forest plots they also confirmed a decreased expected mortality risk for older populations with SO while the prediction interval for the whole population (including studies with mean age of the participants below 65 years) crosses the HR = 1 line. Nevertheless, the larger part of this interval falls below HR= 1, therefore the mortality risk in most study populations are

expected to be higher in the SNO groups. Similarly significant results were obtained from the analysis of the ORs based on another pool of studies that provided binary data of mortality: we found 38% lower mortality risk in the SO vs. SNO community-dwelling older adults (mean age over 65 years).
The follow-up periods ranged from 3 years to 33 years in these studies. We analyzed the follow-up periods in two subgroups: over and under 10-year follow-up duration. The results of these subgroups did not differ significantly (p = 0.772). Only a few publications considered grip strength in the definition of SO. Analyses of their data showed similar tendencies, but the results did not reach statistical significance. The low number of available studies may have contributed to the lack of statistical significance in these analyses. Our observation suggests that additional obesity does not worsen the mortality of sarcopenic adults, it would be rather associated with lower mortality risk, especially in the older adults.
For the assessment of obesity anthropometric measurements (BMI or WC) or different measures of body fat (CT, BIA, DEXA) were applied. Analysis of HR from those studies which assessed obesity by BMI showed similar risk of mortality in the SO compared to the SNO groups of adults (HR: 0.95, 95%CI 0.87, 1.03, p = 0.187; I^2 = 0%, p = 0.525). In contrast, the separate analysis of the older adults (mean age over 65) showed significantly lower risk of mortality in the SO. This result indicates obesity paradox in the older sarcopenic subjects with BMI-based SO definition, i.e. "BMI paradox". Analysis of the three studies which defined obesity on WC measurement showed similar risk of mortality in the community-dwelling older adults with SO compared to the SNO group. Similar result was obtained from the analysis of the ORs based on another pool of studies that provided binary data of mortality. In contrast to these results based only on a few studies, analysis of those studies which provided data on the measurement of body composition (obesity assessed by DEXA or BIA) yielded significant results. . Surprisingly, we found 15% lower mortality risk of sarcopenic groups with obesity compared to those without obesity among the community-dwelling older adults (p = 0.013). Although the prediction interval slightly crosses the HR = 1 line, the larger part of this interval falls below HR= 1, therefore the mortality risk in most study populations are expected to be higher in the SNO groups.

All-cause mortality risk in sarcopenic severely ill or cancer patients with obesity vs. without obesity

In contrast to healthy community-dwelling adults, the separate analysis of the severely ill/hospitalized or cancer patients demonstrated similar risk of mortality (HR) in the SO and the SNO groups (HR: 1,06, 95%CI 0,79–1,42, p = 0,664). Interestingly, in the three oldest severely ill/hospitalized sarcopenic populations (their mean age over 70 years) the mortality ratios reached the lowest values (HR: 0.56, 0.57 and 0.68, respectively), in contrast to other studies involving younger patients. Similar non-significant results were obtained from the analysis of odds ratios (OR) based on another pool of studies that provided binary data of mortality, even when muscle function parameters were considered in the definition of SO. These results did not depend on the tools used for the assessment of obesity as shown by the following analyses. Additional obesity assessed by BMI or measurement of fat mass using DEXA, BIA or CT (HR: 1.00, 95%CI 0.60, 1.66, p = 1.000; I 2 = 48%, p = 0.075) did not increase or decrease the mortality (HR) in severely ill sarcopenic patients. Only two studies on cancer patients assessed obesity by visceral fat area using CT. Their mortality risk did not differ from the overall point estimate of cancer patients.

Age-dependence of the all-cause mortality risk in sarcopenic adults with obesity vs. without obesity

Meta-regression analyses were performed to investigate whether the age could explain the inconsistent

results. Meta-regression analysis of the relationship between mean (or median) age of the analyzed community-dwelling adult populations and the HR of all-cause mortality in SO compared to SNO adults showed a significant negative linear correlation. This result demonstrates, that additional obesity may increase the mortality risk of sarcopenic adults at younger ages (HR above 1), but it does not worsen the mortality in older age. Moreover, higher fat mass seems to be protective (HR below 1) in very old age above 65–70 years (cp. significantly lower relative mortality risk of the SO compared to the SNO groups of older adults. In contrast, meta-regression did not show association between age and HR in severely ill or cancer patients.

Cardiometabolic disorders in sarcopenic populations with obesity vs. without obesity

In the community-dwelling adult SO group, the risk of cardiovascular disease was higher compared to the SNO group, although the difference was not statistically significant ($p = 0.109$), and no differences were observed for other heart diseases or stroke. However, CRP levels, indicative of inflammatory activity, were significantly higher in the SO group in both community-dwelling ($p = 0.001$) and older populations ($p = 0.012$). Regarding metabolic syndrome, the SO group exhibited a 4.59-fold higher risk, and the odds ratios for diabetes, hypertension, and hyperlipidemia were also significantly elevated, particularly in older individuals. Accordingly, in the elderly SO group, significantly higher fasting glucose, triglyceride, and blood pressure values were measured. When sarcopenia was defined based on muscle strength, the cardiovascular risk showed a similar trend, except for hypertension, where the difference was not significant. Sensitivity analyses confirmed the higher risk for diabetes and low HDL levels in the SO group, whereas fasting glucose and triglycerides showed only a trend, likely due to the small sample size.

Physical and mental function in sarcopenic populations with obesity vs. without obesity

Based on the timed up-and-go test, physical performance was worse in the elderly SO group compared to the SNO group, whereas the incidence of falls did not differ between the groups. Obesity increased the risk of arthritis 1.68-fold among older sarcopenic individuals. Hip bone mineral density was higher in the SO group, although the difference was not statistically significant, and the risk of osteoporosis remained comparable. Assessment of mental status using the MMSE and evaluation of depression risk revealed no significant differences between the two groups, and sensitivity analyses based on muscle strength did not alter these findings.

Publication bias

Visual inspection of the funnel plots suggested no signs of small study effect.

3.2.3. Conclusions

Sarcopenic obesity represents the combination of two age-related changes in body composition. Our meta-analysis demonstrated that the concomitant presence of obesity in the elderly, sarcopenic, self-sufficient population is associated with a reduced mortality risk compared to sarcopenic non-obese individuals, thus illustrating the so-called obesity paradox. These findings can primarily be explained by interindividual differences in biological aging processes. Age-related obesity may be associated with slower aging, shifting its impact to later chronological ages; consequently, elderly sarcopenic obese individuals over 65–70 years may be biologically younger than their non-obese sarcopenic counterparts. This biologically younger subgroup exhibits lower mortality risk than older, non-obese sarcopenic individuals.

Our analyses indicate that the presence of obesity in sarcopenic elderly does not further increase the

already elevated risk of falls, cardiovascular disease, or cognitive impairment. Low muscle mass may counterbalance the potentially beneficial effects of higher fat mass on bone health. Obesity in sarcopenic individuals, however, is associated with reduced physical performance and higher risk of arthritis and metabolic syndrome compared to sarcopenic non-obese peers. In severely ill or oncological sarcopenic patients, obesity does not significantly affect mortality risk, as the underlying disease exerts the predominant influence on survival.

These results underscore the critical importance of maintaining muscle mass and strength in the elderly population, as this may prevent or delay the onset of sarcopenic obesity and its associated complications. Given the global increase in sarcopenic obesity prevalence, there is an urgent need for comprehensive and standardized diagnostic criteria. Moreover, appropriate dietary and physical activity interventions should be implemented to slow progression and prevent associated comorbidities.

4. Clinical relevance of the work

Although numerous questions remain regarding the mechanisms underlying age-related changes in body composition and their potential as therapeutic targets, our results have contributed to a better understanding of these age-dependent processes. Furthermore, our findings facilitate the interpretation of body composition alterations observed in human populations and may provide a foundation for the development of pharmacotherapeutic targets.

In addition, the results of my doctoral work have expanded current knowledge regarding obesity in sarcopenic individuals by clarifying the associated mortality and morbidity risks compared to non-obese counterparts. The clinical relevance of this is underscored by the importance of preventing comorbidities in our aging society and maintaining a state in which older adults remain independent, thereby reducing the burden on healthcare systems, the economy, and families. These findings also provide an opportunity to slow the progression of age-related declines through individualized intervention strategies, such as nutrition and physical activity, aimed at preserving muscle mass and strength.

5. New findings

1.) According to our novel experimental animal observations, middle-aged animals exhibiting increased fat accumulation display a hyperactive NPY system, which may contribute to age-related obesity. In the middle-aged group, NPY's orexigenic and hypometabolic effects, as well as its immunoreactivity, were enhanced, but these effects declined in old age, preceding the onset of anorexia and weight loss. These changes may contribute both to age-related obesity and to anorexia and sarcopenia in older age, and should therefore be considered in the development of future NPY-targeting pharmacotherapies. Our findings highlight the age-dependent differences in NPY's anabolic effects, which is particularly relevant for drug dosing considerations.

2.) New findings from our meta-analysis:

- In older adults, sarcopenic obesity is associated with better survival than sarcopenia alone (obesity paradox).
- The impact of obesity on mortality risk is age-dependent.
- Although obesity impairs physical function and exacerbates metabolic syndrome in sarcopenia, it does not significantly affect mortality risk in severely ill sarcopenic patients.

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Publication list
First author's publications on which the thesis is based
Eitmann S, Matrai P, Hegyi P, Balasko M, Eross B, Dorogi K, Petervari E. Obesity paradox in older sarcopenic adults - a delay in aging: A systematic review and meta-analysis. Ageing Res Rev. 2024 Jan;93:102164.
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